Coagulation, fibrinolysis and endothelial cell activation in abdominal aortic aneurysm repair

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Abstract

Background

In patients undergoing ruptured and non-ruptured AAA repair, major cardiac events, multiple organ failure and haemorrhage are responsible for the majority of the morbidity and mortality.

Hypothesis

Deranged coagulation, fibrinolysis and endothelial activation contribute to the thrombotic and haemorrhagic complications associated with AAA repair.

Aims

1. To determine the true hospital-based and overall community-based mortality rate for ruptured AAA within the catchment area served by a regional vascular surgical unit.
2. To examine coagulation, fibrinolysis and endothelial activation in patients undergoing ruptured and non-ruptured AAA repair.
3. To examine the relationship between coagulation, fibrinolysis and endothelial activation and morbidity and mortality in these patients.
4. To investigate possible pathogenetic mechanisms for haemostatic derangement.

Methods and Results

A retrospective study identified 741 patients with ruptured AAA admitted to a regional vascular unit over a 14-year period. Of these, 616 patients underwent attempted repair with an operative mortality rate of 37%. The 'intention to treat' mortality rate was 42%. During the study period, a greater proportion of patients underwent attempted repair

with an associated increase in operative mortality rate but no change in overall mortality. Cardiac events, renal and respiratory failure, haemorrhage and coagulopathy, limb ischaemia, stroke and pulmonary embolism were the major factors contributing to peri-operative mortality.

Of 972 patients diagnosed with ruptured AAA within the catchment area of a regional vascular unit over a 7-year period, only 39% of patients were admitted to the regional unit. Overall only 35% of patients underwent operation, 90% of operations being performed in the regional unit. The overall community-based mortality rate was 79%, similar to that where centralisation of vascular services has not occurred.

Markers of coagulation and fibrinolysis were examined in 10 patients undergoing repair of ruptured and nine patients undergoing repair of asymptomatic non-ruptured AAA. All patients survived to at least 24 hours after operation. Ruptured AAA repair was associated with peri-operative thrombin generation and inhibition of systemic fibrinolysis. Prolonged duration of symptoms before admission was associated with increased thrombin generation; and increased operative blood loss was associated with reduced fibrinogen and platelet count, and prolonged clotting times. In patients undergoing non-ruptured AAA repair, the majority exhibited increased peri-operative thrombin generation and a proportion demonstrated increased systemic fibrinolysis. Increased operative blood loss and prolonged aortic clamp times were associated with reduced fibrinogen and platelet count, prolonged clotting times and inhibition of systemic fibrinolysis.

Haemostatic markers were examined pre-operatively in 22 patients with asymptomatic, seven patients with acutely symptomatic and 37 patients with ruptured AAA. The study confirmed the above findings. Haemostatic markers were not significantly different between normotensive and hypotensive patients with rupture. Acutely symptomatic AAA

was associated with reduced thrombin generation and increased systemic fibrinolysis compared with rupture. There were significant differences in haemostatic markers between symptomatic AAA and normotensive patients with ruptured AAA, such that elevated PAI activity was more accurate than previous experience with emergency computed tomography.

The endothelial marker, von Willebrand Factor, and platelet count were examined in 20 patients undergoing repair of ruptured and 10 patients undergoing repair of asymptomatic AAA. Endothelial activation was present pre-operatively in 60%, and post-operatively in almost 100% of both groups of patients. There was a significant positive association between peri-operative vWF levels and platelet count in both groups. In ruptured AAA, increased operative blood loss was associated with reduced intra-operative vWF levels. In non-ruptured AAA, prolonged aortic clamp time was associated with reduced intra-operative vWF levels and platelet count.

Eight patients with ruptured AAA who developed peri-operative coagulopathy and haemorrhage were studied. All patients had increased thrombin generation but, unlike those who survived to 24 hours, there was evidence of increased systemic fibrinolysis on admission and/or before aortic declamping in five patients, all of whom died, four within the first 24 hours after surgery.

The relationship between haemostasis and myocardial injury was examined in 10 patients undergoing ruptured AAA repair and nine patients undergoing repair of asymptomatic AAA. Cardiac troponin I levels demonstrated peri-operative myocardial injury in 80% of patients with ruptured and 33% with non-ruptured AAA. In ruptured AAA, increased intra-operative inhibition of systemic fibrinolysis was associated with increased post-operative cardiac troponin I levels.

Markers of haemostasis and the endothelial vasopressor agent, endothelin, were examined in 14 patients undergoing ruptured AAA repair. Patients who developed acute renal failure and fatal organ dysfunction had significantly lower peri-operative endothelin levels. Increased peri-operative endothelin levels were associated with reduced thrombin generation.

Markers of haemostasis, endothelial activation and soluble TNF receptors were examined in 16 patients undergoing repair of ruptured and 10 patients undergoing repair of asymptomatic AAA. Ruptured AAA repair was associated with elevated levels of soluble TNF receptors, and elevated levels during early reperfusion were associated with increased post-operative mortality. Increased soluble TNF receptors were associated with increased thrombin generation, inhibition of systemic fibrinolysis and increased endothelial activation.

Declaration of originality

The work in this thesis was carried out while I was a Clinical Research Fellow in the Edinburgh Vascular Surgery Unit. The work described in this thesis is my own, the concept and design of all the studies in this thesis were my own and the thesis was composed by me. I carried out all patient recruitment, data collection, sample handling, processing and storage. Soluble tumour necrosis factor receptor, cardiac troponin I and C-reactive protein assays were performed by myself in the Lister Surgical Research Laboratories, University Department of Clinical and Surgical Sciences, Royal Infirmary of Edinburgh. I carried out all statistical analysis and data interpretation, and created all the tables and figures. The thesis has not been submitted in candidature for any other degree, diploma or professional qualification.

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Dedication

To my wife, Sarah, and my children, Daniel and Anya, for their love and patience.

Abbreviations

AAA	=	abdominal aortic aneurysm
AF	=	atrial fibrillation
aPTT	=	activated partial thromboplastin time
ARDS	=	adult respiratory distress syndrome
ARF	=	acute renal failure
AT	=	antithrombin
BKA	=	below knee amputation
CCF	=	congestive cardiac failure
CK	=	creatine kinase
CK-MB	=	myocardial iso-enzyme of creatine kinase
CLI	=	critical lower limb ischaemia
CRP	=	C-reactive protein
cTn	=	cardiac troponin
CVA	=	cerebrovascular accident
DVT	=	deep venous thrombosis
ECG	=	electrocardiograph
ECLT	=	euglobulin clot lysis time
EDTA	=	ethylene diamine tetra-acetic acid
ELISA	=	enzyme-linked immunosorbent assay
ERVSU	=	Edinburgh Regional Vascular Surgery Unit
ET	=	endothelin
FDPs	=	fibrin/fibrinogen degradation products
FFP	=	fresh frozen plasma
IL	=	interleukin
IPPV	=	intermittent positive pressure ventilation
ITU	=	intensive therapy unit
MI	=	myocardial infarction
MOF	=	multiple organ failure
NO	=	nitric oxide
PAI	=	plasminogen activator inhibitor
PAP	=	plasmin-antiplasmin
PF	=	prothrombin fragment
PT	=	prothrombin time
RCC	=	red cell concentrate
RRT	=	renal replacement therapy
sTNF-R	=	soluble tumour necrosis factor receptor
TAFI	=	thrombin-activatable fibrinolysis inhibitor
TAT	=	thrombin-antithrombin
TEG	=	thromboelastography
TF	=	tissue factor
TFPI	=	tissue factor pathway inhibitor
TM	=	thrombomodulin
TNF	=	tumour necrosis factor
t-PA	=	tissue plasminogen activator
vWF	=	von Willebrand factor

Chapter 1

General Introduction

1.1

Outcome of ruptured AAA

In the UK, infrarenal AAA greater than 4cm in diameter is present in 2.3% of men aged 65 to 74 years (1), and aneurysm rupture is responsible for 1.7% of all deaths in this group (2). The natural history of aortic aneurysms is that they will rupture unless the patient dies from another cause or the aneurysm is repaired. Once rupture has occurred, death inevitably follows unless the aneurysm is successfully repaired.

The true mortality rate for ruptured AAA in hospital-based studies varies considerably and results are often difficult to interpret. Many published series, for example, do not report patients who survive to reach the hospital only to be denied repair, or who succumb during transfer from the resuscitation room to the operating theatre (3). Furthermore, many series, particularly those from North America, describe small numbers of highly selected patients operated upon over long time periods. For example, in a 26-year period, Crawford's group in Houston (4) operated upon 60 patients with ruptured AAA achieving an impressive 30-day mortality rate of 23%. Lawrie *et al* (5) and Cooley and Carmichael (6) are responsible for the lowest published operative mortality rates for ruptured AAA repair; respectively, 15% in 61 patients over a 14-year period, and 21% in 75 patients over a 10-year period. While there are several large multicentre studies reporting operative mortality rates of between 38% and 64% (7-11), there are few reports from single centres which describe experience with more than 100 patients (3,12-23) (Table 1.1).

TABLE 1.1

Single centre experiences with more than 100 patients undergoing ruptured AAA repair.

Author	Study period	Number of patients undergoing operation	Operative mortality rate
DeBakey (12)	1952 - 1963	117	34%
Shumacker (20)	1953 - 1972	158	55%
Hildebrand (21)	1955 - 1973	131	51%
DiGiovanni (22)	1953 - 1974	107	68%
Gaylis (23)	1963 - 1977	105	58%
Wakefield (13)	1964 - 1980	116	52%
Fielding (14)	1960 - 1981	198	43%
Jenkins (15)	1974 - 1984	174	33%
Shackleton (16)	1975 - 1985	106	41%
Johansen (17)	1980 - 1989	180	69%
Gloviczki (3)	1980 - 1989	214	45%
McCready (18)	1980 - 1989	208	49%
Nasim (19)	1979 - 1991	317	58%
		2131	50%

Population studies show that a significant proportion of patients with rupture will either not survive to reach hospital or will not undergo attempted repair, such that the overall community-based mortality rate is between 79% and 94% (24-29).

It is widely believed that surgical and anaesthetic sub-specialisation leads to better patient outcomes. As a result of clinical, political and economic factors, there is an increasing tendency in the UK to centralise vascular surgical services within large regional vascular units. The effect of such centralisation of vascular surgical services on individual patient outcome and the overall prognosis from ruptured AAA remains unknown.

1.2

Complications of ruptured AAA repair

Cardiac events, respiratory failure and renal failure are responsible for the majority of the morbidity and mortality associated with ruptured AAA repair.

Myocardial infarction

Complications of coronary artery disease are the most common causes of early mortality in vascular surgical patients. In patients operated for rupture, MI occurs in 6-24% (3,14,15,30) with a mortality rate of 60-100% (3,10,14,15,30). Myocardial injury is discussed in more detail in section 1.8.

Respiratory failure

This is reported in 25-48% of patients operated for rupture (3,13,14,23) with an associated mortality rate of 34-70% (3,14). Patients at increased risk include the elderly, cigarette smokers and those with chronic obstructive airways disease. Respiratory complications are more frequent in association with emergency procedures, prolonged general anaesthesia, thoracic and upper abdominal incisions, prolonged peri-operative whole body hypoperfusion and massive blood transfusion.

There are several pathophysiological processes which may contribute to peri-operative respiratory failure in patients undergoing aortic surgery. Increased pulmonary capillary permeability secondary to aortic cross-clamping, cytokinaemia secondary to lower torso ischaemia and reperfusion, and excessive peri-operative fluid resuscitation lead to an increase in extravascular lung water and ARDS. Coagulopathy leads to pulmonary microvascular thrombosis and haemorrhage, identical to that seen in ARDS (31).

Myocardial injury and left ventricular dysfunction lead to pulmonary oedema. Post-operative pain and impaired consciousness may lead to shallow tidal breathing and alveolar collapse, with sputum retention and secondary bacterial pneumonia. Impaired consciousness and post-operative ileus may also lead to the aspiration of gastric contents and subsequent pneumonitis.

Acute renal failure

The incidence of ARF in ruptured AAA repair is 18-33% (3,14,30) but may be as high as 72% (13). The associated mortality is 33-76% (3,14). ARF in isolation is associated with a mortality rate of 25-70%. When it occurs in association with MOF involving three or more organ systems, ARF is almost universally fatal.

The patients' age, pre-morbid renal function and the presence of renal artery occlusive disease are important risk factors for the development of ARF. There are several pathophysiological processes which may contribute to the development of ARF in aortic surgery (32). A massive shift of body water from the circulation to the extracellular and third spaces occurs as a consequence of operative dissection, inadequately replaced blood loss, altered acid-base balance in ischaemic tissues, increased inflammatory mediators due to ischaemia and reperfusion, and low cardiac output during aortic declamping. Acute tubular necrosis may occur secondary to renal ischaemia caused by renal hypoperfusion during prolonged suprarenal aortic clamping, whole body hypoperfusion during haemorrhagic shock, and myocardial dysfunction secondary to arrhythmia or MI. Aortic clamping is associated with reduced renal cortical blood flow during the period of ischaemia and immediately on reperfusion, and furthermore, inflammatory mediators released on aortic declamping may damage renal parenchyma. Renal athero-embolism may occur secondary to suprarenal aortic manipulation or

clamping if there is atheroma in the proximal aorta. Finally, post-operative complications such as sepsis and coagulopathy lead to ARF secondary to haemodynamic instability and deposition of fibrin thrombi in the microvasculature.

Stroke

This may occur in approximately 5% of patients and is associated with a mortality rate of 50% (3). The presence of pre-existing carotid artery occlusive disease is an important risk factor. Possible mechanisms include cerebral hypoperfusion secondary to haemorrhagic shock and haemodynamic changes which occur during aortic clamping and declamping, as well as embolisation from intra-cardiac clot secondary to MI or arrhythmia. A procoagulant state has been shown to be associated with CVA (33).

Venous thrombo-embolism

This is a multifactorial condition which occurs as a result of venous stasis and/or vessel wall damage in the presence of a hypercoagulable state. A hypofibrinolytic state has been demonstrated in approximately 30-40% of patients with DVT. Risk factors in patients operated for AAA include age, prolonged surgery and cardiovascular complications. Pulmonary embolism may lead to progressive or sudden cardiorespiratory failure.

Lower extremity ischaemia

Lower limb ischaemia occurs in 1-10% of patients following AAA repair (23,34). This may occur for three principal reasons: distal micro- or macroscopic embolisation of atheromatous debris or thrombus due to aortic manipulation; thrombotic occlusion of pre-existing arterial occlusive disease or popliteal aneurysm secondary to low flow or a hypercoagulable state; and embolisation of intra-cardiac clot secondary to MI or

arrhythmia. Microembolisation obstructing small arterioles leads to a reactive obliterative endarteritis and subsequent tissue ischaemia and infarction. It affects not only the lower extremities (when it is colloquially called 'trash foot') but may involve the kidney, colon and spinal cord.

Bowel ischaemia

This most commonly affects the left colon owing to its precarious blood supply and poor collateral circulation if arterial occlusive disease is present. Transmural infarction may occur in up to 5-10% of patients undergoing ruptured AAA repair and is associated with a mortality rate of approximately 70% (3). Lesser degrees of ischaemia may be present in over 50% of patients. Bowel ischaemia may occur occur due to hypovolaemic shock, local pressure effects from retroperitoneal haematoma, traction on the small bowel mesentery, ligation of the inferior mesenteric artery, bypass or ligation of the internal iliac arteries, reduced cardiac output, ischaemia and reperfusion injury, and the presence of a hypercoagulable state (35).

Multiple organ failure

MOF may account for up to 37% of post-operative deaths (17,35) in ruptured AAA. Failure of two or more organ systems is associated with a mortality rate approaching 100%. Factors contributing to the development of MOF include: a) blood loss, transfusion and malnutrition leading to depressed immune function; b) tissue ischaemia and reperfusion leading to leucocyte activation and the production of cytokines. This results in endothelial, platelet and leucocyte activation, and increased expression of endothelial adhesion molecules, which are involved in the recruitment of leucocytes across the endothelium and into adjacent tissues. Oxygen-derived free radicals and

proteases then damage the endothelium and surrounding tissues; c) bowel ischaemia leading to endotoxaemia, bacterial translocation, and peritoneal contamination and sepsis, all of which lead to further cytokine production; d) generalised procoagulant state and coagulopathy leading to macro- and microvascular thrombosis which compromises end organ perfusion (35-38).

Haemorrhage

This may occur for technical reasons and/or because of abnormal haemostasis. Excessive haemorrhage may occur in 17-33% of patients (3,10,22) and is associated with a mortality rate of 89-100% (3,10,30).

Coagulopathy is an important cause of haemorrhage in ruptured AAA repair. Wakefield *et al* (13) demonstrated coagulopathy in 15 of 24 (63%) patients who died intra-operatively, and 8 of 92 (9%) patients who died post-operatively. Marsh (39) reported coagulopathy during and after operation in 9 of 29 (31%) patients, eight of whom died; and haemorrhage secondary to coagulopathy was a major contributory factor in 25% of all post-operative deaths. The incidence of post-operative haemorrhage due to abnormal haemostasis is 0.8-3.7% (30,40) in elective surgery for non-inflammatory AAA. In thoraco-abdominal aortic aneurysm repair, the incidence of re-operation for bleeding may be as high as 9% in specialist centres with haemorrhage responsible for 12-38% of early deaths (41,42). In ruptured AAA repair, re-exploration for haemorrhage is not uncommon (10,11,14,15) and is associated with poor outcome. For example, Milne *et al* (43) reported re-operation for bleeding in 12 of 262 (4.6%) patients after ruptured AAA repair in the ERVSU with a mortality rate of 50%. An abnormal coagulation screen at the end of the primary operation was a universal finding.

Clinico-pathological variables associated with mortality

Many studies have attempted to identify variables which predict mortality in patients presenting to hospital with rupture, but no single variable has been shown to be 100% predictive in isolation (Table 1.2). Coagulopathy has been reported by several investigators to be associated with poor outcome. Coagulopathy and bleeding requiring re-operation or transfusion are as significant predictors of poor outcome as major cardiac events, respiratory failure, ARF requiring supportive therapy, CVA and distal embolisation (7). More recently, Davies *et al* (44) and Bradbury *et al* (45) have demonstrated a significant association between peri-operative haemostatic derangement and increased morbidity and mortality from haemorrhage, cardiac events, MOF, CVA and venous thrombo-embolism.

TABLE 1.2

Clinicopathological variables associated with poor outcome in ruptured AAA.

Author	Year	Variables
Wakefield (13)	1982	Hypotension; abnormal blood urea nitrogen and creatinine; volume of blood and fluid administered
Fielding (14)	1984	Volume of blood administered
Donaldson (46)	1985	Age > 76 years; haematocrit <0.30; persistent hypotension; acute ECG changes; technical complications; organ failure
Shackleton (16)	1987	Reduced level of consciousness; cardiac failure
Vohra (47)	1988	Volume of blood administered
Amundsen (48)	1989	Age > 72 years; hypotension
Ouriel (49)	1990	Haemodynamically unstable; renal insufficiency; chronic pulmonary disease
Harris (11)	1991	Renal failure
AbuRahma (50)	1991	Loss of consciousness; hypotension; symptom duration > 1 day; delay from emergency to theatre > 2 hours; intraperitoneal rupture
Johansen (17)	1991	Male gender; unstable blood pressure; pre-operative cardiac arrest; volume of blood administered > 15 units
Gloviczki (3)	1992	Hypotension; low haematocrit; chronic lung disease; high APACHE II score
Johnston (7)	1994	Abnormal creatinine; suprarenal aortic clamping; intra-operative urine output < 200ml

1.3

Normal haemostasis

Under normal conditions, blood circulates through the vasculature without appreciable thrombus formation or haemorrhage. Normal haemostasis acts to minimise haemorrhage and maximise perfusion. Vascular injury leads to temporary vasoconstriction and increased tissue tension due to blood loss into adjacent tissues. Subsequent formation of a platelet-fibrin plug (predominantly in the extravascular space) at the site of vessel injury restores the integrity of the circulation. Inhibition of thrombus formation in intact areas ensures that this occurs with minimal interruption to blood flow. The end result is healing of the vascular injury, removal of the blood clot and restoration of function. Normal haemostasis is a dynamic balance between fibrin formation and resolution, and is dependent on interactions between endothelium, platelets, coagulation and fibrinolysis.

Endothelial cells

The vascular endothelium forms the physical interface between blood and the underlying tissues, allows exchange and active transport of substances across the vessel wall, and has an important role in the regulation of haemostasis and the maintenance of vascular tone. Injured, denuded endothelium initiates thrombus formation by exposing the subendothelial surface to platelets and coagulation factors.

Healthy intact endothelium is negatively charged and forms a physical barrier which prevents the interaction between platelets and clotting factors, and subendothelial TF and collagen. Healthy endothelial cells also contain an endogenous heparin-like factor called heparan sulphate which acts to increase the rate of thrombin inactivation by the antithrombins. Thrombin also binds to TM expressed on the surface of endothelial cells,

and the resultant thrombin-TM complex activates protein C which, together with the endothelial product protein S, inactivates coagulation factors Va and VIIIa, and the tenase and prothrombinase complexes. Endothelial cells are the main site of synthesis of TFPI, the principal inhibitor of TF. Endothelial cells also express t-PA which activates fibrinolysis and ensures that coagulation is confined to areas of vessel injury.

Endothelial cells play an important role in the regulation of vascular tone. This is determined, in part, by certain vasodilator and vasoconstrictor substances that control vascular smooth muscle function. Thrombin, aggregating platelets, and increased shear stress stimulate production of NO which maintains arterial relaxation at rest and inhibits platelet aggregation and monocyte and leucocyte adhesion. Prostacyclin is a vasodilator and an inhibitor of platelet activation. The endothelins are the most potent endogenous vasoconstrictors known and expression is induced by thrombin, shear stress and hypoxia. ET-1 is the major isoform in blood vessels and is synthesized by endothelial cells, macrophages and vascular smooth muscle cells. It is mainly secreted abluminally but may enter the circulation if concentrations are high at the endothelial cell-vascular smooth muscle interface (51).

Endothelial cell activation involves a change in function and morphology (52) and consists of; change in phenotype from anticoagulant to procoagulant; loss of vascular integrity; cytokine production; expression of leucocyte adhesions molecules; and upregulation of human leucocyte antigen molecules. Type I endothelial cell activation occurs rapidly and does not involve de novo protein synthesis. Examples include endothelial cell retraction, and expression of P-selectin and vWF. Type II activation requires protein synthesis. An example is expression of cytokines. Endothelial cell activation is induced by a wide range of agents including ischaemia, endotoxaemia and cytokines, such as IL-1 and TNF (52,53). *In vitro*, these cytokines induce TF expression

by endothelial cells (and leucocytes) and upregulate the release of vWF into plasma (53,54) resulting in a procoagulant state. The procoagulant effects of endothelial cell activation consist of increased PAI release which inhibits fibrinolysis; increased platelet activating factor expression; diminished platelet anti-aggregatory effects due to reduced prostacyclin expression; vasodilation secondary to increased NO production; increased TF expression; and shedding of heparan sulphate. There is debate about the significance of shedding of TM from endothelial cells. Unlike vWF, increased soluble TM levels may occur independent of cytokines and reflect endothelial injury rather than activation (55).

Endothelial cell activity may be assessed by monitoring plasma levels of its specific products. vWF is the most commonly used endothelial marker. It is synthesised by vascular endothelial cells and megakaryocytes (56). It mediates platelet adhesion to the subendothelium which is essential for the formation of occlusive platelet thrombi at sites of arterial injury; it also acts as a co-factor for factor VIII (57,58). The vWF-mediated adhesion of platelets to exposed subendothelial collagen is irreversible and of considerable importance in conditions of high shear stress (59). Endothelial cell vWF accounts for 40% of total platelet adhesion, with the remaining 60% provided by plasma vWF (59). Circulating vWF is stored intracellularly in the Weibel-Palade bodies (60), the contents of which are released upon stimulation by factors such as thrombin, fibrin, TNF, IL-1 and vasopressin (54,58,61). Platelet vWF contributes little if any to plasma levels (62). Elevated levels of circulating vWF have been demonstrated as part of the acute phase response (63), and in association with risk factors for atherosclerosis. There is an increased thrombotic tendency with increased platelet adhesion in association with elevated plasma vWF, and elevated plasma levels have been demonstrated in patients with peripheral (64,65) and coronary artery disease (66-68).

Platelets

Activated platelets orchestrate all of the components of the haemostatic system leading to the formation of a stable clot; specifically, they adhere to subendothelial collagen, form aggregates by chemotaxis, promote thrombin generation by releasing factors, and provide negatively charged phospholipid for the prothrombinase and tenase complexes. Endothelial cell injury induces several biochemical mechanisms which lead to platelet activation (adhesion, aggregation, and secretion) (69). Plasma vWF binds to exposed subendothelial collagen and elastin in the damaged vessel wall and undergoes a conformational change to expose binding sites for platelets. Platelet adhesion to vWF occurs via the platelet membrane glycoprotein (GP) Ib, and is dependent on subendothelial fibronectin, and ionised calcium and magnesium. The adherent platelets form pseudopodia and flatten and spread over the injured endothelium. They express glycoprotein receptor sites for coagulation factors, the most significant of which is GP IIb/IIIa which facilitates the binding of other platelets via fibrinogen. The release of platelet components and platelet recruitment are stimulated not only by platelet adhesion, but also by agonists in the vicinity of the injury such as adenosine diphosphate (ADP), adrenaline, platelet activating factor and thrombin. Adherent platelets release ADP, ionised calcium, serotonin and thromboxane A2 which provide a positive feedback pathway for platelet aggregation. They also release large quantities of factor V which increases thrombin generation; and expose large areas of negatively charged phospholipids which are essential for the formation of the tenase and prothrombinase complexes and further increased thrombin generation.

Coagulation

Tissue injury, endothelial activation and injury, and monocyte activation leads to TF expression which triggers the extrinsic coagulation cascade (70) (Figure 1.1). Vessel wall injury leads to the exposure of subendothelial collagen and exposure and release of TF. TF forms a complex with coagulation factor VII on endothelial cells and monocytes which leads to activation of factor VII itself. The TF-activated factor VII complex activates factor X directly when TF concentrations are high, and indirectly via activation of factor IX. Activated factor IX, X and XII act in a positive feedback pathway to increase activation of factor VII. The activation of factor X by activated factor IX (via the intrinsic coagulation pathway) requires calcium ions and is increased almost 1000 times in the presence of activated factor VIII and negatively charged phospholipid. The combination of activated factor IX, vWF, activated factor VIII, calcium and phospholipid constitutes the tenase complex which is present on activated platelets and endothelial cells.

All of the components of the intrinsic coagulation pathway are present in the plasma. When it comes into contact with a foreign surface, the pre-kallikrien-high molecular weight (HMW) kininogen complex is activated to release kallikrien. This serine protease inhibitor stimulates the production of bradykinin from HMW kininogen, and activates coagulation factor XII. Activated factor XII acts in a positive feedback to increase kallikrien release and activate factor XI. Sufficient amounts of thrombin are generated to induce fibrin formation before TFPI is released. Most thrombin formation occurs within the fibrin clot by the activation of factor XI by thrombin via the intrinsic coagulation cascade. Activated factor XI in turn activates factor IX and the extrinsic and intrinsic coagulation pathways unite by the activation of factor X. Activated factor X converts prothrombin to thrombin which, through another positive feedback pathway, activates

factor V (large amounts of which are released from platelets). Activated factor X, activated factor V and calcium bind to phospholipid to form the prothrombinase complex on platelets. This increases the conversion of prothrombin to thrombin at over 300,000 times the rate of activated factor X alone. Thrombin also acts in a positive feedback by increasing the release and activation of factor VIII from its complex with vWF. The serine protease inhibitor TFPI, present in plasma and on the surface of endothelial cells, binds and inhibits activated factor X when small amounts are generated by the extrinsic coagulation cascade. This, in turn, rapidly inhibits the TF-activated factor VII complex. This negative feedback pathway inhibits activation of factor X unless there is massive expression and release of TF.

The conversion of prothrombin to thrombin leads to the release of PF 1+2. Once produced, thrombin cleaves fibrinopeptides A and B from fibrinogen in platelets and plasma, converting it into fibrin monomer. The fibrin monomer polymerises to form strands and thrombin activates factor XIII which, in the presence of calcium, stimulates the formation of a dense open mesh of insoluble cross-linked fibrin. This binds platelets via GP IIb/IIIa to form the platelet-fibrin plug, and also allows plasmin to enter the clot to initiate fibrinolysis.

Thrombin generation adjacent to normal intact endothelium is inactivated by a number of antithrombins, the most important of which is AT III. Thrombin binds to AT III, which is concentrated by intact endothelium, and splits it to form the inactive TAT complex. TM is expressed on the surface of the endothelium and binds thrombin. The thrombin-TM complex inhibits fibrin formation and platelet activation, and activates protein C which inactivates factors V and VIII and decreases thrombin generation. Protein C activity is increased 10 times in the presence of protein S. Coagulation is localised to areas of tissue injury by the fact that damaged endothelium is incapable of neutralising thrombin

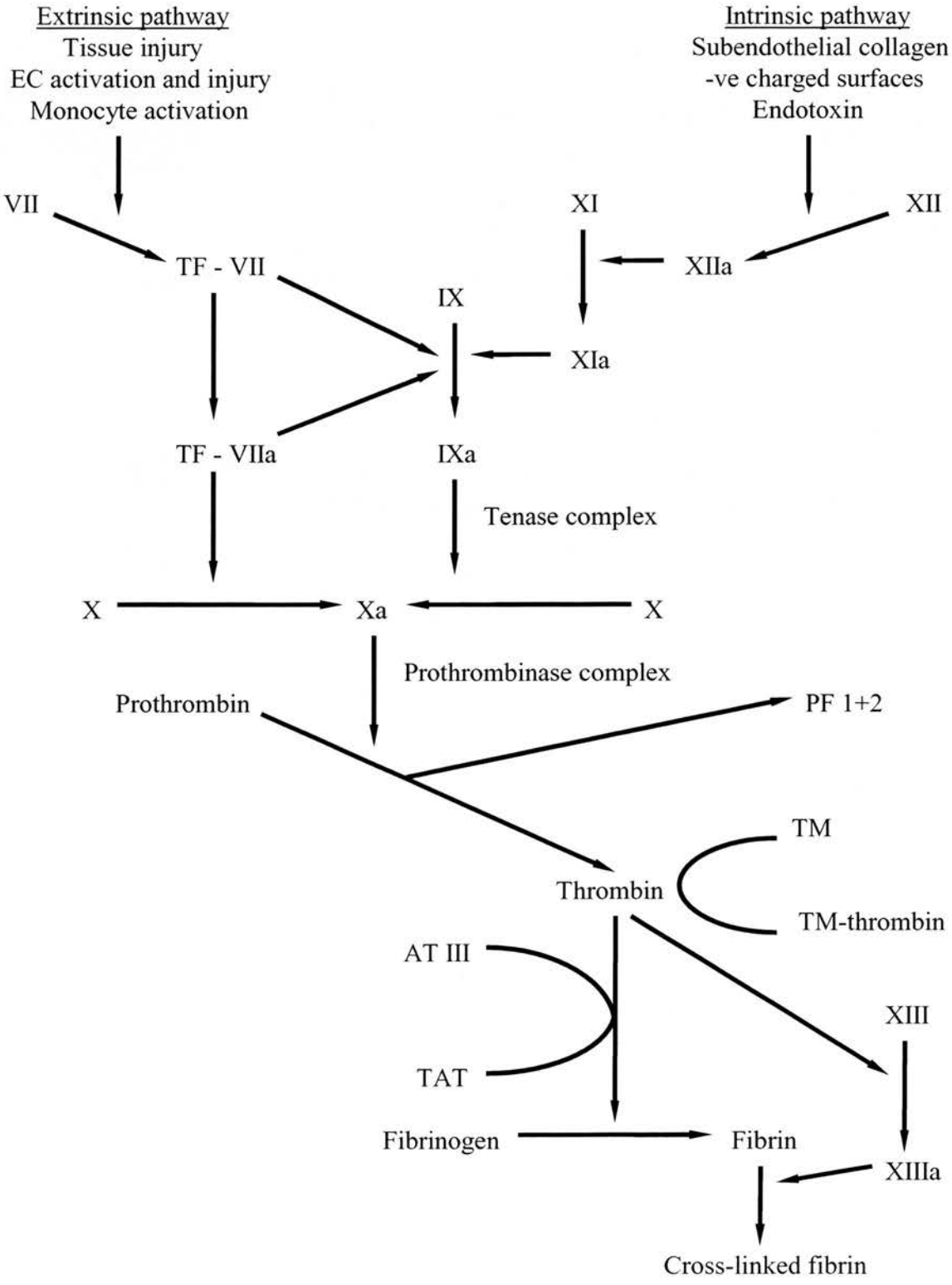
whereas adjacent intact endothelium continues to express agents such as AT III, TM and protein C.

Thrombin has anticoagulant properties at low concentrations by increasing the activation of protein C. At high concentrations, thrombin has a procoagulant and antifibrinolytic effect by overcominf the effect of activated protein C and activating TAFI.

Thrombin cannot be measured directly as, under pathological conditions, less than 1% of circulating prothrombin is transformed to thrombin, and it is rapidly inactivated by AT III. Thrombin generation can, however, be measured indirectly by assessing levels of the cleavage products released in the conversion of prothrombin to thrombin, namely PF 1+2; the activation products of the substrates of thrombin, namely fibrinopeptide A and fibrin monomer; the products generated by secondary fibrinolysis, namely FDPs and D-dimer; or more directly by measuring the amount of thrombin inactivated, namely TAT.

FIGURE 1.1

Extrinsic and intrinsic coagulation systems.



Fibrinolysis

Fibrinolysis is a physiological consequence of fibrin deposition anywhere in the body. At the same time as thrombin generation and fibrin deposition are taking place, the fibrinolytic system is secondarily activated in order to limit clot formation to the site of vessel wall injury and recanalise blood vessels after repair has taken place.

t-PA is the principal endogenous activator of plasminogen (Figure 1.2) and is rapidly released from vascular endothelial and smooth muscle cells in response to thrombin, endotoxin, cytokines and ischaemia (71-77). Plasminogen is concentrated into the forming clot and t-PA binds to the fibrin surface to form the t-PA/plasminogen/fibrin complex. t-PA activates plasminogen to the enzyme plasmin. Plasmin activity is maximal in the clot where it is protected from the circulating plasmin inhibitor, α-2 antiplasmin. Plasmin causes lysis of fibrin, fibrinogen and cross-linked fibrin clot to produce split products including FDP and D-dimer which further stimulate plasmin formation. Plasmin acts specifically in areas of excessive fibrin deposition as tPA is a poor plasminogen activator in the absence of fibrin (78).

The availability and activity of t-PA is dependent, not only on its release from endothelium, but also on neutralisation by its inhibitors, α-2 antiplasmin and PAIs, and first-pass PAI-independent hepatic clearance. PAI is the principal fast-acting inhibitor of t-PA. It is synthesised by vascular endothelial and smooth muscle cells, hepatocytes and is found in the alpha granules of platelets and in normal plasma. PAI is an acute-phase protein produced in response to sepsis, endotoxin, cytokines, thrombin, ischaemia, surgery and trauma (71,76-83). The main role of PAI would appear to be in the regulation of plasma tPA activity. PAI rapidly complexes with tPA to reduce its activity in plasma, prevent excessive plasmin formation and thus localise fibrinolysis to fibrin deposits. The binding of PAI to clot-bound tPA however is much slower. High plasma

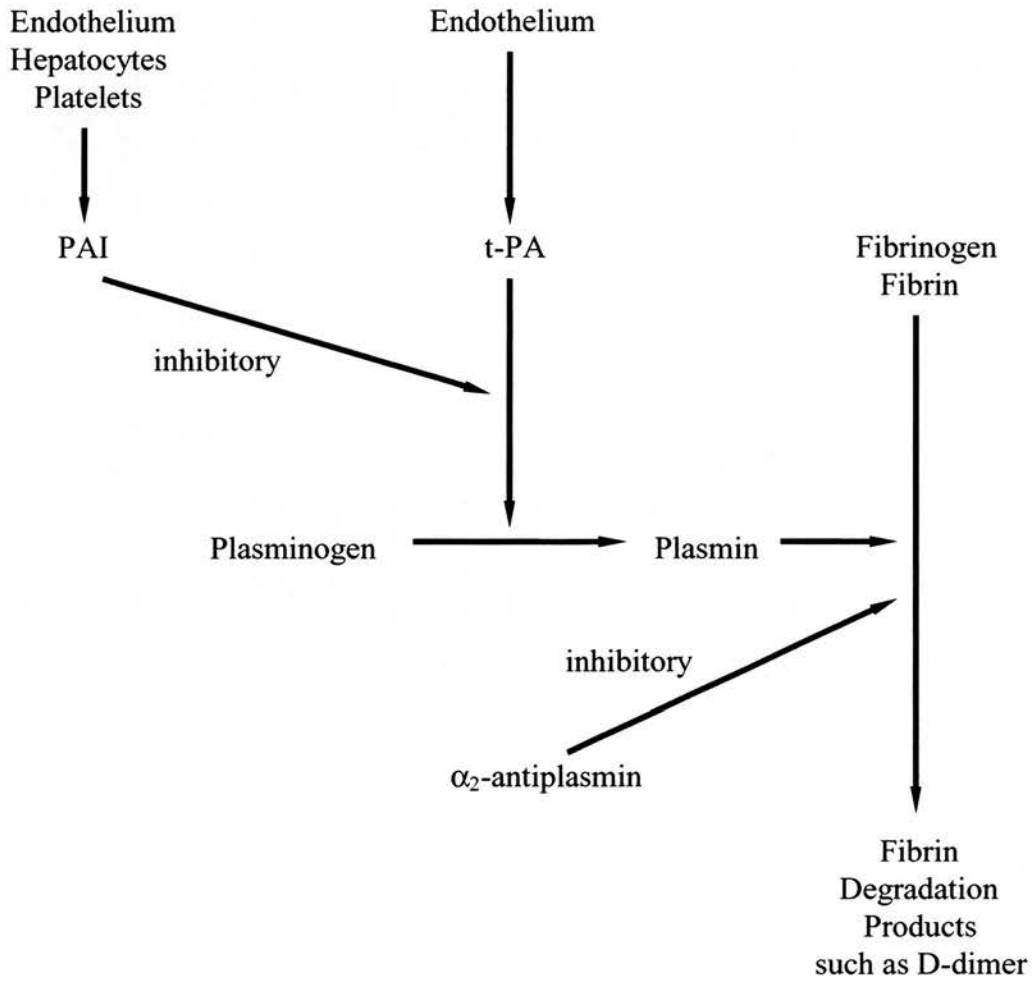
levels of t-PA antigen reflect inactive t-PA/PAI complexes rather than free active t-PA. If there is a large excess of PAI activity, then the plasma levels of t-PA/PAI complex and t-PA antigen are raised but t-PA activity is reduced. Increased PAI activity and increased t-PA antigen levels reflect reduced fibrinolytic activity. High concentrations of thrombin activate TAFI which acts on partially degraded fibrin to prevent formation of the t-PA/plasminogen/fibrin complex and thus limit plasmin formation.

Under resting conditions, platelets do not appear to contribute to plasma PAI levels. Plasma PAI has a short half-life (2 hours) and so, to maintain constant levels, there must be rapid synthesis and release into the circulation (84). Stimulation and aggregation of platelets can lead to a transient and rapid increase in PAI levels (78,85). High concentrations of platelets in blood clot may provide high PAI levels which prevent fibrinolysis. PAI's short half-life, its inactivation by activated protein C and thrombin, and the diffusion of t-PA into fibrin clot all favour fibrinolysis.

Elevated fibrinogen is a risk factor for the development of cardiovascular (86-88) and peripheral arterial disease (64,89,90), and predicts death in patients with intermittent claudication (91,92). Elevated fibrinogen may disrupt the normal equilibrium between coagulation and fibrinolysis (93). Increased FDP levels are associated with peripheral vascular disease and are predictive of coronary thrombosis in patients with peripheral vascular disease (92). Low fibrinolytic activity is associated with ischaemic heart disease and thromboembolic disease (94-99). t-PA antigen levels predict risk of MI and stroke (100,101), elevated PAI levels occur in young survivors of MI (102,103), and patients with peripheral vascular disease (64,104,105) and predict future events (103, 105).

FIGURE 1.2

Fibrinolytic system.



1.4

Abnormal haemostasis

Definition

Abnormal haemostasis or coagulopathy occurs due to a disturbance in the physiological balance between coagulation and fibrinolysis. It is a consequence of inappropriate and excessive activation of the extrinsic and/or intrinsic coagulation pathway in the general circulation rather than within the confines of an area of tissue damage.

Causes

Coagulopathy has a large number of underlying causes which may be acute or chronic (Table 1.3). TF release is the responsible for the majority of acute coagulopathies. Hypothermia and acidosis have a major impact on haemostasis as coagulation factors are enzymes which function optimally within a narrow range of temperature and pH. A chronic sub-clinical 'compensated' coagulopathy may exist in patients with aortic aneurysms. Decompensation may occur if the haemostatic system is stressed by, for example, the development of renal or hepatic impairment, rapid aneurysm expansion, aneurysm repair, or the onset of rupture. This will be discussed in more detail in subsequent sections.

TABLE 1.3

Causes of abnormal haemostasis.

Acute	Chronic
Ischaemia and acidosis	Carcinomatosis (esp. adenocarcinoma)
Hypotensive shock	Retained dead foetus syndrome
Hypothermia	Haemangioma
Trauma	Empyema thoracis
Infection, septic shock (esp. Gram -ve bacteraemia, endotoxaemia)	
Massive blood transfusion	
Burns	
ABO incompatible blood transfusion (immune complex formation)	
Massive head injury	
Placental abruption, amniotic fluid embolism	
Hepatic failure (hypersplenism, reduced vitamin K absorption, reduced clotting factor and inactivator synthesis, reduced activated clotting factor clearance)	

Pathophysiology

As platelets are consumed at sites of endothelial injury and form procoagulant surfaces and/or express TF, thrombocytopenia coincides or precedes activation of the coagulation cascade. Activated coagulation factors are relatively protected from endogenous inhibitors while they are attached to procoagulant surfaces, and therefore excess thrombin generation spreads proximally and distally from the site of injury. Fibrin microthrombi lodge within the vascular beds of end organs (especially kidney, lung, adrenals and brain) and polymerisation of fibrin monomer occurs. This stimulates secondary fibrinolysis which acts to restore microvascular patency. Secondary fibrinolysis, however, results in the production of FDPs and D-dimer which are anticoagulants, potentiate fibrinolysis and interfere with formation of fibrin clot. Excess fibrinolytic activation overcomes the endogenous inhibitors and leads to the destruction of coagulation factors themselves. Unrestricted activation and destruction of fibrin, fibrinogen and coagulation factors VII and VIII occur until the reserves are exhausted and *de novo* synthesis is inadequate. This is known as consumptive coagulopathy.

Primary systemic fibrinolysis occurs when excess plasmin saturates PAI and α-2 antiplasmin leading to increased free circulating plasmin. Free plasmin consumes coagulation factors, and plasmin within clot and on endothelial cells and platelets causes lysis of haemostatic plugs. Compensatory thrombin generation and platelet activation occur in an attempt to restore haemostasis. In both consumptive coagulopathy and primary fibrinolysis, microvascular thrombosis and haemorrhage occur once the reticulo-endothelial system and the phagocyte system are overwhelmed.

The primary mechanism in most cases of coagulopathy is considered to be widespread deposition of fibrin thrombi within the microvasculature with consumption of

coagulation factors and platelets. There is considerable debate, however, as to whether fibrinolysis and fibrinogenolysis in coagulopathy is a primary phenomenon or secondary to thrombin generation and intravascular fibrin deposition. Hyperfibrinolysis, however, remains the major cause of haemorrhage in patients with coagulopathy. Depletion of coagulation factors and platelets, and the inhibitory effect of FDPs and D-dimer further contribute to haemorrhage.

There is evidence to suggest that endothelial activation and injury has a key role in the development of MOF and mortality in patients with coagulopathy. Wada *et al* (36) demonstrated that elevated plasma levels of TAT, PAP and D-dimer predicted the development of coagulopathy. Furthermore, MOF and/or death were associated with a hypofibrinolytic state as demonstrated by elevated plasma t-PA antigen, PAI antigen and soluble TM, and reduced PAP:TAT ratio.

Clinical presentation

Coagulopathy is a syndrome of clinical and laboratory findings. The clinical presentation is variable and dependent on the haemostatic stimulus; the activity of the coagulation and fibrinolytic pathways; bone marrow function; and liver and reticulo-endothelial system function. Coagulopathy may be asymptomatic or it may cause severe bleeding or thrombosis, or both simultaneously. Haemorrhage, however, is the commonest presentation and manifests clinically as; spontaneous bleeding from multiple sites with petechiae, ecchymoses, mucosal oozing, prolonged bleeding from puncture sites, and excessive secondary haemorrhage into wounds during and after procedures; hypovolaemia, hypotension and haemorrhagic shock; and organ dysfunction, particularly affecting lung, kidney and liver. Haemorrhage is associated with hypovolaemia,

hypotension and shock due to activation of the intrinsic coagulation pathway and bradykinin production and interaction between IL-1 and TNF (53). ARF is typical in patients with coagulopathy and occurs due to hypotension and reduced renal blood flow, and also deposition of fibrin thrombi in the microvasculature. Respiratory failure occurs due to microvascular thrombosis and haemorrhage and is identical to that seen in patients with ARDS; 20% of patients with coagulopathy and ARDS share the features of both (31).

Laboratory findings

The diagnosis of coagulopathy consists of thrombocytopenia, prolonged clotting times (PT, aPTT), hypofibrinogenaemia and elevated levels of FDPs. Thrombocytopenia due to platelet consumption consequent on thrombin generation is almost universal. Prolonged clotting times occur in 50-70% of patients and are due to consumption of prothrombin and the tenase and prothrombinase complexes. Hypofibrinogenaemia occurs in less than 50% of patients and elevated FDPs occur in 85% of patients. PT assesses the extrinsic and aPTT the intrinsic coagulation pathway with the exception of factor VII. These tests must be interpreted with caution in hypothermic patients as they are performed in the laboratory at 37 C. If the clotting times are normal, however, then it suggests that sufficient coagulation factors are present to sustain haemostasis once hypothermia is reversed.

Summary

Abnormal haemostasis or coagulopathy occurs due to a disturbance in the physiological balance between coagulation and fibrinolysis in the general circulation as well as within the confines of an area of tissue damage. Stimuli include hypotension, ischaemia, acidosis, hypoxia, hypothermia and massive blood transfusion. Coagulopathy may be asymptomatic or manifest as severe bleeding or thrombosis, or both simultaneously. The primary mechanism in most cases is considered to be widespread deposition of fibrin thrombi within the microvasculature of end organs with resultant consumption of coagulation factors and platelets. Haemorrhage is the commonest presentation and occurs due to depletion of coagulation factors and platelets secondary to thrombus formation, (primary or secondary) hyperfibrinolysis and the inhibitory effect of FDPs. Organ dysfunction occurs due to haemorrhage, reduced blood flow secondary to hypotension and deposition of fibrin thrombi within the microvasculature. The laboratory diagnosis consists of a) thrombocytopenia which is almost universal, b) prolonged clotting times which occur in 50%-70% of patients, c) hypofibrinogenaemia which occurs in less than 50% of patients and d) elevated levels of FDPs which occur in 85% of patients.

1.5

Abnormal haemostasis and aortic aneurysmal disease

Previous studies

Siebert and Natelson (106) proposed four criteria to ensure that intact AAA was the cause of any coagulopathy after exclusion of other possible causes: 1) presence of a chronic acquired bleeding diathesis; 2) laboratory evidence of coagulopathy; 3) correction of the abnormality by aneurysm repair; and 4) maintenance of normal coagulation for a minimum of three months after repair. If adhered to, these criteria effectively exclude patients who do not undergo aneurysm repair from having the diagnosis of coagulopathy secondary to non-ruptured AAA. Despite this, several interesting studies suggest that platelet activation and increased fibrinolysis may be responsible for coagulopathy associated with intact non-operated aortic aneurysms.

In 1971, Biegler and colleagues (107) were the first to report a case of coagulopathy associated with a thoracic aortic aneurysm. A 73 year old patient with an aortic arch aneurysm presented with haematuria, abdominal pain, and dyspnoea with opacification of the left hemithorax on chest x-ray presumed secondary to haemothorax. The patient had laboratory evidence of coagulopathy and his condition improved spontaneously without operation. In 1973, Schnetzer and Penner (108) reported the first case of chronic coagulopathy (of two and a half years duration) secondary to a large thoraco-abdominal aortic aneurysm which responded to heparin therapy without repair.

Fouser *et al* (109) demonstrated laboratory evidence of coagulopathy as well as acquired platelet dysfunction in an 82 year old man with an 8cm diameter AAA and a one year history of a bleeding disorder. Booth *et al* (110) demonstrated increased systemic

fibrinolysis, rather than platelet or coagulation cascade activation, in two patients with large AAAs who developed major haemorrhage after minor surgery. More recently, Micallef-Eynaud and Ludlam (111) reported three patients with non-ruptured abdominal (n=2) and thoracic aortic aneurysm (n=1) who presented with bleeding and laboratory evidence of coagulopathy. Bone marrow aspirate in one patient revealed large numbers of megakaryocytes suggesting that the thrombocytopenia was due to excessive peripheral destruction of platelets. Indium-labelled platelet scanning in two patients demonstrated increased uptake in the region of the aneurysm indicative of increased platelet destruction. Mamiya *et al* (112) reported one patient with a thoraco-abdominal aneurysm and coagulopathy which responded to antifibrinolytic therapy without repair.

Proposed mechanisms

Many patients with AAA take regular antiplatelet or anticoagulant medication, or non-steroidal anti-inflammatory drugs. All of these medications may lead to clinically significant haemostatic derangement and bleeding.

Straub and Kessler (113) were the first to demonstrate an accumulation of labelled fibrinogen within AAA before operation and in the resected aneurysm tissue, and ten Cate reported a similar finding in a patient operated for rupture (114). Fibrin thrombus within the aneurysm sac may lead to increased fibrin turnover and elevated levels of FDPs (115) which, in turn, may stimulate fibrinogen synthesis leading to hyperfibrinogenaemia (116). The finding of focal fibrinolytic activity and fibrinolytic gene expression within the aortic wall of patients with asymptomatic AAA (117,118) suggests that the fibrinolytic system is inappropriately activated in this group.

Fouser *et al* (109) were the first to report abnormal platelet function associated with AAA and proposed that this may occur due to; inhibition of platelet retention and aggregation by FDPs; mechanical damage to platelets due to turbulent blood flow adjacent to the aneurysm thrombus; or prolonged survival of platelets which have already underwent aggregation and partial/complete storage granule release in response to exposure to thrombin within the aneurysm. Using platelet scintigraphy, several authors (111,119,120) have demonstrated increased radio-activity over the aortic aneurysm suggesting that increased platelet destruction may occur due to incorporation in the aneurysm thrombus (111).

Several early studies in the 1960s and early 1970s demonstrated that the aortic adventitia had high fibrinolytic activator activity, that the aortic media and intima had weak prothrombotic properties (121), and that ulcerated atheromatous aorta and aortic subendothelial tissues induced platelet adhesion and thrombin activation (122,123). ten Cate *et al* (114) hypothesised that damage to the aortic wall might lead to; exposure of subendothelial collagen and induction of the intrinsic coagulation pathway; TF exposure and induction of the extrinsic coagulation pathway; exposure of aortic adventitia and induction of fibrinolysis; and platelet consumption secondary to coagulation and adhesion to collagen.

1.6

Abnormal haemostasis and aortic aneurysm surgery

Thrombotic and haemorrhagic complications are responsible for the majority of the major morbidity and mortality associated with aortic aneurysm surgery, and in particular ruptured AAA repair. There is increasing evidence that haemostatic derangement is central to the pathogenesis of these complications. Many studies have examined haemostasis in animal models of aortic surgery, some have included patients who have and have not undergone operation, and others have studied patients undergoing reconstruction for aneurysmal and occlusive disease but have not differentiated between the two. Haemostasis before and after aortic surgery has been the subject of several studies, but there are few detailed investigations of peri-operative changes in the haemostasis in patients undergoing elective AAA repair. To our knowledge, there are no previously published reports in patients undergoing ruptured AAA repair.

Previous studies of thoraco-abdominal aneurysm repair

While coagulopathic bleeding is a common problem during and after surgery for thoracic and thoraco-abdominal aortic aneurysm (41,42), there are few studies examining the pathophysiology of this complication. Cohen *et al* (124-126) studied peri-operative haemostasis in a canine model of supracoeliac aortic cross clamping. Increasing duration of aortic cross-clamping was associated with a significant decrease in platelet count and fibrinogen and significantly prolonged clotting times possibly due to ischaemic hepatic damage and intestinal bacterial translocation.

Previous studies of non-ruptured and ruptured AAA repair

As early as 1955, Krevans and Jackson (127) reported two patients who received massive blood transfusions during elective AAA repair and developed fatal post-operative bleeding with laboratory evidence of coagulopathy. Since then, there have been many case reports of abnormal coagulation in patients undergoing abdominal aortic surgery, and of patients with AAA and coagulopathy who have undergone successful repair (106, 112, 114, 119, 120, 127-136).

In a retrospective study, Mulcare *et al* (137) reported laboratory evidence of coagulopathy and increased fibrinolysis in blood sampled within 12 hours of operation in five patients who received massive blood transfusion and developed excessive bleeding during or after repair of ruptured (n=4) and non-ruptured AAA (n=1). Getaz and Louw (138) demonstrated an abnormal standard coagulation screen in 10 of 18 patients presenting with ruptured AAA, of whom 10 had thrombocytopenia and two had coagulopathy. Thrombocytopenia alone was present in 11 of 39 patients with non-ruptured AAA. Fisher *et al* (139) performed coagulation studies in 76 patients with aortic aneurysm. Elevated FDPs alone were present in 8 of 22 patients with infrarenal AAA, and 14 of 32 patients with thoraco-abdominal aortic aneurysm. Elevated FDP levels were not useful in diagnosing coagulopathy and were not associated with increased operative blood loss.

Mulcare *et al* (140) performed coagulation studies pre-operatively, and immediately and 24 hours post-operatively in 32 patients with non-ruptured AAA and/or aorto-iliac occlusive disease. All patients received intra-aortic heparin before aortic cross clamping. As a function of time, there was a significant increase in clotting times and levels of fibrin monomer and FDPs; and a significant decrease in platelet count, fibrinogen, plasminogen

and fibrinolytic inhibitors. The authors suggested that elective aortic surgery may produce low-grade local or disseminated coagulopathy with secondary fibrinolysis. In 33 patients undergoing aorto-bifemoral bypass, Mashiah *et al* (141) also reported a significant fall in fibrinogen and plasminogen levels intra-operatively but were unable to demonstrate laboratory evidence of increased coagulation or fibrinolysis. Although the authors suggested that fibrinogen and plasminogen may have been removed by physical means, this may have occurred secondary to peri-operative haemodilution. De Mol Van Otterloo *et al* (142) demonstrated increased platelet activation and increased FDPs before and after operation in patients undergoing aorto-iliac reconstruction for aneurysmal and occlusive disease. Brothers *et al* (143) also reported post-operative increased fibrinolysis as demonstrated by elevated FDPs in 10 patients which was confirmed by TEG in only 25% of samples. In 10 patients undergoing elective aorto-bifemoral bypass for occlusive disease (n=8) and non-ruptured (n=2), von Sommoggy *et al* (144) reported a significant increase in ECLT and decrease in AT III and α_2 - antiplasmin before aortic clamping which was possibly secondary to haemodilution. During aortic clamping, there was a significant increase in FDPs suggesting increased fibrinolysis. Interestingly, there authors reported no significant difference in clotting tests from femoral and central venous blood suggesting that lower limb ischaemia does not contribute directly to haemostatic derangement.

In 19 patients undergoing elective aortic surgery for aneurysmal and occlusive disease, Gibbs *et al* (145) demonstrated an early post-operative hypercoagulable state with elevated levels of fibrinogen, factor VIII and vWF and reduced levels of protein C, AT III and α_2-macroglobulin. The authors were the first to speculate that this procoagulant

state might contribute to peri-operative MI. The same authors later confirmed this hypercoagulable state using TEG in 30 patients (146) and failed to demonstrate a beneficial effect of epidural and general anaesthesia on this procoagulant state over general anaesthesia alone in 20 patients undergoing elective abdominal aortic surgery (147). Aramato *et al* (148) measured FDP, D-dimer, TAT and PAP levels in 41 patients undergoing elective surgery for AAA and 30 patients for aorto-iliac occlusive disease. Levels of all markers were elevated in AAA patients. Intra-operatively, the levels of conventional haemostatic markers fell suggesting consumption, and post-operatively returned to normal or increased consistent with a hypercoagulable state.

Gomez et al were the first to report the results of detailed investigation of the fibrinolytic pathway in aortic surgery. In a study comparing patients undergoing thoracic, abdominal and peripheral vascular surgery, the authors demonstrated peri-operative inhibition of fibrinolysis in patients undergoing aortic surgery with a significant increase in PAI levels (80). The same authors (78) measured PAI activity in 25 patients undergoing aortic surgery for aneurysmal (n=14) or occlusive disease (n=11). Plasma PAI levels were not elevated before, but increased during, the operation. Levels peaked at 8 hours post-operatively and returned to baseline by 48 hours. Eriksson and Rosberg (149) confirmed post-operative inhibition of systemic fibrinolysis, as demonstrated by significantly elevated PAI levels and undetectable t-PA activity, in 12 patients undergoing infrarenal aortic reconstruction for occlusive disease. In a study of 31 patients operated for AAA (n=18) and aorto-iliac occlusive disease (n=13), Welch *et al* (150) reported low PAI activity before and during surgery which increased post-operatively to peak 6 hours after declamping. In a rat model of infrarenal aortic cross clamping and isolated lower body ischaemia, Schneiderman *et al* (71) demonstrated a significant increase in plasma t-PA

activity and decrease in PAI activity after 30 minutes of ischaemia with a further increase in both PAI and t-PA activities after 90 and 120 minutes of ischaemia, respectively. Although PAI synthesis increased during ischaemia there was a greater elevation in t-PA synthesis leading to a net increase in fibrinolytic activity. The first rise in t-PA activity was due to release of stored t-PA from the ischaemic vascular bed, and the second rise in t-PA and PAI was associated with increased expression of t-PA and PAI mRNA in non-ischaemic viscera, muscle and fat presumably due to the effect of humoral mediators.

In a prospective study, Davies *et al* (44) reported an abnormal coagulation screen on admission to the resuscitation room in 20 of 43 patients who underwent attempted repair of ruptured AAA. The coagulation screen was abnormal in 13 of 15 patients who died in the peri-operative period from haemorrhage, MI, cardiac arrest, cardiac failure, MOF and CVA. Thrombocytopenia, prolonged PT and hypofibrinogenaemia were individually and collectively associated with significantly increased risk of peri-operative death. More recently, Bradbury *et al* (45) demonstrated a significant relationship between low pre-operative platelet count and mortality, and low post-operative platelet count and mortality, development of MOF, days of ventilatory support, and days spent in the ITU in 65 patients operated for ruptured AAA. Platelet count remained low after operation in non-survivors who died from continuing haemorrhage. Almost 40% of survivors developed post-operative thrombocytosis which was strongly associated with the development of venous thrombo-embolism.

Proposed mechanisms

The pathophysiology of haemorrhage and thrombosis in patients with aortic aneurysm is multifactorial. It has been suggested by many investigators that, in most patients with non-ruptured AAA, there exists a compensated state between the production and localised consumption of platelets, fibrinogen, and clotting factors within the aneurysm sac which amounts to a low-grade and subclinical form of coagulopathy (45,137). This may render the patient particularly sensitive to the effects of profound or prolonged hypotension, hypothermia, ischaemia, acidosis, endothelial cell damage, haemodilution and depressed reticulo-endothelial cell function (45,79,137), such that the consumptive process overwhelms production and clinically apparent coagulopathy occurs (139). As a result, the degree of coagulopathy may be out of proportion to the degree of hypotension and blood loss.

Several mechanisms have been proposed for the abnormalities in coagulation and fibrinolysis associated with AAA repair. Mild platelet dysfunction, prolonged bleeding times and moderate thrombocytopenia occur due to platelet activation and consumption in surgical wounds and on the prosthetic graft (151,152). Coagulation factor and platelet consumption within the haematoma in ruptured AAA (39). Coagulopathy in sepsis, endotoxaemia and atherosclerosis is associated with TF expression (153-155) and, in a similar manner, the pathophysiology of ruptured AAA repair may be thrombogenic by the increasing TF exposure on endothelial cells and monocytes. Patients with rupture have higher levels of elastase in the aortic wall than patients with non-ruptured AAA (156,157). Although no significant difference in circulating elastase activity has been demonstrated between AAA patients and controls (158), it is possible that aortic wall

disruption in ruptured AAA may release elastase and other proteases into the circulation leading to deranged coagulation and fibrinolysis (144).

Consumption of coagulation factors and activation of the fibrinolytic system have been reported in patients with septic shock (82,159), and bacterial translocation has been implicated in the development of coagulopathy (160). Endotoxaemia occurring due to increased intestinal permeability consequent on bowel ischaemia (150) may stimulate the procoagulant effects of endothelial and leucocyte activation, either directly or indirectly, through the synthesis and release of cytokines (52-54,72-75,77,82,83,150). Intestinal ischaemia and bacterial translocation may contribute to the increased incidence of coagulopathy in patients undergoing thoracoabdominal aortic aneurysm repair (124,125). Elevated levels of cytokines such as TNF have been demonstrated in human and animal studies of infrarenal aortic cross-clamping (161-164), and it has been suggested that TNF and IL-1 may be responsible for fibrinolytic activation in lower torso ischaemia (71).

Coagulopathy has been linked to neutrophil activation and endothelial dysfunction (165). Whole body or local hypoperfusion with reperfusion of large ischaemic areas leads to acidosis, hyperkalaemia and myoglobinaemia which contribute to abnormal haemostatic function. Hypoxia combined with reperfusion induces procoagulant activity in vascular endothelium (166). Prolonged supracoeliac aortic cross-clamping is associated with an increased incidence of coagulopathy suggesting that the greater the ischaemic insult, the greater the haemostatic derangement (42,124). The exposure of endothelial cells *in vitro* to hypoxia is associated with a procoagulant state (167) and stimulation of the synthesis of PAI-1 (168). Morphological evidence of endothelial cell activation has been demonstrated before operation in patients with ruptured AAA (169). Increased PAI synthesis occurs during isolated lower torso ischaemia but a greater elevation in t-PA

synthesis leads to a net increase in fibrinolytic activity (71). Similar findings have been demonstrated in humans and animals exposed to endotoxin, where an early increase in t-PA activity was associated with increased t-PA and PAI mRNA expression (72-74). Others investigators, however, have demonstrated increased PAI release and down-regulation of t-PA release in animal studies and in cultured endothelial cells (83). Platelet activation may also lead to the release of PAI (78).

In patients undergoing elective aortic surgery, blood loss and crystalloid/colloid replacement may have a dilutional effect on levels of coagulation factors and platelets (144). Massive transfusion of RCC leads to critically low levels of circulating fibrinogen, platelet and clotting factors such that dilutional coagulopathy invariably occurs. The degree and length of shock, as well as the number of units of RCC transfused are strong predictors of outcome in patients with massive blood loss. Hypothermia occurs as a consequence of whole body hypoperfusion, prolonged complicated surgery, increased blood loss and resuscitation with large volumes of non-warmed intravenous fluid. It is a common finding in patients undergoing aortic surgery (43,170). Hypothermia may itself cause further blood loss by both potentiating platelet activation by plasmin (171) and causing reversible platelet dysfunction (117,172). In patients undergoing elective AAA repair, Kahn *et al* (172) demonstrated a significant association between change in body core temperature and bleeding time suggesting a marked effect on platelet function which was greater than the effect of intra-operative heparinisation.

Summary

The pathophysiology of haemorrhage and thrombosis in patients with aortic aneurysm is multifactorial. Antiplatelet and anticoagulant medication are associated with an increased incidence of bleeding and impaired platelet function. Mural thrombus within the aneurysm sac leads to increased consumption of fibrin, platelets and coagulation factors. Increased secondary fibrinolysis consequent on increased thrombin generation within the aneurysm leads to elevated FDP levels that may, in turn, stimulate fibrinogen synthesis leading to hyperfibrinogenaemia. A compensated state may exist between the production and localised consumption of haemostatic components within the AAA sac.

Focal fibrinolytic activity and fibrinolytic gene expression have also been demonstrated within the aortic wall of patients with AAA suggesting that the fibrinolytic system is inappropriately activated. Disruption of the aortic wall secondary to rupture might release proteases and TF into the circulation and trigger the coagulation pathways, and stimulate fibrinolysis and platelet consumption. Further consumption of coagulation factors and platelets occurs within the retroperitoneal haematoma.

Operative factors are important in the development of coagulation disorders. Whole body or local hypoperfusion with reperfusion of large ischaemic areas leads to acidosis, hyperkalaemia and myoglobinaemia which contribute to abnormal haemostatic function. Prolonged duration of aortic cross-clamping is associated with an increased incidence of coagulopathy. While lower torso ischaemia and endotoxaemia have been reported to be associated with increased fibrinolytic activity, others have demonstrated inhibition of fibrinolysis. Blood loss, massive blood transfusion and resuscitation with large volumes of crystalloid and/or colloid may have a dilutional effect on levels of coagulation factors and platelets. Hypothermia may cause blood loss by both potentiating platelet activation

by plasmin and causing reversible platelet dysfunction. Mild platelet dysfunction, prolonged bleeding times and moderate thrombocytopenia may also occur due to platelet activation and consumption in surgical wounds and on the aortic prosthetic graft. Bacterial translocation and endotoxaemia, which occur secondary to bowel ischaemia and increased intestinal permeability, may stimulate the procoagulant effects of endothelial and leucocyte activation, either directly or indirectly through the synthesis and release of cytokines. Cytokines have been shown to contribute to fibrinolytic system activation in lower torso ischaemia and endotoxaemia. Morphological evidence of endothelial cell activation has been demonstrated in patients with ruptured AAA. As in sepsis and endotoxaemia, haemostatic derangement in ruptured AAA repair may occur secondary to neutrophil and endothelial cell activation and TF expression.

1.7

Prevention and management of haemostatic complications

Surgical bleeding occurs for two main reasons; inadequate surgical haemostasis due to a large defect in a vessel and where the treatment is surgical; and failure of the physiological mechanisms of haemostasis where there is microvascular bleeding and the treatment is correction of the underlying disorder. Vascular patients may have a number of concomitant disorders responsible for increased bleeding such as pre-existing liver and renal impairment, antiplatelet and anticoagulant therapy, dilutional coagulopathy due to massive blood transfusion and fluid resuscitation, and coagulopathy secondary to haemorrhagic and hypovolaemic shock.

The definitive management of coagulopathy is removal of the underlying cause. In addition the patient may require supportive therapy and regular laboratory assessment of the coagulation status to guide further management (Table 1.4).

TABLE 1.4

Management of coagulopathy.

Therapy	Action
Treat the underlying cause	
Circulatory and ventilatory support including RCC transfusion, inotropic support, IPPV, RRT	Maintain end organ perfusion and oxygenation
Replacement of deficient factors including FFP, platelets, cryoprecipitate	Replete endogenous stores
Exogenous administration of modulators of coagulation and fibrinolysis	Modify coagulation, fibrinolysis and platelet reactions
Vitamin K administration	Promotes synthesis of endogenous liver-derived coagulation factors

Replacement therapy.

This will replete endogenous stores of red blood cells, coagulation factors and platelets.

a) RCC is used to restore the oxygen carrying capacity of the circulation

b) FFP is used if there are multiple coagulation factor deficiencies and consumptive coagulopathy. It replaces most factors with the exception of factors V and VIII.

c) Platelets are transfused if there is active bleeding and thrombocytopenia. Pooled platelets also contain FFP and coagulation factor V.

d) Cryoprecipitate is used in consumptive coagulopathy. It contains fibrinogen, vWF and coagulation factor VII.

Administration of agents which modify haemostasis

Thrombin inhibitors

Heparin is the most frequently used thrombin inhibitor. There are no prospective randomised trials of the use of intravenous or low-dose heparin in patients with coagulopathy and there is no conclusive evidence that it improves outcome. It may be ineffective in patients with coagulopathy as it requires AT III for its action and this is reduced due to binding with thrombin. Antithrombin (AT) III infusion, with or without heparin, may be associated with some improvement in coagulopathy and reduced mortality in patients with septic shock (173). Recombinant activated protein C inhibits generation of thrombin and TAFI and has been shown to reduce mortality in patients with severe sepsis. Gabexate mesylate is a synthetic inhibitor of thrombin and plasmin and a non-randomised study has reported some improvement in coagulopathy (174).

Plasmin inhibitors

ε-aminocaproic acid (EACA), tranexamic acid and aprotinin inhibit plasminogen directly or inhibit the action of plasmin on fibrinogen and fibrin. Aprotinin is the most frequently used antifibrinolytic agent and much of the work on its applicability has occurred in the context of cardiopulmonary bypass. Proposed mechanisms of action include; inhibition of the intrinsic coagulation pathway and complement cascade; antifibrinolysis; hypercoagulability; reduced endothelial adhesion molecule expression; and preservation of platelet adhesion molecule expression. Antifibrinolytic agents are advocated in primary systemic fibrinolysis and aprotinin may be beneficial in the treatment of aspirin-induced platelet abnormalities. In consumptive coagulopathy, secondary fibrinolysis acts to restore microvascular patency and should not be inhibited. Antifibrinolytic agents may lead to a hypercoagulable state with increased microvascular thrombosis and organ failure (31,175) and some have recommended that their use in aortic surgery is contra-indicated (137,140) for this reason. In non-cardiac surgery, randomised clinical trials of aprotinin have shown a reduction in bleeding and blood requirement in thoracic and thoraco-abdominal aortic aneurysm surgery using left heart bypass (176) and orthotopic liver transplantation (177).

Desmopressin (DDAVP)

This is a synthetic analogue of vasopressin which leads to an acute increase in circulating coagulation factor VIII and vWF levels and increased platelet adhesiveness to injured endothelium. It may have a role in the treatment of aspirin-induced platelet abnormalities.

Specific management of haemostatic complications in association with AAA.

There are many reports of the use of blood product replacement and intravenous/low-dose heparin (106-108,111,114,120,130,135,137,140,178), antifibrinolytic agents (108,112) or warfarin (111) in patients with coagulopathy presumed secondary to AAA, and in whom aortic surgery is not performed. Pre-operative heparin therapy (119,128,132) and infusion of gabexate mesylate (119) have been used to correct the underlying coagulation abnormalities and reduce intra-operative blood loss in patients who have chronic coagulopathy and AAA.

In ruptured AAA, delaying fluid resuscitation until haemorrhage is controlled by aortic cross-clamping may be associated with a reduction in requirement for blood product replacement. Most of the blood loss incurred during aortic aneurysm surgery is due to technical manoeuvres involving large vessels and can be minimised by careful and minimal dissection, the use of blended electrocautery for dissection, avoidance of excess heparin administration, selection of a woven or sealed prosthetic graft and the use of deep sutures. There is no evidence that the retroperitoneal approach to AAA repair is associated with less blood loss than the transperitoneal approach. Short aortic clamp times may also reduce the risk of coagulopathy (42,124). If major intra-operative blood loss is anticipated, the use of auto-transfusers has been advocated (131,139) but these devices may wash platelets and coagulation factors from the blood and cause mechanical trauma to these elements. Prevention of severe hypothermia may minimise blood loss and can be achieved by resuscitation with warm intravenous fluids, short operating times, maintenance of optimal tissue perfusion and the use of warming devices (43,172). If six or more units of RCC are transfused or there is on-going bleeding, then FFP and platelet transfusion are advocated to reduce the risk of dilutional coagulopathy. Cryoprecipitate

may also be required if the fibrinogen level is less than 1g/dl. Due to the increased incidence of severe coagulopathy in ruptured AAA, transfusion of platelets and/or FFP has been recommended either pre-operatively (179) or after aortic cross clamping (45,138). Thrombocytopenia and deranged coagulation at the end of the operation should prompt further transfusion of platelets and/or FFP to reduce the risk of continuing haemorrhage. Mulcare *et al* (137) have also recommended heparin therapy and the avoidance of re-operation in this situation. Intravenous DDAVP has no beneficial effect on haemostatic function or clinical outcome in elective aortic surgery (180).

The high incidence of post-operative thrombotic events after ruptured AAA repair prompted Bradbury *et al* (45) to recommend the use of post-operative low-dose heparin therapy in the absence of an abnormal coagulation screen or continuing haemorrhage. Intravenous heparinisation in patients undergoing elective aortic surgery is not associated with increased blood loss and reduces peri-operative fatal and non-fatal acute coronary events (181). In patients undergoing ruptured AAA repair, peri-operative intravenous heparinisation is mentioned in only one larger multicentre study. Hoffman *et al* (10) reported that over 40% of 152 patients operated for rupture were given intravenous heparin and peri-operative haemorrhage was the principal cause of death. These anecdotal data suggest that heparinisation may be detrimental, but as no other studies of ruptured AAA repair mention the use of intra-operative heparinisation, no firm conclusion can be reached.

1.8

Myocardial injury

Patients with peripheral arterial disease have a high prevalence of symptomatic and asymptomatic coronary artery disease which is associated with a significantly increased risk of post-operative MI (182,183). There are multiple patient- and procedure-related factors that might contribute to the development of peri-operative cardiac events. Patient factors include age over 70 years, previous MI, cigarette smoking, previous coronary artery interventions and co-existing cardiac failure, cardiac valve disease, angina pectoris, arrhythmia and diabetes mellitus. In patients undergoing elective aortic surgery, 75-95% have significant double or triple vessel coronary artery disease (184,185). In aortic surgery, cardiac events are considered to occur secondary to hypotension associated with anaesthetic induction, blood loss, fluid shifts and declamping; during the period of lower torso ischaemia; and secondary to acidosis and metabolic defects which occur during reperfusion. Cardiac morbidity is often preceded by myocardial ischaemia. However, this is often difficult to detect as it occurs frequently, is typically asymptomatic, and may only be suggested by persistent tachycardia. Myocardial ischaemia occurs in up to 30% of patients undergoing aortic surgery, and is clinically 'silent' in 98%.

Atherosclerotic plaque rupture with subsequent occlusive thrombus formation generally results in Q-wave infarction on ECG (186). A generalised procoagulant state has been shown to be associated with myocardial injury (68,95-100,102,103,187-189). Peri-operative MI in vascular and non-vascular patients is most often of the non-Q-wave type (190,191) and is frequently preceded by periods of ST segment depression on ECG, rather than ST segment elevation, indicating cyclical changes in coronary artery

bloodflow. This implies that peri-operative MI is not solely attributable to main stem plaque rupture and thrombotic occlusion. Although haemodynamic instability may result in sustained subendocardial ischaemia, a procoagulant state may lead to macro- and microvascular thrombosis of the coronary circulation.

In contemporary practice, the reported median (range) incidence of peri-operative MI and fatal MI in patients undergoing elective infrarenal aortic surgery is 3% (range, 0-10%) and 1.3% (range, 0-4.7%), respectively (192). The incidence of MI, however, largely depends on how enthusiastically the clinician investigates the patient. The MI rate in retrospective studies is approximately 3%, whereas in prospective studies using ECG and CK-MB analyses, this approaches 10-15%. As a marker of myocardial injury, cTn I has major advantages over CK-MB and cTn T in patients undergoing major peripheral vascular surgery. CK-MB is a non-structural cardiac enzyme which is largely, but not absolutely, specific for myocardial injury and is usually elevated only when myocardial cell necrosis occurs. CK-MB persists in the circulation for 24-36 hours after myocardial injury. cTn I is a structural myocardial protein which modulates the interaction of the contractile proteins, actin and myosin. cTn I is found in larger quantities in the myocardial cell than CK-MB. Thus, when the myocardial cell is injured due to ischaemia, cTn I is liberated into the circulation in much larger quantities than CK-MB. It is detectable between 2 and 6 hours after the injury and persists for up to 7-10 days. cTn I levels usually begin to rise while the patient is asymptomatic and precedes clinically significant cardiac events by 24-48 hours in 50% of patients. Although small areas of myocardial injury, rather than actual necrosis, may liberate CK-MB in quantities too small to be detected, cTn I is liberated in relatively greater quantities and is more likely to be detectable. Unlike CK-MB and cTn T, cTn I is entirely cardiospecific, does not

accumulate in patients with renal failure, has not been shown to be released from skeletal muscle. Furthermore, cTn I can be detected after myocardial ischaemia and minor myocardial injury whereas CK-MB is usually only elevated secondary to myocardial necrosis. For these reasons, cTn I is currently the most specific and sensitive marker for myocardial injury (193-197). The size of cTn I rise has been shown to be proportional to the size of the area of myocardial injury (197). In patients with acute chest pain, cTn I is highly sensitive for the early detection of myocardial cell injury (198) and can predict the risk of acute MI and death in patients with unstable angina (199-201).

1.9

Endothelin

The vascular endothelium has an important role in the regulation of haemostasis and the maintenance of vascular tone. Vasoconstriction in response to vascular injury is the initial step in the process leading to haemostasis.

Endothelin (ET)-1 is the most potent known vasoconstrictor. It is synthesized by endothelial cells as well as macrophages and vascular smooth muscle cells. It is principally secreted abluminally from vascular endothelial cells but may enter the circulation if concentrations are high at the endothelial cell-vascular smooth muscle interface (51). Expression is induced by haemorrhage, haemodilution, hypoxia, acidosis, shear stress, catecholamines, cytokines, endotoxin and thrombin.

Big ET-1 is the immediate precursor of ET-1, and its conversion to biologically active ET-1 by endothelin-converting enzymes occurs mainly in the vessel wall (202,203). Big ET-1 is detectable in the plasma for considerably longer than ET-1 which has a half-life of approximately five minutes (204), and increased plasma levels of big ET-1 are considered to represent increased ET-1 generation rather than reduced clearance.

ET-1 leads to vasoconstriction in resistance vessels, especially the coronary, cerebral and renal circulation by acting on ET_A receptors in vascular smooth muscle cells, and ET_B receptors on vascular smooth muscle and endothelial cells. ET-1 stimulates thrombin generation *in vitro* as demonstrated by elevated TAT levels and reduced levels of AT III and fibrin monomer (205,206). ET-1 leads to vasoconstriction and a local inflammatory response both of which facilitate intravascular thrombosis (205-208).

Elevated plasma ET levels may form part of a homeostatic response to maintain systemic blood pressure (204), and have been demonstrated in critically ill patients with MI (209), cardiogenic shock (210), septic shock (211), ARDS (212), cardiac failure (213,214), and ARF (215). ET levels are increased in patients with coagulopathy, particularly if associated with sepsis or MOF (208,216). To date, several animal and human studies of septic shock have suggested that ET release may be pathological (217-222) or homeostatic (222,223). Increased plasma ET levels have been demonstrated in patients undergoing non-ruptured AAA repair with infrarenal (224,225) and supracoeliac aortic cross-clamping (226), as well as in one animal model of infrarenal aortic clamping and subsequent exsanguination (227).

1.10

Tumour necrosis factor

The pro-inflammatory cytokine, tumour necrosis factor (TNF), has been implicated in the pathophysiology of the systemic inflammatory response syndrome and MOF (35,228). Increased levels of TNF, occurring directly or in response to endotoxaemia, have a pivotal role in endothelial cell activation (52,53). TNF causes leucocyte activation, increased phagocytosis, and neutrophil chemotaxis and tissue sequestration. TNF can also induce a procoagulant and hypofibrinolytic state by stimulating TF expression by endothelial cells (and leucocytes), upregulating the release of vWF into plasma (53,54), and upregulating PAI and downregulating t-PA release (72-75,77,82,83,150).

TNF acts by binding to target cells at specific TNF receptors (TNF-R) of 55 kDa and 75 kDa molecular mass (p55 and p75), respectively. Binding of TNF leads to cleavage of these receptors from target cells and their release into the circulation as soluble TNF receptors (sTNF-Rs) (229). Circulating TNF is frequently difficult to demonstrate. This may be because of its short half-life, or because of TNF binding to plasma proteins, difficulties with the numerous methods of measuring TNF in the circulation, or the fact that it is mainly produced in tissues and only occasionally enters the circulation. It has been suggested, therefore, that sTNF-R levels may better reflect the degree of TNF-induced tissue injury (230,231). The finding that high concentrations of the sTNF-Rs act as endogenous TNF antagonists, and that low concentrations may slow down TNF clearance so prolonging its activity (231), has led to the suggestion that administration of exogenous sTNF-Rs may ameliorate the adverse effects of TNF. Experimental data suggest that administration of exogenous sTNF-R before aortic cross-clamping is

associated with a reduction in circulating TNF and subsequent lung injury in an animal model (232). However, human studies have shown that elevated levels of endogenous sTNF-Rs are actually associated with the development of MOF and increased mortality in sepsis (233), pancreatitis (234) and multiple trauma (235). Circulating TNF has been demonstrated in elective and emergency aortic surgery (75,161,163,236-241). Elevated levels of sTNF-Rs have also been detected in patients undergoing repair of non-ruptured (241,242), and post-operatively in patients with ruptured AAA (241).

Chapter 2

Hypothesis and Aims

Activation of the coagulation cascade and the fibrinolytic system contribute to the pathophysiology of haemorrhagic and micro- and macrovascular thrombotic events such as coagulopathy, myocardial infarction and multiple organ dysfunction. Although deranged haemostatic function has been described in patients with asymptomatic AAA, careful review of the literature reveals that there are no detailed studies of peri-operative haemostatic function in patients undergoing emergency AAA surgery for rupture.

The hypothesis of this thesis is that whole body hypoperfusion due to haemorrhagic shock in ruptured AAA, and lower body ischaemia and reperfusion injury during AAA repair all contribute to the development of abnormal haemostatic function. In turn, this state of haemostatic derangement is responsible for haemorrhagic and thrombotic complications which account for the majority of the morbidity and mortality associated with elective and emergency AAA repair.

The principal aims of this thesis were four-fold. The first aim was to determine the hospital-based and overall community-based mortality rate for patients with ruptured AAA within the catchment area served by a regional vascular surgical unit in order to assess whether advances in anaesthesia, surgical techniques and critical care, as well as the centralisation of vascular surgical services have altered the outcome for patients with ruptured AAA in the past two decades. In addition, the aetiology of fatal peri-operative complications was examined to determine the contribution of haemostatic derangement to outcome in this group of patients.

The second aim was to examine, for the first time, the pathophysiology of haemostatic derangement in patients undergoing ruptured AAA repair by measuring peri-operative changes in circulating markers of coagulation, fibrinolysis and endothelial cell activation. The findings in patients with ruptured AAA were compared with patients undergoing

repair of asymptomatic and symptomatic non-ruptured AAA to assess the effect of haemorrhagic shock and other clinicopathological variables such as operative blood loss and aortic clamp time on haemostastic function.

The third aim was to relate the observed peri-operative changes in coagulation, fibrinolysis and endothelial cell activation to clinical end-points including death, myocardial injury, haemorrhage and organ dysfunction.

The final aim of this thesis was to investigate possible pathogenetic mechanisms for the observed changes in haemostatic function by determining the relationship between circulating markers of haemostatic function, endothelial cell activation and cytokines.

Chapter 3

Methodology

Ethical and consent issues

Lothian Region Ethical Committee approval was obtained for all aspects of the work within this thesis. Fully-informed consent was obtained from all patients or next of kin. When a patient was admitted with ruptured AAA and written consent could not be obtained, verbal consent was obtained from the patient or the next of kin. All patients studied were admitted to the ERVSU between February 1996 and December 1997.

Patients

Sixty-six patients who underwent operation for infrarenal AAA were prospectively studied. Twenty two patients (19 men and 3 women of median age 69, range 56 - 81, years) underwent elective repair of asymptomatic AAA. Seven patients (7 men of median age 68, range 65 - 74, years) underwent emergency repair of acutely symptomatic non-ruptured AAA. Thirty seven patients (33 men and 4 woman of median age 74, range 63 - 87, years) underwent attempted repair of ruptured AAA. The overlap of patients studied in chapters 6-12 are illustrated in Tables 3.1 and 3.2.

TABLE 3.1

Patients with asymptomatic and symptomatic non-ruptured AAA.

Patient	AAA	Ch 6	Ch7	Ch8	Ch9	Ch10	Ch11	Ch12
1	A	Y	Y		Y	Y		Y
2	A	Y	Y		Y	Y		Y
3	A		Y					
4	A		Y					
5	A		Y					
6	A		Y					
7	A	Y	Y		Y	Y		Y
8	A		Y		Y	Y		Y
9	A		Y					
10	A		Y					
11	A		Y					
12	A		Y					
13	A		Y					
14	A	Y	Y		Y	Y		Y
15	A		Y					
16	A		Y					
17	A		Y					
18	A	Y	Y		Y	Y		Y
19	A	Y	Y		Y	Y		Y
20	A	Y	Y		Y	Y		Y
21	A	Y	Y		Y	Y		Y
22	A	Y	Y		Y	Y		Y
23	S		Y					
24	S		Y					
25	S		Y					
26	S		Y					
27	S		Y					
28	S		Y					
29	S		Y					

KEY: A = asymptomatic, S = symptomatic

TABLE 3.2

Patients with ruptured AAA.

Patient	Ch 6	Ch7	Ch8	Ch9	Ch10	Ch11	Ch12
1		Y					
2	Y	Y		Y	Y	Y	Y
3		Y					
4		Y	Y				
5		Y					
6		Y	Y	Y		Y	Y
7	Y	Y		Y	Y	Y	Y
8		Y	Y	Y			
9		Y					
10		Y	Y	Y		Y	Y
11	Y	Y		Y	Y	Y	Y
12	Y	Y		Y	Y		Y
13		Y					
14	Y	Y		Y	Y	Y	Y
15	Y	Y		Y	Y	Y	Y
16		Y					
17	Y	Y		Y	Y		Y
18		Y	Y				
19		Y	Y				
20		Y					
21		Y		Y		Y	Y
22		Y		Y		Y	Y
23		Y					
24		Y					
25		Y		Y		Y	Y
26	Y	Y		Y	Y	Y	Y
27	Y	Y		Y	Y	Y	Y
28	Y	Y		Y	Y	Y	Y
29		Y					
30		Y		Y		Y	Y
31		Y					
32		Y		Y			
33		Y		Y			
34		Y		Y			
35		Y	Y				
36		Y	Y				
37		Y					

Data collection

The following clinicopathological and operative data were collected prospectively:

a) Patient demography - age and sex; co-morbidity and medications; type of presentation (asymptomatic, acutely symptomatic non-ruptured or ruptured AAA); and antero-posterior diameter of the aneurysm measured by ultrasonography in asymptomatic AAA.

b) Pre-operative data - delay between the onset of symptoms of rupture and hospital admission; documented episodes of hypotension, loss of consciousness and cardiac arrest; and resuscitative measures.

c) Operative data - type of aneurysm (intraperitoneal and/or retroperitoneal rupture, inflammatory AAA, suprarenal AAA); pharmacological intervention (including heparin, protamine sulphate, mannitol, adrenaline and dopamine); aortic clamp time and operation time; measured blood loss and fluid administration (including crystalloid, colloid, RCC and blood products); intra-operative episodes of hypotension; complications; and graft configuration (aorto-aortic, aorto-bi -iliac or aorto-bifemoral).

d) Post-operative data - duration of ITU stay, ventilatory support and hospital stay; complications and mortality; and therapeutic intervention.

Operative methods

Ruptured AAA was defined by the presence of fresh retroperitoneal and/or intraperitoneal blood in the presence of an aortic aneurysm with no other identifiable cause for the findings at operation. Acutely symptomatic non-ruptured AAA was defined by the acute onset of abdominal and/or back pain in the presence of a tender AAA which was found to be intact at emergency operation. Sudden expansion or impending rupture

were presumed to be responsible for the clinical findings as there were no other identifiable causes.

Patients with rupture had general anaesthesia, and patients with non-ruptured AAA had combined general and epidural anaesthesia. All patients underwent AAA repair through a transverse supra-umbilical incision with infra-renal aortic clamping. No patients required suprarenal or supracoeliac aortic clamping. No patients with ruptured AAA were systemically heparinised. Patients with non-ruptured AAA were given an intravenous bolus of 5000 units of heparin immediately before aortic clamp placement. The dose of heparin was not varied according to the weight of the patient.

Definition of peri-operative complications

1. Major peri-operative cardiac complications were defined as:

a) Acute MI diagnosed by two of the following three criteria: history of ischaemic-type chest pain, evolving ECG changes, and a rise and fall in serial serum cardiac enzymes.

b) Cardiac failure associated with chest x-ray evidence of pulmonary oedema.

c) Cardiac arrhythmias requiring therapeutic intervention to maintain cardiovascular stability.

2. Peri-operative organ failure:

a) Cardiac failure was defined as sustained periods of hypotension (mean arterial pressure less than or equal to 60 mmHg) requiring fluid resuscitation and inotropic support and/or cardiac arrhythmia requiring pharmacological treatment to maintain cardiovascular stability

b) Respiratory failure was defined as hypoxia requiring mechanical ventilatory support for more than four days.

c) ARF was defined as elevated serum creatinine greater than or equal to 250 μmol/l and/or the requirement for renal replacement therapy.

d) Coagulopathy was defined as clinical evidence of haemorrhage accompanied by laboratory evidence of thrombocytopenia, prolonged clotting times, hypofibrinogenaemia and elevated levels of D-dimer.

Blood sample collection

Arterial blood was sampled from an indwelling radial arterial line. The first 10ml of blood were discarded. Blood was collected in a standard syringe without the application of suction, and then transferred to specific tubes. After sampling, the arterial line was flushed with heparinised saline. A 2.7 ml sample was collected into EDTA anticoagulant (1.6mg/ml). A 3ml sample was collected into sodium citrate anticoagulant (0.106 mol/l). A 9ml sample was collected into lithium heparin. A 9ml sample was collected into a tube containing clot activator. All of the above tubes were manufactured by Monovette ®, Sarstedt. A 4.5 ml sample was collected into strong acid citrate (Stabilyte ®, Biopool, Sweden). Samples were placed immediately on ice and transferred to the laboratory where they were centrifuged within 30 minutes of collection at 3,000 revolutions per minute for 30 minutes at a temperature of 4 C (1400g). Plasma and serum were separated and stored at - 70 C for later batch analysis.

Assay methods

All commercially available assays were performed according to the manufacturer's instructions. Prior to each assay run, a standard reference curve was constructed from standards provided by the supplier. All determinants were performed in duplicate.

1. Standard coagulation screen

Haematocrit (normal range, 0.37 - 0.54) and whole blood platelet count (normal range, 150 - 350 x 10^9/l) was determined on an EDTA sample using the fully-automated Sysmex NE 8000 analyser. Fibrinogen (normal range, 1.5 - 4.0 g/l), PT (normal range, 10.5 - 14.5 seconds) and aPTT (normal range, 28 - 40 seconds) were determined on a sodium citrate anticoagulant sample using the fully-automated Sysmex CA 6000 analyser.

2. Markers of thrombin generation

a) PF 1+2 (normal range, 0.4 - 1.1 nmol/l) was determined on a sodium citrate anticoagulant sample by sandwich ELISA (Enzygnost ® F 1+2 micro, Behring Diagnostics, USA). During the first incubation period (30 mins at 37C), PF 1+2 antigen in a 50µl serum sample binds to rabbit anti-human PF 1+2 antibodies fixed to the surface of a microtitration plate. Unbound constituents are removed by washing the plate and 100µl of peroxidase-conjugated rabbit anti-human prothrombin antibody is added to the plate. During the second incubation period (15 mins at 37C), these enzyme-conjugated antibodies bind to PF 1+2 antigen that was bound by the first layer of antibody. The plate is rinsed to remove excess enzyme-conjugated antibodies and a chromogen solution is added. The plates are incubated protected from light for 15 mins at 20-25C. The enzymatic reaction between hydrogen peroxide and chromogen is terminated by adding diluted sulphuric acid. The resulting colour intensity, which is proportional to the concentration of PF 1+2, is determined photometrically against distilled water at 492nm. The lower limit of detection is 0.04 nmol/l.

b) TAT (normal range, 1.0 - 4.1 µg/l) was determined on a sodium citrate anticoagulant sample by sandwich ELISA (Enzygnost ® TAT micro, Behring Diagnostics, USA). During the first incubation period (15 mins at 37C), TAT antigen in a 50µl serum sample binds to rabbit anti-human thrombin antibodies fixed to the surface of a microtitration plate. Unbound constituents are removed by washing the plate and 100µl of peroxidase-conjugated rabbit anti-human ATIII antibody is added to the plate. During the second incubation period (15 mins at 37C), these enzyme-conjugated antibodies bind to TAT antigen that was bound by the first layer of antibody. The plate is rinsed to remove excess enzyme-conjugated antibodies and a chromogen solution is added. The plates are incubated protected from light for 30 mins at 20-25C. The enzymatic reaction between hydrogen peroxide and chromogen is terminated by adding diluted sulphuric acid. The resulting colour intensity, which is proportional to the concentration of TAT, is determined photometrically against distilled water at 492nm. The lower limit of detection of the assay is 0.5 µg/l.

3. Markers of fibrinolysis

a) t-PA activity (normal range, 0.2 - 2.0 IU/ml) was determined on a strong acid citrate sample by chromogenic assay (amidolytic method) (Coaset ® t-PA, Chromogenix, Sweden). The blood sample for estimation of t-PA activity is collected into strong acid citrate (Stabilyte ®, Biopool, Sweden) and this immediately stops the *in vitro* inhibition of t-PA by PAI. The acidified plasma sample is thawed and 100µl is diluted with 3.5ml sterile water. 200µl of this diluted sample is placed in a test tube to which 1 volume of human plasminogen, 1 volume of chromogenic substrate S-2251 and 3 volumes of Tris

buffer working solution are added. Human t-PA stimulator and Tris buffer solution are then added and this markedly increases the conversion of plasminogen to plasmin by t-PA in the plasma sample. The contents of the test tube are mixed and incubated at 37C for 135-240 mins. The reaction is terminated by adding 20% acetic acid or 10% citric acid. The amount of t-PA activity is determined by measuring the amidolytic activity of plasmin on the chromogenic substrate and the release of p-nitroanaline is determined photometrically against distilled water at 405nm. The lower limit of detection of the assay is 0.1 IU/ml.

b) t-PA antigen (normal range, 1 - 12 ng/ml) was determined on a sodium citrate sample by ELISA (Coaliza ® t-PA, Chromogenix, Sweden). During the first incubation period (60 mins at 37C), t-PA antigen in the sample binds to two mouse anti-human t-PA monoclonal antibodies fixed to the surface of a microtitration plate. Unbound constituents are removed by washing the plate and 200µl of horseradish peroxidase-conjugated anti-human t-PA monoclonal antibody is added to the plate. These enzyme-conjugated antibodies bind to t-PA antigen that was bound by the first layer of antibody. A further wash removes unbound peroxidase and the plates are incubated for a second period (60 mins at 37C). The plates are then washed again and 200µl of tetramethylbenzidine dissolved in dimethyl sulphoxide is added. This chromogen acts on the peroxidase to form a blue colour. The plates are incubated for a third time for 30 mins at room temperature and the enzymatic reaction is terminated by adding dilute sulphuric acid. The colour then turns yellow and the intensity is proportional to the concentration of t-PA antigen in the sample. This is determined photometrically by a microplate reader with 450nm and 620nm or 690nm filter. The lower limit of detection of the assay is 0.5 ng/ml.

c) PAI activity (normal range, < 15 AU/ml) was determined on a sodium citrate sample by chromogenic assay (amidolytic method) (Coatest ® PAI, Chromogenix, Sweden). To a 25µl sample of plasma, 25µl of 40IU/ml t-PA is added such that inactive t-PA:PAI complexes form. The sample is mixed and incubated at 20-24C for 10 mins. The sample is diluted with 4ml of sterile water and 200µl of this diluted sample is placed in a test tube to which 1 volume of human plasminogen, 1 volume of chromogenic substrate S-2403 and 3 volumes of Tris buffer working solution are added. Human fibrinogen fragments stimulator and Tris buffer solution are then added and this markedly increases the conversion of plasminogen to plasmin by residual t-PA in the plasma sample. The contents of the test tube are mixed and incubated at 37C for 50 mins. The reaction is terminated by adding 20% acetic acid or 2% citric acid. PAI activity is determined by measuring the amidolytic activity of plasmin on the chromogenic substrate and the release of p-nitroanaline is determined photometrically against distilled water at 405nm. The amount of plasmin formed is proportional to the residual t-PA activity and inversely proportional to the PAI activity. The lower limit of detection is 5 AU/ml.

d) FDP D-dimer (normal range, 630 - 850 ng/ml) was determined on a sodium citrate sample by ELISA (Asserachrom ®, D-Di, Diagnostica Stago, France). During the first incubation period (60 mins at 18-25C), D-dimer antigen in the sample binds to mouse anti-human D-dimer monoclonal antibody fixed to the surface of a microtitration plate. Unbound constituents are removed by washing the plate and 200µl of peroxidase-conjugated rabbit anti-fragment D antibody is added to the plate and incubated for 60 mins at 18-25C. These enzyme-conjugated antibodies bind to D-dimer antigen that was bound by the first layer of antibody. After incubation, a further wash removes unbound peroxidase and the bound peroxidase acts on 200µl of ortho-phenylenediamine and

hydrogen peroxide which is added. The plates are incubated for 3 mins at room temperature and the enzymatic reaction is stopped with dilute sulphuric acid. The intensity of the colour change is determined photometrically at 492nm and is proportional to the D-dimer concentration. The lower limit of detection is 5 ng/ml.

4. Markers of endothelial cell activation

a) Big ET-1 (normal range, 10 - 60 pg/ml) and ET-1 (normal range, 1.5 - 4.5 pg/ml) concentrations were determined on a lithium heparin plasma sample using an acetic acid extraction technique and a modified commercial radio-immunoassay using rabbit anti-human big ET-1 or ET-1 (Peninsula Laboratories Europe, UK). Sample extract was incubated with either big ET-1 or ET-1 antibody for 24 hours at 4 C. Following incubation, 125 I-labelled big ET-1 (Peninsula Laboratories Europe) or ET-1 (NEN Life Science Products, Boston, MA, USA) was added and incubation was continued for an additional 20 minutes at 4 C. Complexes were precipitated with Amerlex ® donkey anti-rabbit antibody (Amersham Life Sciences Limited, UK) and counted for radioactivity. The lower limit of detection for big ET-1 and ET-1 is 1 pg/ml and 0.25 pg/ml, respectively.

b) vWF antigen (normal range, 0.42 - 1.22 IU/ml) was determined on a sodium citrate sample by ELISA developed and validated in the Department of Haematology, Royal Infirmary of Edinburgh. During the first incubation period (120 mins at room temperature), vWF antigen in the sample binds to rabbit anti-human vWF monoclonal antibodies fixed to the surface of a microtitration plate. Unbound constituents are removed by washing the plate and 100µl of peroxidase-conjugated anti-human vWF antibody is added to the plate. These enzyme-conjugated antibodies bind to vWF antigen

that was bound by the first layer of antibody. The plates are incubated for 60 mins at room temperature and a further wash removes unbound peroxidase. The plates are then washed again the bound peroxidase acts on 100µl of ortho-phenylenediamine and hydrogen peroxide which is added. The plates are placed in the dark for 7 minutes and the enzymatic reaction is terminated with dilute sulphuric acid. The colour intensity is proportional to the concentration of vWF antigen in the sample. This is determined photometrically by a microplate reader at 492nm. The lower limit of detection is 0.5 ng/ml.

c) Soluble thrombomodulin (sTM) (normal range, < 25 ng/ml) was determined on a sodium citrate sample by ELISA (Asserachrom Thrombomodulin, Diagnostica Stago, France). During the first incubation period (120 mins at 18-25C), sTM antigen in the sample binds to mouse anti-thrombomodulin monoclonal antibody fixed to the surface of a microtitration plate. Unbound constituents are removed by washing the plate and 200µl of peroxidase-conjugated mouse anti-thrombomodulin monoclonal antibody is added to the plate and incubated for 120 mins at 18-25C. These enzyme-conjugated antibodies bind to sTM antigen that was bound by the first layer of antibody. After incubation, a further wash removes unbound peroxidase and the bound peroxidase acts on 200µl of ortho-phenylenediamine and urea peroxide which is added. The plates are incubated for 8 mins at room temperature and the enzymatic reaction is stopped with dilute sulphuric acid. The intensity of the colour change is determined photometrically at 492nm and is proportional to the sTM concentration. The lower limit of detection is 5 ng/ml.

5. Markers of myocardial injury

a) CK (normal range, 30 - 150 U/l) and its myocardial iso-enzyme CK-MB were determined on a clotted serum sample by colourimetric assay with dry slide technology (Vitros CK and CK-MB slides, Johnson and Johnson, USA) using the fully-automated Kodak 250 analyser. The lower limit of detection for CK is 20 U/l. If total CK was elevated above the normal range, then CK-MB was measured. CK-MB level less than 16 U/l is negative for CK-MB. CK-MB greater than 16U/l is positive and the proportion of CK-MB relative to CK was calculated. A value for CK-MB between 4 and 25% of the total CK is considered positive for myocardial infarction.

b) cTn I was determined on a clotted serum sample by ELISA (OPUS Troponin I, Dade Behring Inc, USA) using the fully-automated OPUS II analyser. The lower limit of detection is 0.5 ng/ml and a value greater than or equal to this is considered positive for myocardial injury.

6. C-reactive protein

CRP (normal range, < 10 mg/l) was determined on a clotted serum sample by sandwich ELISA using rabbit anti-human-CRP antibody and peroxidase-conjugated rabbit anti-human-CRP antibody (DAKO Rabbit Anti-human CRP, High Wycombe, UK). Prior to each assay run, a standard reference curve is constructed from standards provided by the supplier. All determinants are performed in duplicate. The lower limit of detection is 0.39 mg/l.

7. Soluble Tumour Necrosis Factor Receptors p55 and p75

Serum levels of sTNF-Rs p55 and p75 were determined on a clotted serum sample by sandwich ELISA using polyclonal and monoclonal anti-sTNF-R55 and sTNF-R75 antibodies. The assays were developed and validated by Dr WA Buurman, University of Maastricht, Netherlands who kindly donated them for use in this thesis. Prior to each assay run, a standard reference curve is constructed from standards provided by the supplier. All determinants are performed in duplicate. The lower limit of detection for sTNF-R p55 and p75 is 0.2 ng/ml and 2 ng/ml, respectively.

Statistical analysis

The Mann-Whitney U test (MW), χ^2 test and Fisher's exact test were used to compare groups of patients. The Kruskal-Wallis one-way analysis of variance was used to examine whether assay levels changed significantly between sampling points. As the data were not normally distributed, the Spearman rank test was used to correlate clinicopathological variables and haemostatic data. A probability value of less than 0.05 was regarded as statistically significant.

Chapter 4

Outcome of ruptured AAA in the Edinburgh Regional Vascular Surgery Unit

4.1 Introduction

Careful review of the literature reveals few reports from single centres which describe the results of surgery for more than 100 patients with ruptured AAA (Table 1.1). Many series describe relatively small numbers of highly selected patients operated upon over long study periods, and patients who survive to reach the hospital only to be denied repair, or succumb during transfer to the operating theatre, are often not included (3).

In 1983, three consultants with a special interest in vascular surgery combined formally to create the ERVSU. At the time of writing, the ERVSU was the sole provider of 24-hour, 365-day vascular surgical services for a population of 1.2 million living in an area of approximately 4,500 square miles in south-east Scotland. In this unit, almost 50% of all the AAA repairs are performed for rupture and this has resulted in considerable experience in the management of this group of patients (15).

4.2 Aims

To determine the operative mortality rate, and the incidence and aetiology of fatal complications in patients who underwent ruptured AAA repair in the ERVSU during the 14-year period ending 31st December 1996. As patient selection impacts on operative mortality, the characteristics of those patients who were admitted to this unit with rupture and did not undergo attempted repair were also examined.

4.3 Methods

The prospective Lothian Surgical Audit data-base was interrogated to identify all patients admitted to the ERVSU with a diagnosis of ruptured AAA during the 14-year period between 1st January 1983 and 31st December 1996. Hospital discharge summaries were reviewed and cause of death recorded.

A patient was considered to have had an operation if an anaesthetic was administered with the intention of repairing the ruptured AAA regardless of whether a graft was successfully inserted. Ruptured AAA was defined by the presence of fresh retroperitoneal and/or intraperitoneal blood in the presence of an aortic aneurysm and with no other identifiable cause for the findings. Operative mortality has been defined as death within 30 days of operation or within the same hospital admission as the initial operation.

Statistical analysis

The Mann-Whitney test (MW), χ^2 test and Fisher's exact test were used.

4.4 Results

A total of 1381 patients had surgery for AAA during the 14-year study period. Of these, 616 patients (45%) (505 men and 111 women of median age 72, range 46-86, years) underwent operation for ruptured AAA. A tube graft was inserted in 311 patients and a bifurcated graft in 240 patients. In 65 patients, no graft was inserted at operation and all of these patients died. There was no significant difference in the age of patients who had a graft inserted compared with those who did not. The operative mortality rate was 230 of 616 (37.3%) patients. Survivors were significantly younger than non-survivors (median age 71, range 46-85 years vs. non-survivors: median age 74, range 46-86 years; $p<0.001$, MW). One hundred patients died during attempted repair and 130 patients died in the post-operative period. The factors contributing to the death of these 230 patients are shown in Table 4.1. A further 125 patients (73 men and 52 women of median age 79, range 54-93 years) were admitted but did not undergo operation. Non-operated patients were significantly older than operated patients ($p<0.001$, MW). There were usually multiple factors which influenced the decision not to operate (Table 4.2).

During the study period, the operative mortality increased (Figure 4.1) but there was no significant difference in the operative mortality for patients who had a graft inserted, or the overall mortality rate (including operated and non-operated patients). A significantly greater proportion of patients underwent operation (Figure 4.2) but a significantly greater proportion did not have a graft inserted (Figure 4.3). Significantly more patients had a tube graft (Figure 4.4) and there was no difference in mortality for patients who underwent repair with a tube (88 of 310, 28%) or bifurcated graft (77 of 240, 32%).

TABLE 4.1

Fatal complications in patients undergoing ruptured AAA repair.

Complication contributing to death	No. of patients
Intra-operative death	100
Cardiac dysfunction (MI, cardiac arrest, hypotension)	63
Uncontrollable haemorrhage	39
Coagulopathy	19
Post-operative death	130
Cardiac dysfunction (MI, cardiac failure, cardiogenic shock)	62
MI	26
ARF	53
Respiratory failure	28
Pneumonia	15
Intra-abdominal haemorrhage	30
Coagulopathy	19
CLI	14
CVA	12
Sepsis syndrome	8
Bowel ischaemia	6
Pulmonary embolism	5
Graft infection	2
Ruptured thoracic aortic aneurysm	1

TABLE 4.2

Reasons for patients with ruptured AAA not undergoing attempted repair

Reason	No. of patients
Decision to operate had been reached but patient died *en route* to the operating theatre	48
Poor clinical condition on admission to hospital	
Unresponsive shock	32
Loss of consciousness	10
Cardiac arrest	8
MI	4
Significant co-morbidity	
Extreme age	45
Ischaemic heart disease	12
Chronic obstructive airways disease	8
Previous disabling CVA	5
Chronic renal failure	4
Co-existing inoperable malignancy	3
Dementia	3
Parkinson's disease	2
Co-existent severe acute pancreatitis	1
Paraplegia	1
Previously assessed by anaesthetist as unfit for elective aneurysm repair	11
Known inoperable thoraco-abdominal aortic aneurysm	5
Patient declined the offer of operation	3

FIGURE 4.1

Operative mortality rate (%) for ruptured AAA repair in the ERVSU between 1983 and 1996.

Non-survivors are represented by the red column, and survivors by the yellow column.

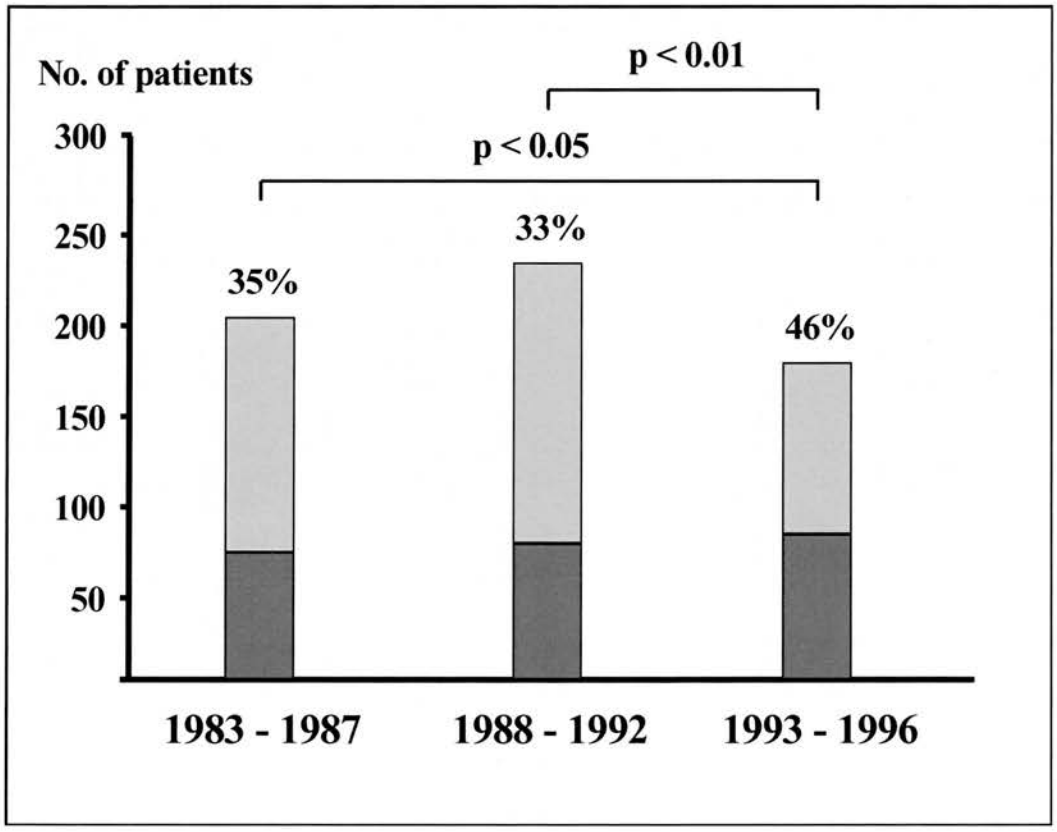


FIGURE 4.2

Patients operated (%) for ruptured AAA in the ERVSU between 1983 and 1996.

Operated patients are represented by the red column, and non-operated patients by the yellow column.

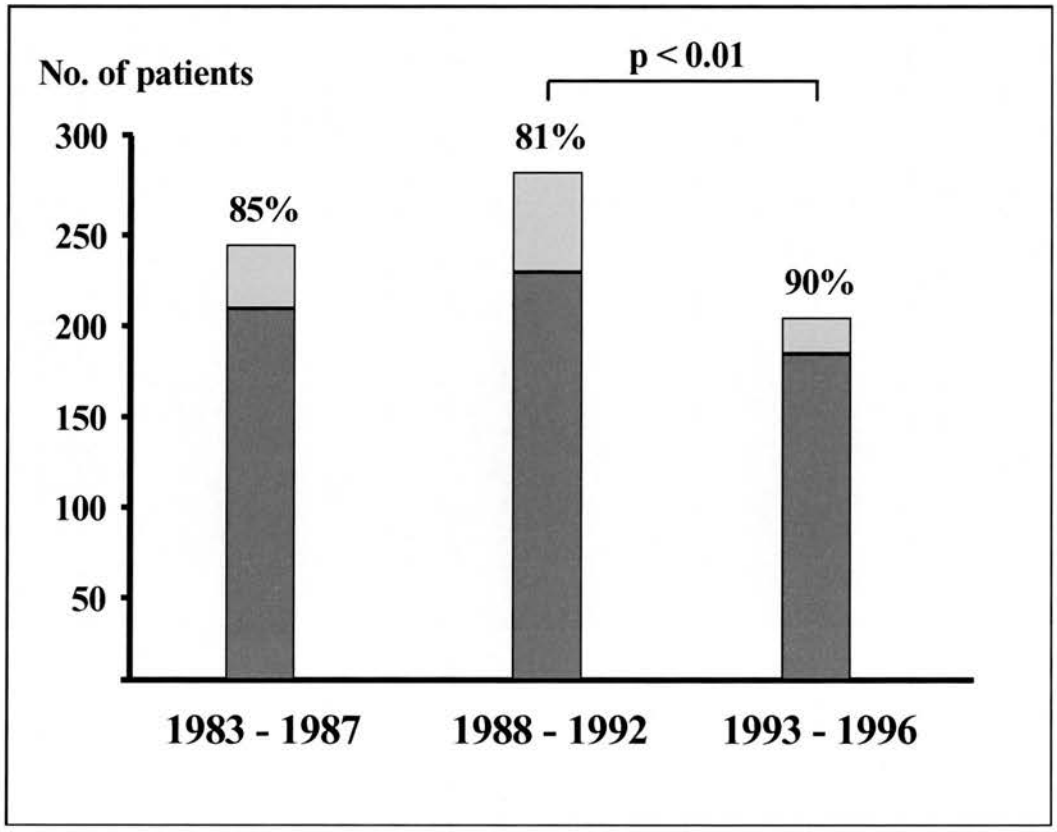


FIGURE 4.3

Proportion of operated patients who received a graft (%) in the ERVSU between 1983 and 1996.

Patients receiving a graft are represented by the red column, and those not receiving a graft by the yellow column.

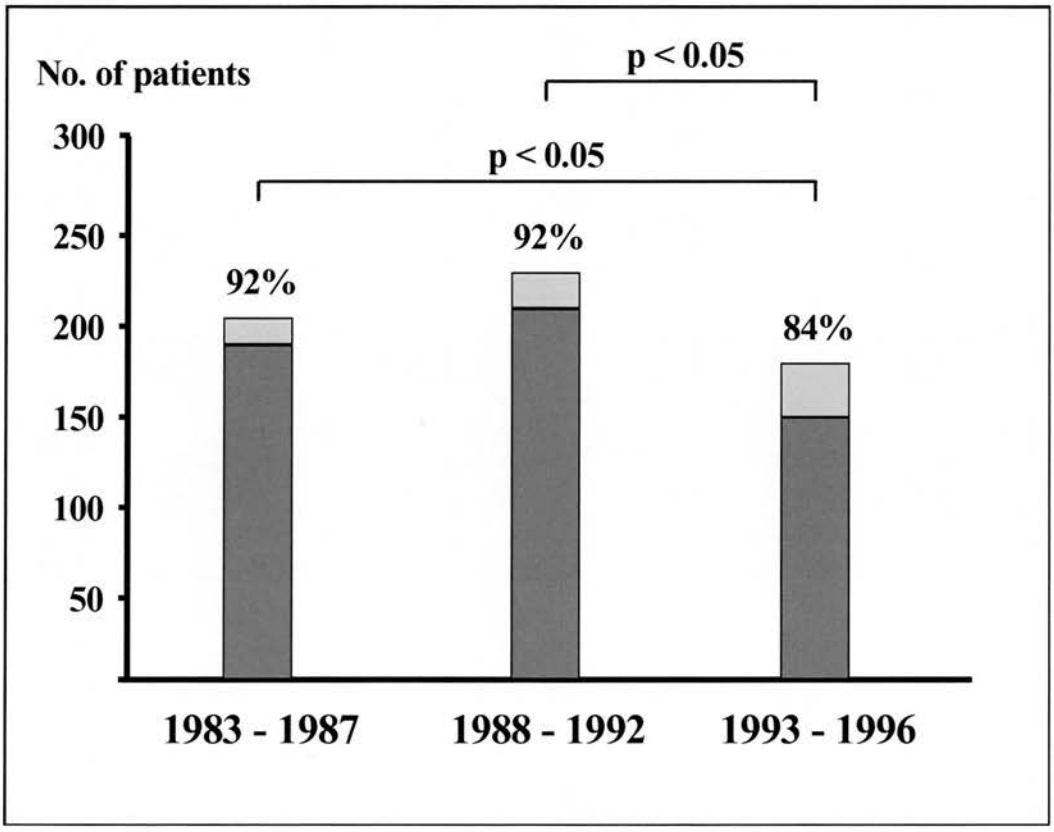
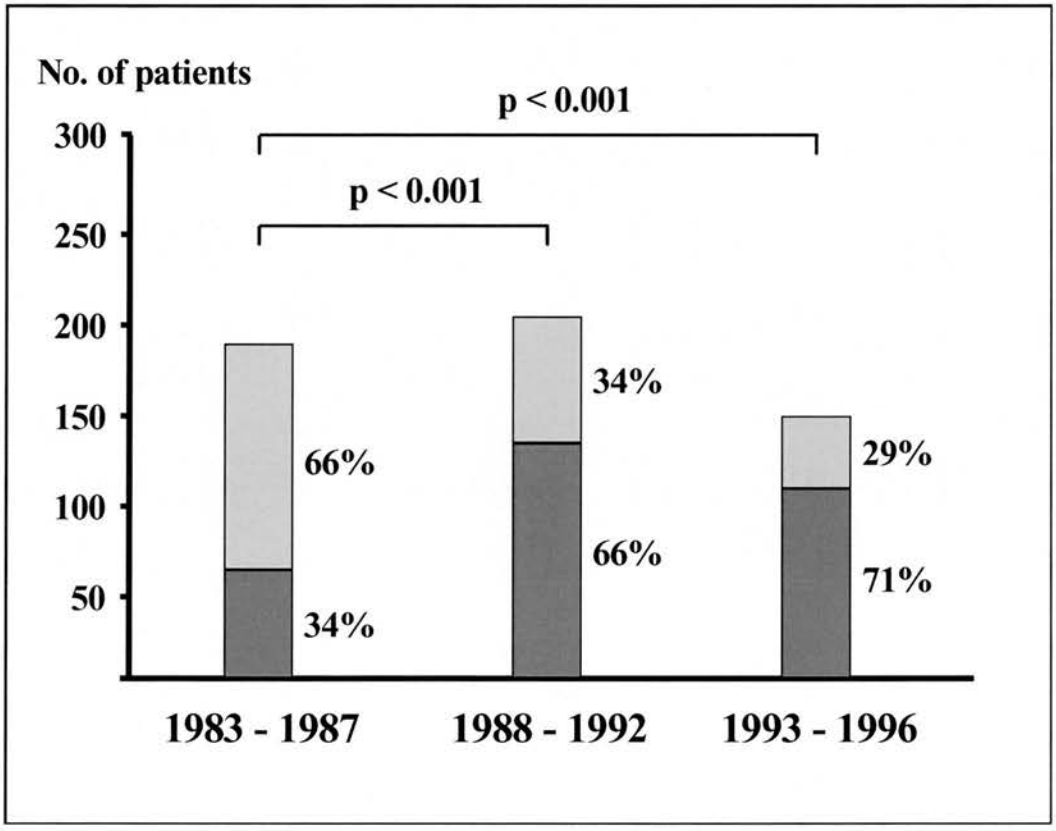


FIGURE 4.4

Patients operated who received a tube or bifurcated graft in the ERVSU between 1983 and 1996.

Patients receiving a tube graft are represented by the red column, and those receiving a bifurcated graft by the yellow column.



4.5 Discussion

This study reports the largest single centre experience of ruptured AAA repair. Unlike other reports, the present study also describes those patients who were admitted and did not undergo operation. The operative mortality rate for rupture was 230 of 616 (37.3%) patients. By including the 48 patients who died during transfer to the operating theatre, the 'intention to treat' mortality rate was 278 of 664 (41.9%) patients. Those patients who did not undergo an operation were significantly older than those who were operated upon, and survivors of operation were significantly younger than non-survivors.

Over time, attempted repair was undertaken in an increasing proportion of patients, but an increasing proportion did not have a graft inserted. This, combined with the fact that there was no improvement in the outcome for patients who had a graft inserted, appears to explain the increase in operative mortality rate during the study period. A more aggressive surgical approach in recent years has not had a positive impact on the overall mortality rate (operated and non-operated patients) in this unit.

Cardiac events, ARF, respiratory failure and haemorrhage contributed to the majority of the peri-operative mortality. Major cardiac events contributed to 48%, and ARF and respiratory failure to 41% and 22% of post-operative deaths, respectively. Coagulopathy was recorded in 19% of intra-operative deaths. Intra-abdominal haemorrhage occurred in 23% of post-operative deaths but coagulopathy was present in only 15%. This may be an underestimate as coagulopathy is present in almost all patients with rupture who are re-operated for bleeding (43). Lower limb ischaemia, CVA and pulmonary embolism contributed to 11%, 9% and 4% of post-operative deaths, respectively.

Despite advances in anaesthesia, surgical techniques and critical care, there has been no improvement in the outcome for patients presenting with ruptured AAA and admitted to the ERVSU in the past two decades. This study did not include patients who died from rupture at home or in hospital, or patients in whom the diagnosis was reached in other hospitals in our catchment area but were not referred, transferred or operated upon. While centralisation of vascular surgical services has not been associated with an improvement in mortality rate for patients presenting to this unit, the effect on the overall community-based outcome for ruptured AAA is unknown and will be addressed in the next chapter.

4.6 Summary

- The operative mortality rate for ruptured AAA was 37%.
- The 'intention to treat' mortality rate was 42%.
- Attempted repair was undertaken in an increasing proportion of patients over time.
- This was associated with an increase in the operative mortality rate.
- Thrombotic and haemorrhagic events (MI, ARF, coagulopathy, CLI, CVA and PE) contributed to the majority of the peri-operative mortality.
- Centralisation of vascular surgical services has not been associated with an improvement in mortality for patients presenting to this unit. Further study is required to assess the effect of centralisation on the overall community-based outcome for ruptured AAA.

Chapter 5

Community outcome from ruptured AAA in the catchment area of the Edinburgh Regional Vascular Surgery Unit

5.1 Introduction

As a result of clinical, political and economic factors, there is an increasing tendency in the UK to centralise vascular surgical services within large regional units. However, to concentrate vascular expertise within a small number of individuals at limited geographical sites inevitably leads to depletion, even an absence, of expertise elsewhere. For example, at the time of writing only one of eight peripheral hospitals conducting 'general' surgery in the catchment area of the ERVSU is staffed by vascular surgeons. While specialisation may improve individual patient outcomes for specific procedures performed within specialist units, it is equally important to demonstrate that centralisation does not prejudice equality of access to specialist care and, therefore, the overall, community-based, outcome for the underlying condition. Ruptured AAA is a major problem in the UK and decisions on how vascular services are to be distributed must be based, at least in part, upon consideration of how these unstable, high risk patients are most appropriately managed.

5.2 Aims

To examine the patterns of referral, management and outcome of patients identified as having ruptured AAA within the catchment area served by this regional vascular unit.

5.3 Methods

This study period was 1st January 1989 to 31st December 1995 (the most recent year for which complete population data were available). All residents of the catchment area of the ERVSU who were admitted to any hospital in the catchment area with a diagnosis of ruptured AAA (International Classification of Diseases ninth revision (ICD-9) codes 441.3, 441.5, and 441.1 if coded in addition to 441.3 or 441.5), or who were certified deceased as a result of ruptured AAA, either in hospital or in the community, were identified through the Information and Statistics Division (ISD) of the National Health Service in Scotland using the Scottish Morbidity Records 1 (SMR1) (hospital discharge records) and General Registrar Office (Scotland) (GRO (S)) mortality records. SMR1 records are linked to each other, and to the GRO (S) mortality records by ISD using probability matching and provide a patient database that includes hospital admission and mortality data. The prospective Lothian Surgical Audit data-base was interrogated to identify all residents from the catchment area who were admitted to this unit with ruptured AAA. Residents of the catchment area who were admitted to hospitals outwith the catchment area (n=32), and residents of other catchment areas who were admitted to hospitals within the area (n=20), were excluded from analysis. Patterns of referral and management, as well as outcome data and post-codes were retrieved for each patient.

As in most instances it was not possible to ascertain the patient's precise location at the time of rupture, it was assumed that rupture had occurred near to their home address rather than at a distant site. Travelling distance for patients admitted directly to this unit was, therefore, defined as the distance by land from the centre of the individual's postcode region of residence to this unit. For those admitted indirectly, travelling

distance was defined as the distance from the centre of the individual's postcode region of residence to the referring hospital and then onto this unit. All patients in the present study were transferred by land ambulance.

Statistical analysis

The Mann-Whitney test (MW), χ^2 test and Fisher's exact test were used.

5.4 Results

The patterns of referral, management and outcome for 972 patients who were identified as having ruptured AAA during the 7-year study period are shown in Figure 5.1. Two hundred and nineteen (22.5%) patients were certified dead in the community without being admitted to hospital, 551 (56.7%) were certified dead in hospital, and 202 (20.8%) survived. The community mortality for ruptured AAA was, therefore, 770 of 972 (79%) patients. The diagnosis was confirmed at operation in 340 (35%) patients, and at post-mortem examination in 268 (28%), of whom 175 died in the community, and 93 died in hospital without transfer to the ERVSU. In the remaining 364 patients, ruptured AAA was diagnosed and recorded on the death certificate on the basis of clinical examination and/or investigation.

Three hundred and seventy two (38%) patients were admitted to other units within or outwith Edinburgh and were not transferred to the ERVSU. Of these, 24 (6.4%) patients underwent operation of whom 14 (3.8%) survived. No data are available to explain the decisions to transfer, operate or treat conservatively this group of patients. Three hundred and eighty one (39%) patients (304 men and 77 women of median age 73, range 46 to 93 years) were admitted to this unit. Of these, 65 (17%) patients did not undergo operation because they were considered unfit for surgery due to severe co-morbidity and/or extreme age, their clinical condition had deteriorated such that they were considered unfit for repair, a decision was made to operate but death occurred before surgery could commence, or the offer of operation was declined. Of 316 (83%) patients who were operated, a graft was inserted in 277 (88%). The overall mortality for all patients admitted to the ERVSU was 193 of 381 (51%), and the operative mortality

was 128 of 316 (41%). There was no significant difference in the overall mortality between patients transferred from units outwith Edinburgh (25 of 43, 58%), those transferred from units within Edinburgh (16 of 28, 57%), and those admitted directly to the ERVSU (152 of 310, 49%) ($p=0.41$, χ^2). Overall, 316 operated patients travelled significantly further than 65 non-operated patients ($p<0.001$, MW). There was no significant difference in travelling distance between 188 (59%) operated patients who survived and the 128 who did not (Table 5.1). Of 310 patients who were admitted directly to this unit, 262 who were operated travelled significantly further than 48 who were not ($p<0.001$, MW), and there was no significant difference in travelling distance between 160 operated patients who survived and 102 (40%) who did not.

FIGURE 5.1

Management of patients diagnosed as ruptured AAA

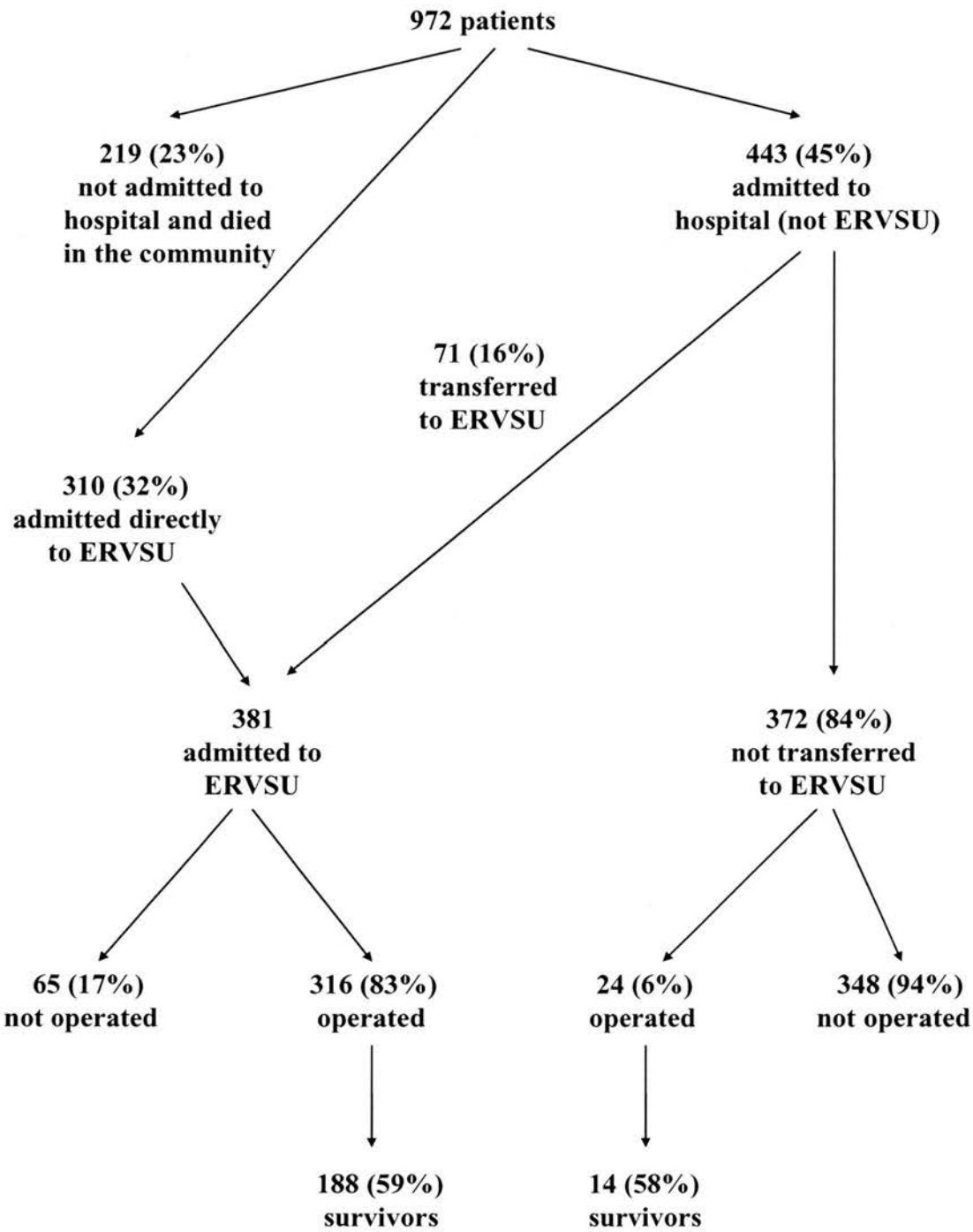


TABLE 5.1

Transfer distance and outcome in patients with ruptured AAA admitted (directly and indirectly) to the ERVSU

Distance travelled (miles)	No. of patients	Not operated	Operated		Operative mortality rate	Overall mortality rate
			graft inserted	no graft inserted		
0 - 5	152	41	95	16	44/111 (40)	85/152 (56)
5 - 10	74	9	58	7	26/65 (40)	35/74 (47)
10 - 15	48	9	34	5	19/39 (49)	28/48 (58)
15 - 20	44	3	39	2	14/41 (34)	17/44 (39)
20 - 25	32	1	27	4	14/31 (45)	15/32 (47)
25+	31	2	24	5	11/29 (38)	13/31 (42)
	381	65	277	39	128/316 (41)	193/381 (51)

Values in parentheses are percentages

5.5 Discussion

The first principal finding of the present study was that there was no significant difference in travelling distance between the operated patients who survived and those who did not; but that patients who were not operated travelled significantly shorter distances to hospital than those who were. One explanation for this may be the pre-selection of 'good risk' patients for transfer over longer distances. In addition, a proportion of patients sustaining rupture in the immediate vicinity of the Edinburgh Vascular Unit may have been moribund on arrival and thus not operated.

Several studies have attempted to determine whether travelling distance and transfer time has an effect on operative mortality in ruptured AAA. Butler *et al* (243) showed no significant difference in operative mortality between patients admitted from the local catchment area (28 of 48, 58%) and those transferred from other centres (13 of 24, 54%). In 183 patients, Fielding *et al* (244) reported no significant difference in operative mortality between those transferred less than five miles (43 of 85, 50.5%) and those transferred farther than five miles (39 of 97, 40.2%) and, similarly, Barros D'Sa (245) demonstrated no significant correlation between travelling distance and outcome in 187 operated patients. While Yashar *et al* (246) reported a mortality rate of 27% for patients operated within four hours of onset of symptoms compared with 80% for those operated beyond four hours, van Heeckeren (247) was unable to demonstrate a significant correlation between duration of symptoms and mortality in 57 operated patients, and Amundsen *et al* (248) failed to demonstrate any correlation between transport time and overall mortality for 114 patients (including 30 who were not operated). Meyer *et al* (249) compared 48 patients admitted to a community hospital and 49 admitted to a

municipal hospital, and demonstrated that while significantly more stable patients were operated more than two hours after diagnosis in the community hospital, significantly more shocked patients underwent immediate operation, and consequently mortality was significantly higher, in the municipal hospital. Ouriel *et al* (49), however, demonstrated no significant difference in the delay from the onset of symptoms to hospital arrival for patients admitted to a university or community facility, as well as no significant relationship between operative mortality and the delay between arrival in hospital and the start of the operation. In a study of 122 patients, Farooq *et al* (250) also demonstrated no relationship between operative mortality and duration of symptoms and delay between hospital arrival and the start of the operation. Although more hypotensive patients were operated upon within two hours of onset of symptoms, this was not associated with a significant increase in mortality.

At first sight, these and present data suggest that centralisation does not prejudice the community outcome for ruptured AAA. However, in this 7-year study, 93% of survivors were operated upon in this regional vascular unit, fewer than 40% were transferred to this unit, and only 6% of those treated outwith this unit underwent operation. The operative mortality outwith the ERVSU was a very acceptable 14 of 24 (58%). However, almost all of these operations were performed in one peripheral hospital by two general surgeons with a major vascular interest. None of the other seven hospitals were staffed by surgeons with vascular expertise which presumably explains the very low operation rate outside the Edinburgh Vascular Unit and the other peripheral hospital.

The present study, indeed all community studies of ruptured AAA, have limitations. The diagnosis of rupture was confirmed by operation or post-mortem examination in only 63% of patients. It is not known what proportion of patients who were not operated

upon were diagnosed as having ruptured AAA in life. It is likely that there were patients who died suddenly from rupture in whom the diagnosis was not made, and perhaps a few who did not die from rupture but in whom this was the certified cause of death.

The important question raised by these data is whether a broader provision of vascular surgical expertise would have increased the proportion of patients offered and surviving surgery and whether this, in turn, would have positively impacted the community survival from the condition. Although the community outcome in the present series is similar to that reported in earlier studies from regions where centralisation has not occurred (Table 5.2), centralisation of vascular surgical services may be associated with an inappropriately low operation and survival rate for the majority of patients who are not transferred to the regional centre.

TABLE 5.2

Reported studies estimating the community outcome from ruptured AAA

Author	No. of patients	Died outside hospital	Died in hospital		Survivors
			not operated	operated	
Armour (24)	25	11 (44)	9	1	4 (16)
Ingoldby (25)	260	158 (61)	1	49	52 (20)
Johansson (26)	88	24 (27)	51	8	5 (6)
Mealy (27)	265	169 (64)	18	48	30 (11)
Thomas (28)	183	64 (35)	44	41	34 (19)
Semmens (29)	873	379 (43)	211	102	181 (21)
Present study	972	219 (23)	413	138	202 (21)

Values in parentheses are percentages

5.6 Summary

- There was no significant difference in overall mortality between patients who were admitted directly to this unit, and those who were transferred from elsewhere.
- Operated patients travelled significantly further than non-operated patients, but there was no significant difference in travelling distance between operated patients who survived and those who did not.
- The overall community-based mortality rate was 79%, similar to that reported from where centralisation of vascular surgical services has not occurred.
- Despite considerable expertise in the management of ruptured AAA, centralisation of vascular surgical services has not been associated with an improvement in individual patient or community-based outcome. By contrast, this situation is associated with an inappropriately low operation and survival rate for patients who are not transferred to the regional centre.
- Fundamental basic science research into the pathophysiology of ruptured AAA repair is required to improve patient care and clinical outcome.

Chapter 6

Serial peri-operative markers of coagulation and fibrinolysis in ruptured and non-ruptured AAA repair

6.1 Introduction

Repair of ruptured and non-ruptured abdominal aortic aneurysm (AAA) is associated with an operative mortality rate of 33% to 69% (Table 1.1), and 3% to 15% (4,6,9,30,251), respectively. The great majority of the morbidity and mortality is due to MI and MOF, which may be related to micro- and macrovascular thrombosis developing as a result of a procoagulant state (Chapter 1.2). It is perhaps surprising, therefore, that previous workers have suggested that supracoeliac aortic cross-clamping and thoraco-abdominal aortic aneurysm repair (252,253), as well as animal studies of infrarenal aortic clamping and isolated lower body ischaemia (71), are associated with increased fibrinolysis. Studies of elective infrarenal aortic reconstruction are few and contradictory (78,80,140,141,144,149,150,254,255). Although data such as these have been used to support the use of antifibrinolytic agents in patients undergoing operation for ruptured AAA (256), careful review of the literature reveals that the precise nature of the haemostatic derangement in such patients has not previously been studied.

6.2 Aims

To examine serial markers of thrombin generation and fibrinolysis during the course of emergency surgery for ruptured AAA and to compare this with patients undergoing elective repair of non-ruptured AAA.

6.3 Methods

Patients

Ten patients (8 men and 2 women of median age 76, range 71-86, years) operated for ruptured and 9 patients (8 men and 1 woman of median age 69, range 58-80, years) operated for asymptomatic non-ruptured infrarenal AAA were prospectively studied.

In patients operated for rupture, the median (range) delay between the onset of symptoms of rupture and hospital admission was 5 (3-14) hours. All patients had at least one documented episode of hypotension (systolic blood pressure less than 100mmHg) prior to surgery. In patients undergoing non-ruptured AAA repair, the median (range) antero-posterior diameter of the aneurysm measured by ultrasonography was 6.5 (5.5-8.0) cm. No patient had liver disease. Co-morbidity data are shown in Table 6.1.

Operative methods

The operations were performed as previously described. Rupture was retroperitoneal in all patients. No patient received protamine sulphate. A dacron tube graft was inserted in 13 patients (9 rupture, 4 non-rupture), an aorto-bi-iliac graft in five (1 rupture, 4 non-rupture) and an aorto-bifemoral graft in one patient with non-ruptured AAA.

Definition of peri-operative complications

These were defined as described above.

Assays of haemostatic function

The extrinsic coagulation and fibrinolytic systems are summarized in Figure 6.1 and 6.2, respectively. Plasma levels of PF 1+2 and TAT were assayed as markers of thrombin generation, and t-PA activity, t-PA antigen, PAI activity, and fibrin degradation product D-dimer as markers of fibrinolysis. Haematocrit, platelet count, fibrinogen, PT, aPTT, and CRP were also measured. Assays were performed as described above.

Sample collection

The pathophysiology of ruptured AAA repair can be divided into three phases (Figure 6.3). Firstly, there is a period of whole body hypoperfusion due to hypovolaemic shock. Secondly, there is a period of lower body ischaemia following aortic clamp placement. Finally, if repair is successful, there is a period of reperfusion. The sampling points were chosen to reflect the maximum effect of each of the three phases. Blood was sampled from an indwelling arterial line immediately prior to the induction of anaesthesia (sample A); immediately before aortic clamp release (sample B); five minutes (sample C) and 24 hours (sample D) after aortic clamp release; and on post-operative days 2, 3 and 5. t-PA antigen, D-dimer and TAT were measured at sample point A. Haematocrit, platelet count, fibrinogen, PT, aPTT, CRP, t-PA activity, PAI activity and PF 1+2 were measured at all sample points. Samples were prepared as described above.

Statistical analysis

The Mann-Whitney U test (MW) and Spearman rank tests were used.

TABLE 6.1

Co-morbidity in patients operated for ruptured and non-ruptured AAA.

	Ruptured AAA (n=10)	**Non-ruptured AAA** (n=9)
Co-morbidity		
None	2	1
Myocardial infarction	2	-
Angina pectoris	3	2
Coronary artery bypass graft	-	1
Hypertension	3	2
Congestive cardiac failure	1	-
Stroke	1	-
Peripheral arterial occlusive disease	1	2
Venous thrombo-embolism	-	1
Chronic obstructive airways disease	1	1
Non-insulin dependent diabetes mellitus	-	1
Cigarette smoking		
Non-smoker	7	1
Ex-smoker	2	5
Current smoker	1	3
Medications		
None	3	3
Aspirin	3	4
Diuretic	3	-
Nitrate	1	1
Angiotensin converting enzyme inhibitor	1	-
Beta-adrenoceptor blocker	-	2
Bronchodilator	1	1

FIGURE 6.1

Overview of the extrinsic coagulation system.

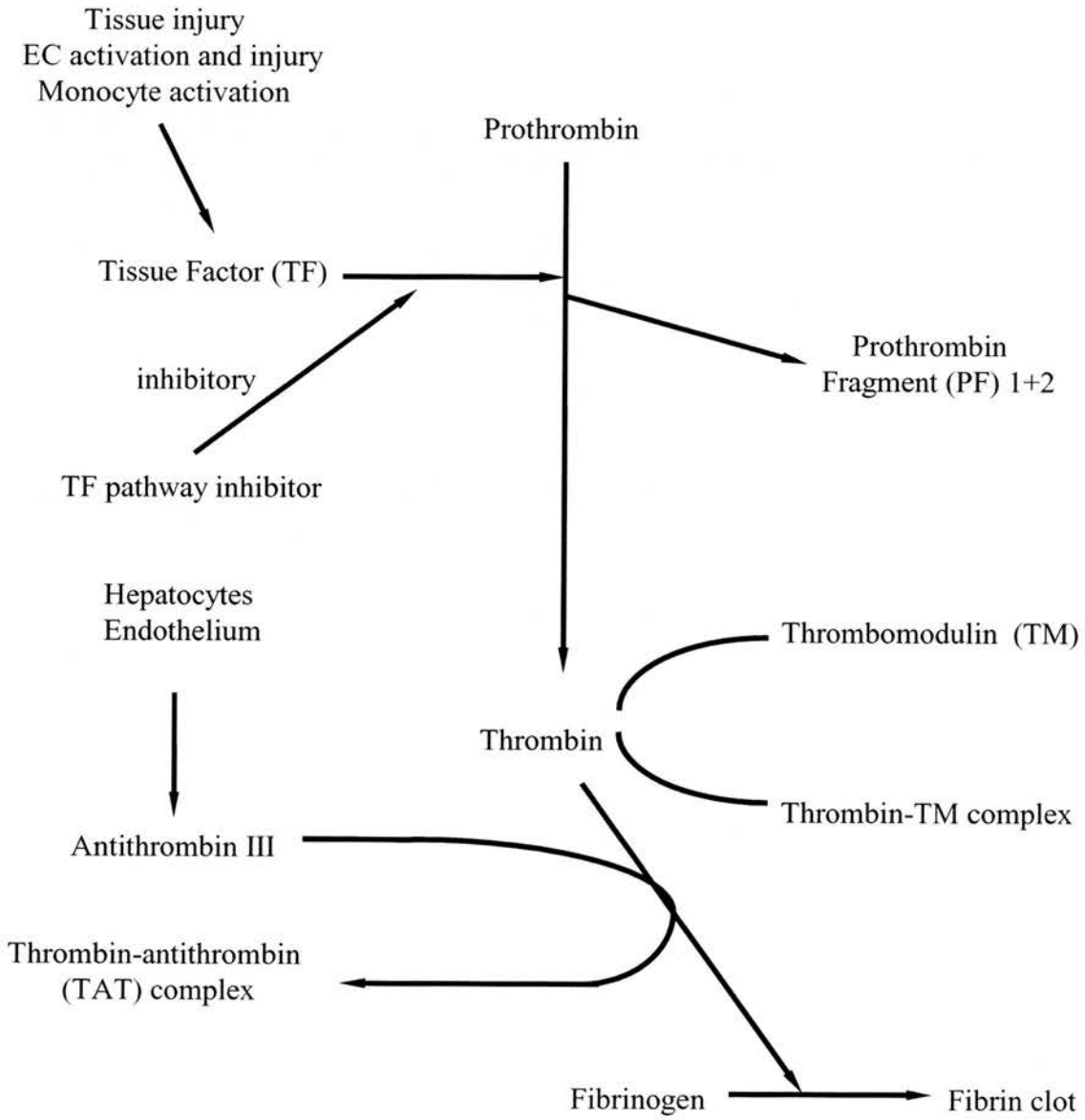


FIGURE 6.2

Overview of the fibrinolytic system.

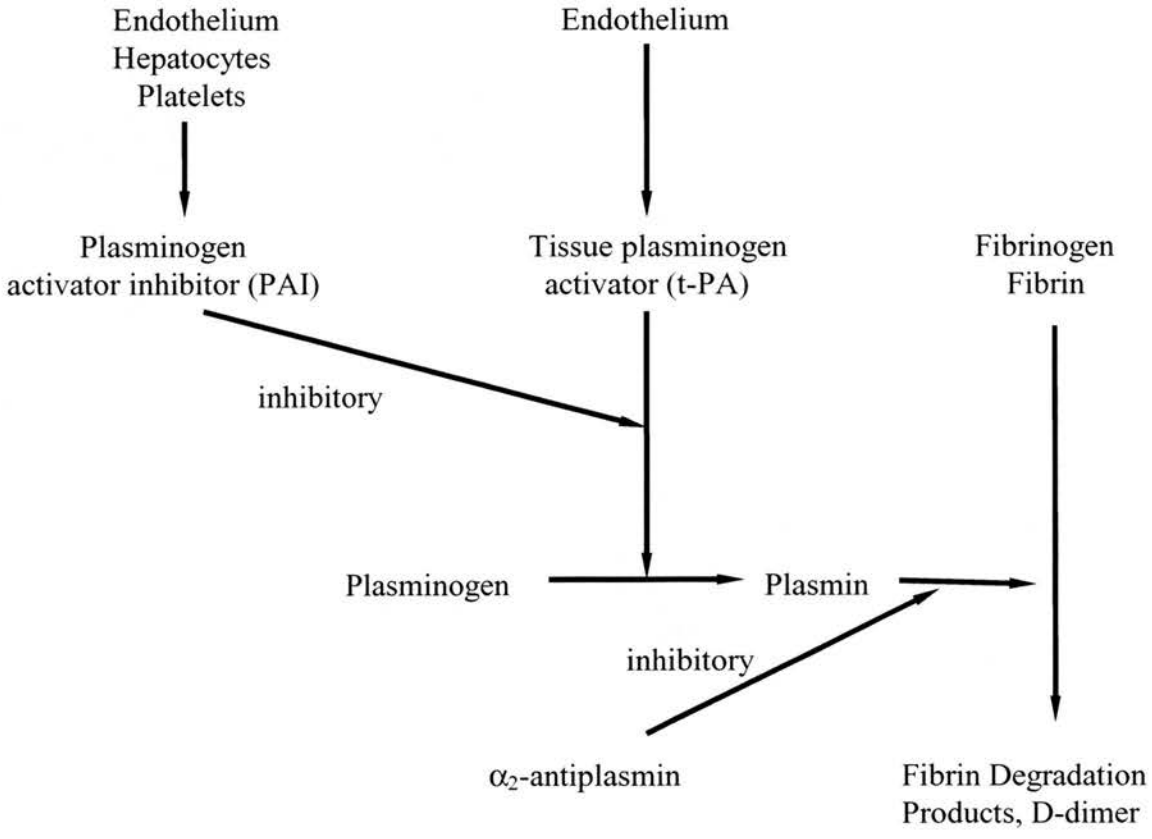
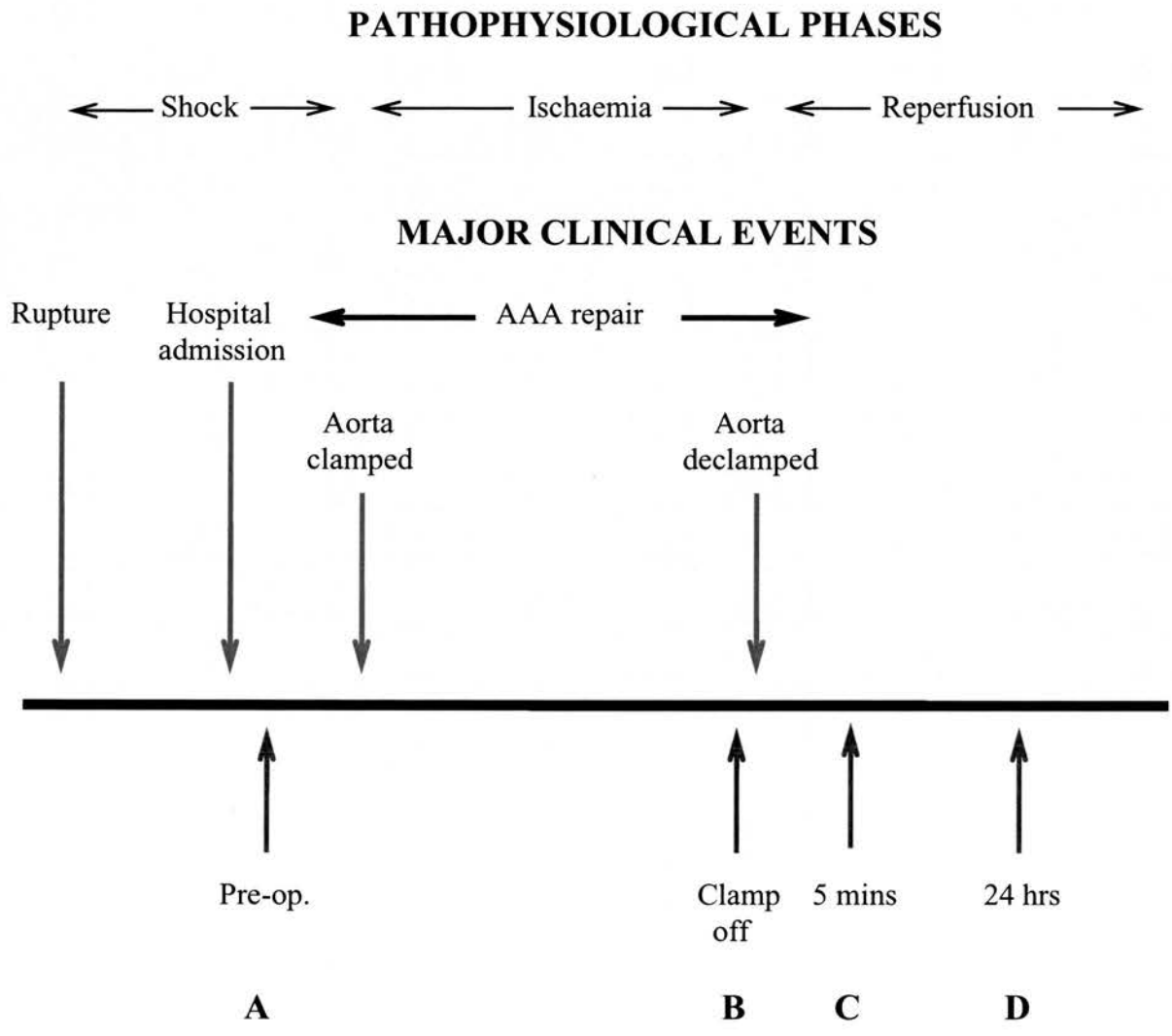


FIGURE 6.3

Schematic view of the pathophysiology of ruptured AAA repair.



6.4 Results

Clinical data

Clinical and operative data for both groups of patients are summarized in Table 6.2. During operation, no patients with non-ruptured AAA received inotropic support. Three patients with rupture received adrenaline, and five received dopamine, infusion. All patients with rupture were admitted to the Intensive Therapy Unit (ITU) post-operatively for ventilatory support. All patients operated for non-ruptured AAA were admitted to the High Dependency Unit post-operatively and no patient was admitted to ITU or required ventilatory support. Seven patients operated for rupture and four operated for non-ruptured AAA developed major post-operative complications (Table 6.3). All patients survived to 24 hours after repair. Two patients with rupture died in hospital from acute respiratory distress syndrome and ARF on post-operative day 10, and from pneumonia and critical lower limb ischaemia on post-operative day 21. There were no deaths after repair of non-ruptured AAA.

TABLE 6.2

Clinical and operative data in ruptured and non-ruptured AAA.

	Ruptured AAA median (range) (n=10)	**Non-ruptured AAA** median (range) (n=9)	**p value** *
Pre-operative			
Crystalloid administration (l)	0.5 (0.1 - 4.0)	-	
Colloid administration (l)	0 (0 - 1.5)	-	
Intra-operative			
Operation time (minutes)	105 (70 - 205)	160 (85 - 285)	NS
Aortic clamp time (minutes)	60 (30 - 125)	70 (25 - 150)	NS
Measured blood loss (l)	2.3 (1.0 - 6.4)	2.8 (1.0 - 6.0)	NS
Crystalloid administration (l)	2.0 (0.5 - 3.5)	2.0 (1.0 - 4.0)	NS
Colloid administration (l)	1.5 (0 - 2.3)	2.0 (0.5 - 3.8)	NS
RCC administration (units) [1]	8 (6 - 11)	4 (0 - 10)	0.02
FFP administration (units) [2]	2 (0 - 6)	0 (0 - 2)	NS
Platelet administration (bags) [3]	1 (0 - 1)	0 (0 - 1)	NS
Post-operative			
Duration of ITU stay (hrs)	72 (13-244)	-	
Duration of IPPV (hrs)	19 (9-142)	-	

KEY: * Mann-Whitney U test, [1] RCC = 300 ml, [2] FFP = 300ml, [3] one bag of platelet transfusion = 4 pooled units (250 ml)

TABLE 6.3

Post-operative complications and procedures in ruptured and non-ruptured AAA.

	Ruptured AAA (n= 7 / 10)	**Non-ruptured AAA** (n= 4 / 9)
Cardiovascular		
AF	4	1
CCF	4	3
MI	2	0
CVA	1	0
CLI	2	1
DVT	0	1
Respiratory		
Chest infection	6	2
Respiratory failure	3	0
ARDS	1	0
Acute Renal Failure	2	1
Coagulopathy	1	0
Sepsis syndrome	1	0
Colon ischaemia	1	0
Total parenteral nutrition	3	0
Inotropic support		
Adrenaline	3	0
Renal dose dopamine	6	0
Re-operation	1 [1]	1 [2]

KEY: [1] = laparotomy for intra-abdominal hemorrhage, femoral thrombectomy, Hartmann's procedure for colon ischaemia, drainage of infected pelvic haematoma, [2] = popliteal embolectomy and fasciotomies

Haematocrit, platelet count, fibrinogen, PT, aPTT and CRP

The median (range) values for haematocrit, the standard tests of haemostasis (platelet count, fibrinogen, PT and aPTT) and CRP to 24 hours post-operatively are shown in Table 6.4. There was no significant difference between rupture and non-rupture with regard to any of the assays on post-operative days 2,3 and 5.

In ruptured AAA, there was a significant negative correlation between operative blood loss and fibrinogen level immediately before ($r = - 0.694$, $p = 0.026$) and five minutes after aortic clamp release ($r = - 0.75$, $p = 0.012$), and also platelet count five minutes after aortic clamp release ($r = - 0.726$, $p = 0.018$). There was a significant positive correlation between operative blood loss and PT immediately before aortic clamp release ($r = + 0.823$, $p = 0.003$) and aPPT immediately before ($r = + 0.787$, $p = 0.007$) and five minutes after aortic clamp release ($r = + 0.64$, $p = 0.046$).

In non-ruptured AAA, there was a significant negative correlation between operative blood loss and fibrinogen immediately before ($r= -0.678$, $p= 0.045$) and 5 minutes after aortic clamp release ($r= -0.711$, $p= 0.032$); and platelet count immediately before aortic clamp release ($r= -0.728$, $p= 0.026$). There was a significant positive correlation between operative blood loss and PT 5 minutes after aortic clamp release ($r= +0.728$, $p= 0.026$). There was a significant negative correlation between clamp time and fibrinogen immediately before ($r= -0.812$, $p= 0.008$) and 5 minutes after aortic clamp release ($r= -0.711$, $p= 0.032$), and platelet count immediately before aortic clamp release ($r= -0.678$, $p= 0.045$). There was a significant positive correlation between aortic clamp time and PT immediately before ($r= +0.72$, $p= 0.029$) and 5 minutes after aortic clamp release ($r= +0.828$, $p= 0.006$).

TABLE 6.4

Haematocrit, platelet count, fibrinogen, PT, aPTT and CRP to 24 hours post-operatively.

Assay (normal range)	**Sample point**	**Ruptured AAA** median (range) (n=10)	**Non-ruptured AAA** median (range) (n=9)	**p value** *
Haematocrit	A	0.31 (0.13 - 0.34)	0.42 (0.33 - 0.47)	0.0004
(0.37-0.54)	B	0.27 (0.18 - 0.44)	0.30 (0.25 - 0.37)	NS
	C	0.28 (0.22 - 0.42)	0.30 (0.23 - 0.35)	NS
	D	0.34 (0.26 - 0.42)	0.34 (0.25 - 0.39)	NS
Platelet count	A	230 (119 - 303)	182 (75 - 744)	NS
(150-350 x 10^9/l)	B	120 (81 - 189)	132 (103 - 541)	NS
	C	108 (59 - 146)	135 (91 - 577)	NS
	D	97 (50 - 133)	127 (85 - 604)	NS
Fibrinogen	A	2.27 (0.86 - 3.75)	2.80 (1.59 - 6.02)	NS
(1.5-4.0 g/l)	B	1.12 (0.88 - 2.51)	1.68 (0.72 - 5.39)	NS
	C	0.97 (0.46 - 1.82)	1.45 (0.36 - 5.44)	NS
	D	3.29 (1.76 - 4.63)	3.70 (2.50 - 8.98)	NS
PT	A	14 (11 - 35)	12 (11 - 14)	0.009
(10.5-14.5 s)	B	20 (15 - 26)	17 (15 - 26)	NS
	C	20 (17 - 31)	20 (14 - 23)	NS
	D	16 (13 - 18)	14 (12 - 21)	NS
aPTT	A	32 (28 - 126)	31 (25 - 49)	NS
(28-40 s)	B	50 (34 - 210)	176 (56 - 240)	0.006
	C	55 (42 - 210)	210 (79 - 240)	0.008
	D	39 (32 - 76)	36 (29 - 39)	0.02
CRP	A	6.7 (2.6 - 178.3)	4.3 (0.3 - 18.6)	NS
(less than 10 mg/l)	B	3.0 (0.9 - 116)	5.0 (1.1 - 13.4)	NS
	C	3.8 (1.9 - 11.4)	2.0 (1.3 - 8.2)	NS
	D	105 (41.8 - 141.8)	92.4 (41.5 - 180.8)	NS

KEY: * = Mann-Whitney U test

Markers of thrombin generation

The median (range) values for PF 1+2 to 24 hours post-operatively are shown in Table 6.5. Before operation, TAT levels were elevated above the normal range in all patients. Levels were significantly higher in patients with ruptured AAA than in those with non-ruptured AAA ($p<0.02$, Mann-Whitney U test) (Figure 6.4). Before operation, 7 of 9 patients with non-ruptured AAA had elevated PF 1+2 levels. Before and during operation, PF 1+2 levels were significantly higher in patients undergoing repair of ruptured AAA when compared to those undergoing repair of non-ruptured AAA. At 24 hours and beyond, there was no significant difference in PF 1+2 levels between the groups (Figure 6.5).

In ruptured AAA, there was no significant relationship between operative blood loss or aortic clamp time and any of the markers of thrombin generation. There was, however, a significant positive correlation between the duration of symptoms of rupture and pre-operative PF 1+2 ($r= +0.717$, $p= 0.02$).

In non-ruptured AAA, there was no significant relationship between operative blood loss or aortic clamp time and any of the markers of thrombin generation.

TABLE 6.5

PF 1+2 to 24 hours post-operatively.

Assay (normal range)	**Sample point**	**Ruptured AAA** median (range) (n=10)	**Non-ruptured AAA** median (range) (n=9)	**p value** *
PF 1+2	A	9.0 (5.4 - 11.6)	2.2 (0.7 - 7.1)	0.0008
(0.4-1.1 nmol/l)	B	6.7 (3.3 - 8.9)	1.0 (0.9 - 4.0)	0.0003
	C	6.5 (4.2 - 9.6)	2.0 (1.0 - 4.9)	0.0007
	D	3.5 (1.9 - 11.4)	1.9 (1.3 - 5.6)	NS

KEY: * = Mann-Whitney U test

FIGURE 6.4

Individual data points for pre-operative TAT level.

Patients with ruptured AAA are represented by the red squares and
patients with non-ruptured AAA by the yellow circles.
The upper limit of the normal range for TAT (1-4.1 microg/l) is shown by the horizontal line.

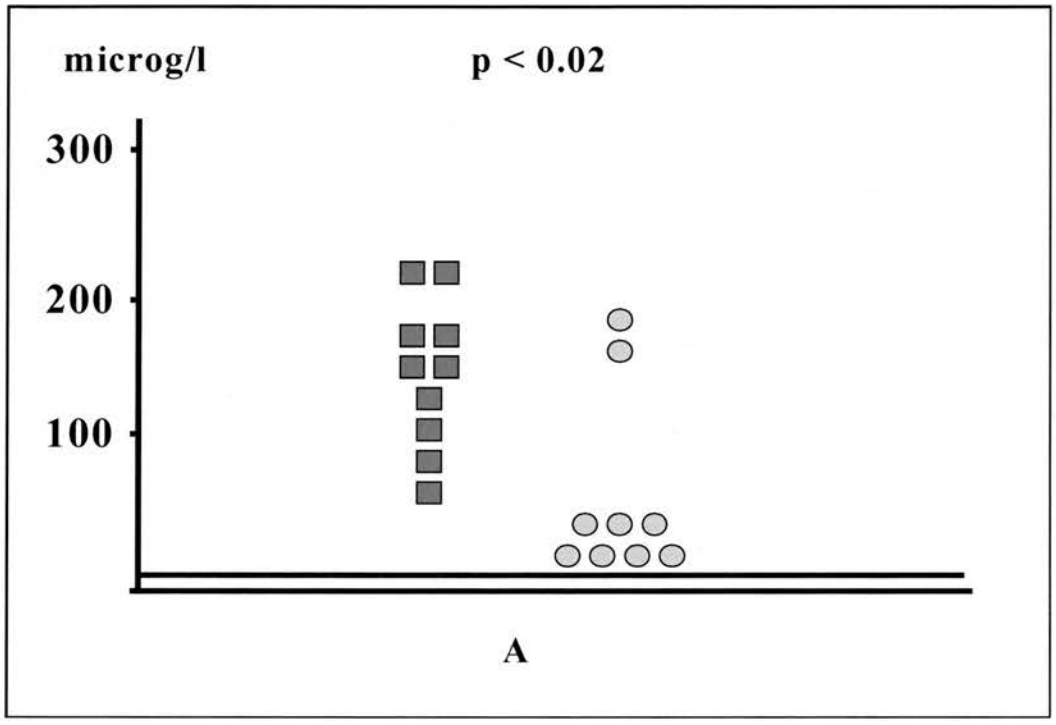
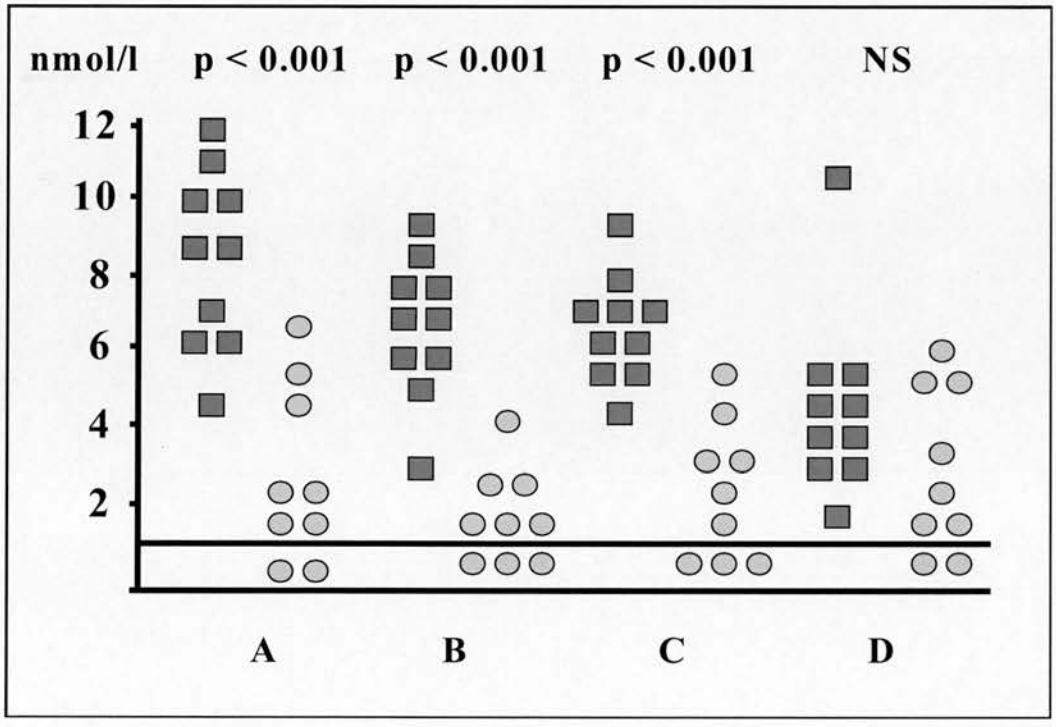


FIGURE 6.5

Individual data points for PF 1+2 immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 24 hours (sample D) after aortic clamp release.

Patients with ruptured AAA are represented by the red squares and patients with non-ruptured AAA by the yellow circles.
The upper limit of the normal range for PF 1+2 (0.4-1.1 nmol/l) is shown by the horizontal line.



Markers of fibrinolysis

The median (range) values for t-PA activity and PAI activity to 24 hours post-operatively are shown in Table 6.6. Before operation, t-PA antigen levels were significantly higher in patients with ruptured AAA compared with those with non-ruptured AAA ($p<0.005$, Mann-Whitney U test) (Figure 6.6). Before operation, there was no significant difference in the D-dimer levels between ruptured and non-ruptured AAA (Figure 6.7). Before and during operation, t-PA activity was significantly lower in patients undergoing repair of ruptured AAA when compared to those undergoing repair of non-ruptured AAA (Figure 6.8). At 24 hours and beyond, there was no significant difference in t-PA activity between the groups. During the operation, four patients with non-ruptured AAA had elevated t-PA activity. Before and during operation, PAI activity was significantly higher in patients undergoing repair of ruptured AAA when compared to those undergoing repair of non-ruptured AAA. At 24 hours and beyond, there was no significant difference in PAI activity between the groups (Figure 6.9). Coagulation and fibrinolytic data for two patients who died after ruptured AAA repair compared with eight who survived are shown in Table 6.7. In ruptured AAA, there was no significant relationship between the duration of symptoms, operative blood loss or aortic clamp time and any fibrinolytic markers. In non-ruptured AAA, there was a significant negative correlation between operative blood loss and t-PA activity 5 minutes after aortic clamp release ($r= -0.753$, $p= 0.019$). There was a significant negative correlation between aortic clamp time and t-PA activity immediately before ($r= -0.837$, $p= 0.005$) and 5 minutes after aortic clamp release ($r= -0.72$, $p= 0.029$); and a significant positive correlation with PAI activity 5 minutes after aortic clamp release ($r= +0.686$, $p= 0.041$).

TABLE 6.6

t-PA and PAI activity to 24 hours post-operatively.

Assay (normal range)	**Sample point**	**Ruptured AAA** median (range) (n=10)	**Non-ruptured AAA** median (range) (n=9)	**p value** *
t-PA activity	A	0.12 (0.06 - 0.43)	0.49 (0.14 - 3.2)	0.009
(0.2-2.0 IU/ml)	B	0.27 (0.08 - 0.8)	0.91 (0.34 - 4.65)	0.0014
	C	0.32 (0.09 - 4.53)	1.06 (0.19 - 5.62)	0.034
	D	0.41 (0.15 - 2.1)	0.46 (0.21 - 1.45)	NS
PAI activity	A	36.5 (20.6 - 38.8)	8.2 (3.2 - 21.7)	0.0003
(less than 15 AU/ml)	B	38.6 (13.0 - 39.4)	10.8 (2.8 - 38.9)	0.0042
	C	37.2 (10.6 - 39.4)	12.6 (2.2 - 28.7)	0.0055
	D	18.1 (5.0 - 35.3)	14.7 (5.7 - 22.3)	NS

KEY: * = Mann-Whitney U test

FIGURE 6.6

Individual data points for pre-operative t-PA antigen.

Patients with ruptured AAA are represented by the red squares and
patients with non-ruptured AAA by the yellow circles.
The upper limit of the normal range for t-PA antigen (1-12 ng/ml) is shown by the horizontal line.

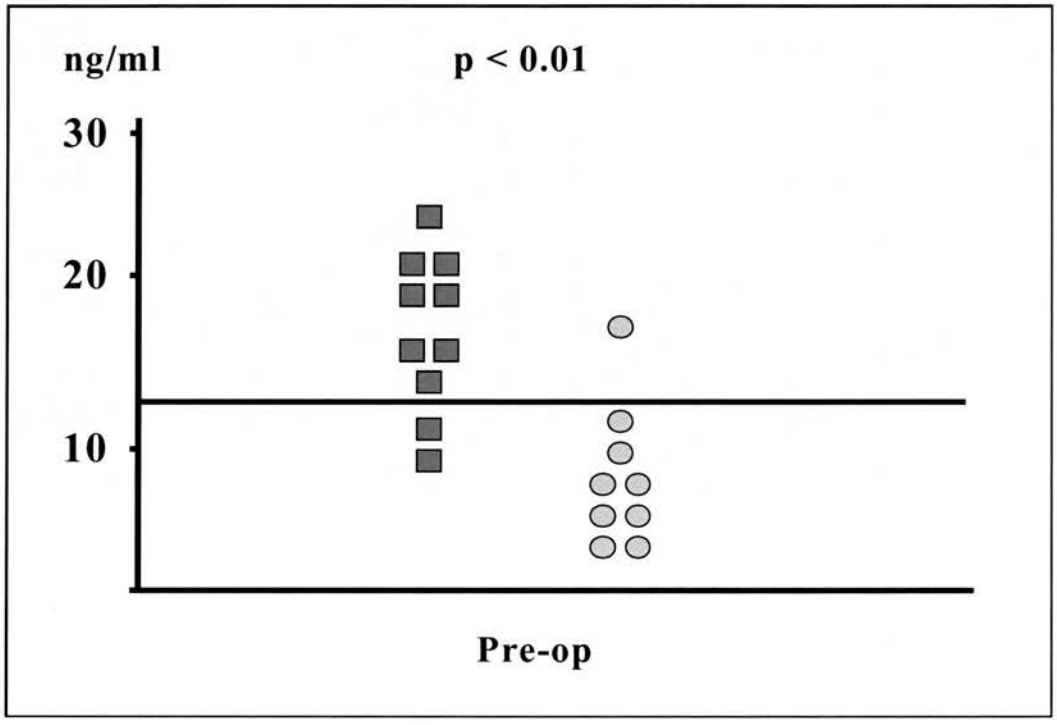


FIGURE 6.7

Individual data points for pre-operative D-dimer

Patients with ruptured AAA are represented by the red squares and patients with non-ruptured AAA by the yellow circles.
The upper limit of the normal range for D-dimer (630-850 ng/ml) is shown by the horizontal line.

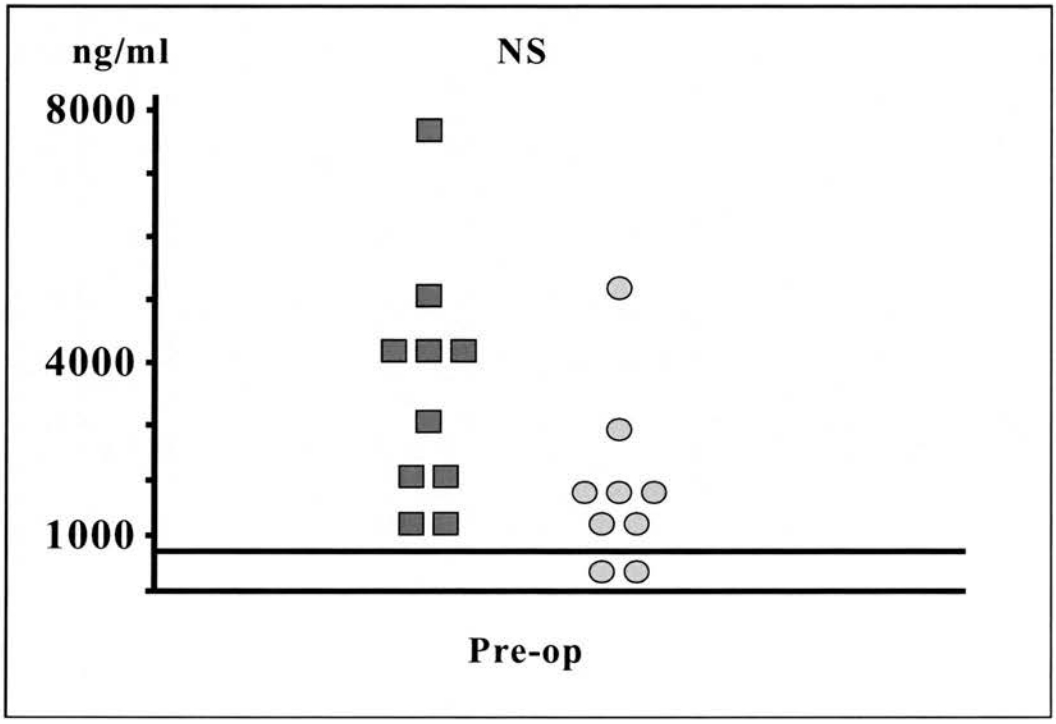


FIGURE 6.8

Individual data points for t-PA activity immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 24 hours (sample D) after aortic clamp release.

Patients with ruptured AAA are represented by the red squares and patients with non-ruptured AAA by the yellow circles.
The upper limit of the normal range for t-PA activity (0.2-2.0 IU/ml) is shown by the horizontal line.

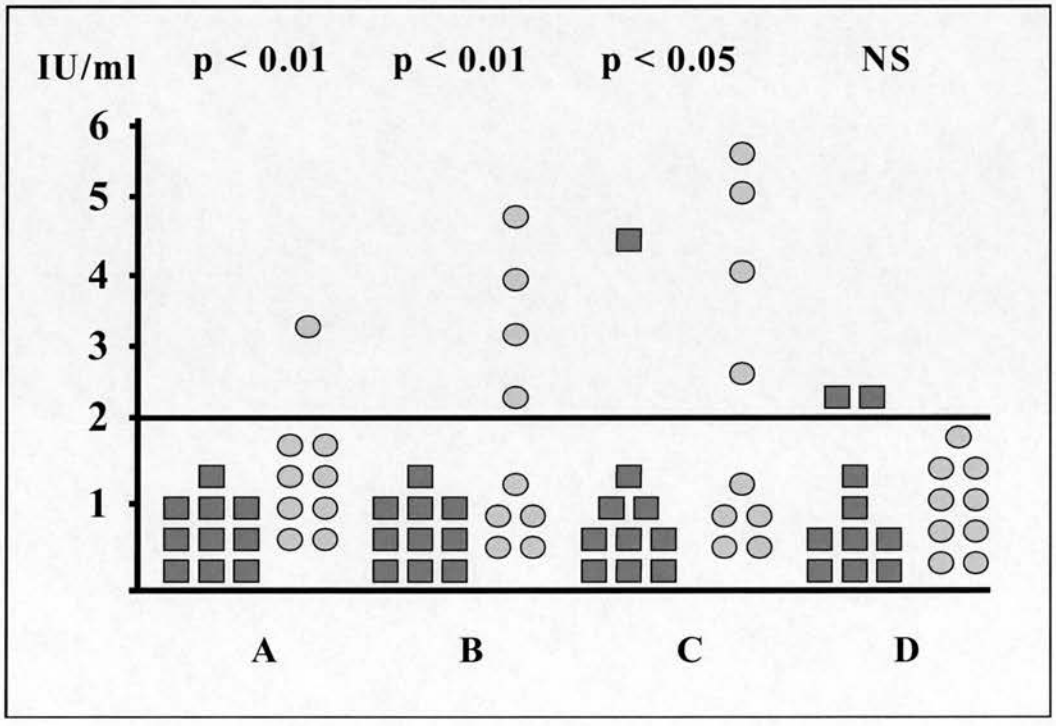


FIGURE 6.9

Individual data points for PAI activity immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 24 hours (sample D) after aortic clamp release.

Patients with ruptured AAA are represented by the red squares and patients with non-ruptured AAA by the yellow circles.
The upper limit of the normal range for PAI activity (< 15 AU/ml) is shown by the horizontal line.

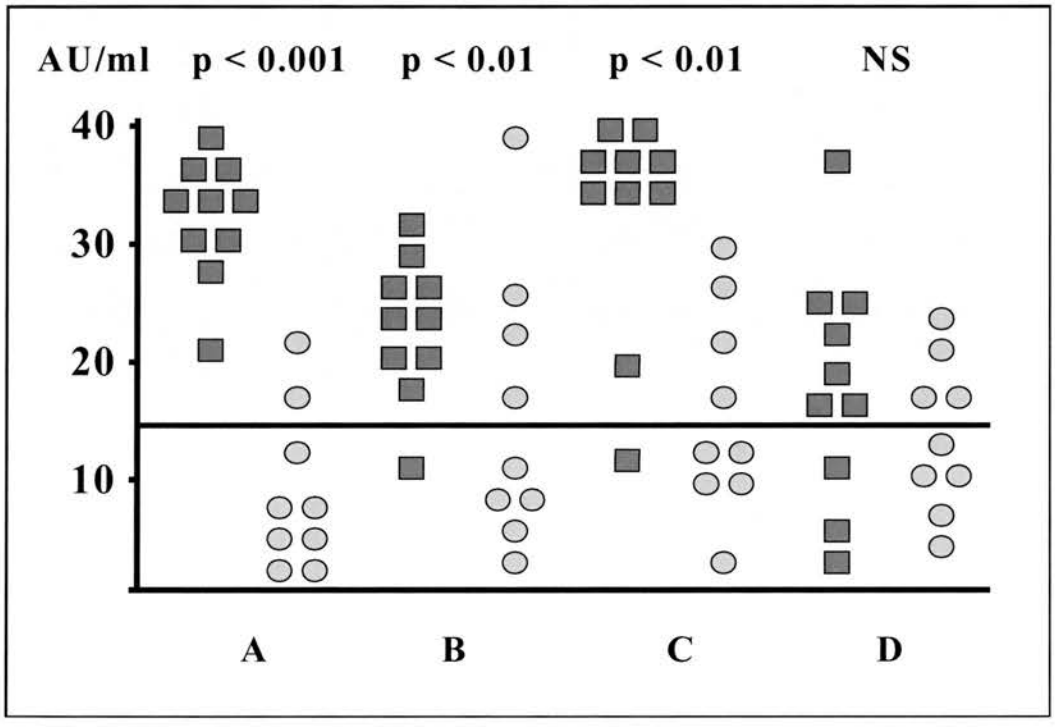


TABLE 6.7

Haemostatic data for 2 non-survivors and 8 survivors of ruptured AAA repair.

Assay (normal range)	**Sample point**	**Non-survivor** (ARDS, ARF)	**Non-survivor** (pneumonia, CLI)	**Survivors** median (range)
Platelet count	A	119	178	249 (156 - 303)
(150-350 x 10^9/l)	B	81	116	125 (96 - 189)
	C	66	84	117 (59 - 146)
	D	86	72	102 (50 - 133)
Fibrinogen	A	2.18	1.87	2.52 (0.86 - 3.75)
(1.5-4.0 g/l)	B	1.2	0.94	1.20 (0.88 - 2.51)
	C	0.77	0.85	1.20 (0.46 - 1.82)
	D	1.87	2.57	3.35 (1.76 - 4.63)
PT	A	16	14	14 (11 - 35)
(10.5-14.5 s)	B	23	18	20 (15 - 26)
	C	27	24	20 (17 - 31)
	D	18	15	16 (13 - 18)
aPTT	A	40	35	30 (28 - 126)
(28-40 s)	B	120	52	53 (34 - 210)
	C	108	210	55 (42 - 123)
	D	51	49	39 (32 - 76)
PF 1+2	A	10.1	9.3	7.7 (5.4 - 11.6)
(0.4-1.1 nmol/l)	B	7.0	8.9	6.2 (3.3 - 8.4)
	C	4.8	9.6	6.5 (4.2 - 8.0)
	D	11.4	4.8	3.4 (1.9 - 4.4)
t-PA activity	A	0.13	0.11	0.17 (0.06 - 0.43)
(0.2-2.0 IU/ml)	B	0.8	0.12	0.27 (0.08 - 0.41)
	C	4.53	0.43	0.28 (0.09 - 1.27)
	D	2.1	0.25	0.41 (0.15 - 2.08)
PAI activity	A	31.0	36.4	36.7 (20.6 - 38.8)
(< 15 AU/ml)	B	13.0	38.9	38.6 (29.4 - 39.4)
	C	10.6	38.2	37.2 (19.4 - 39.4)
	D	5.9	22.2	18.1 (5.0 - 35.3)

6.5 Discussion

The principal finding of the present study is that emergency repair of ruptured AAA is associated with intense thrombin generation (as demonstrated by elevated TAT and PF 1+2 levels) and inhibition of systemic fibrinolysis (as demonstrated by elevated t-PA antigen, reduced t-PA activity and elevated PAI activity). This procoagulant state is present prior to surgery, persists throughout the period of operation, but has largely resolved 24 hours post-operatively. The majority of patients undergoing elective repair of non-ruptured AAA also exhibit increased thrombin generation, but this is of a much lesser magnitude than in rupture. Furthermore, a proportion have evidence of increased systemic fibrinolysis associated with ischaemia and early reperfusion.

Previous studies examining coagulation and fibrinolysis in aortic surgery are contradictory. Mulcare *et al* (140) were the first to demonstrate a significant peri-operative decrease in platelet count, fibrinogen, plasminogen and fibrinolytic inhibitors in patients undergoing elective aortic reconstruction for non-ruptured AAA and aorto-iliac occlusive disease. Mashiah et al (141) later confirmed an intra-operative fall in fibrinogen and plasminogen, but were unable to demonstrate evidence of increased coagulation or fibrinolysis in patients undergoing aortic reconstruction. Gomez and colleagues (78,80), however, demonstrated peri-operative inhibition of fibrinolysis in patients undergoing elective aortic surgery for aneurysmal and occlusive disease by demonstrating a gradual increase in PAI levels during operation, peaking 8 hours post-operatively. Eriksson and Rosberg (149) confirmed elevated PAI levels in patients undergoing aortic reconstruction for occlusive disease, and Welch *et al* (150) demonstrated minimal intra-operative PAI activity with post-operative increase peaking 6 hours after declamping in patients undergoing elective surgery for AAA or occlusive disease. By contrast, in an

animal model of infrarenal aortic cross clamping and isolated lower body ischaemia, Schneiderman *et al* (71) demonstrated a net increase in fibrinolytic activity during ischaemia. Two studies have demonstrated the confounding effect of haemodilution in the interpretation of coagulation studies in elective infrarenal aortic surgery. In a study by von Sommoggy *et al* (144), almost 2 litres of intravenous crystalloid were administered before aortic clamping, which was sufficient to cause a fall in haematocrit and total protein levels. Vahl *et al* (254) reported a decrease in fibrinogen, plasminogen, α_2-antiplasmin and antithrombin III during the operation; after correction for the effects of blood loss and haemodilution, there was no significant change in fibrinogen or plasminogen levels but a significant decrease in α_2-antiplasmin and antithrombin III, the latter of which may have been due to heparinisation. There was, however, an increase in PF 1+2 levels indicating thrombin activation. The authors concluded that haemodilution was an important variable as it reduced coagulation and fibrinolytic factors by 50% while subsequent activation reduced the loss to 25% overall. In the present study, patients with ruptured AAA received a median of 500ml of intravenous crystalloid and/or colloid before induction of anaesthesia and both groups of patients received a median of 3.5-4.0 litres of crystalloid and/or colloid intra-operatively. The haemodilution effect may have contributed to the intra-operative fall in platelet count and fibrinogen, but it is unlikely to have had been responsible for the elevated levels of markers of thrombin generation and fibrinolytic inhibition. Furthermore, given the fact that there was no significant difference in the volume of crystalloid and/or colloid transfusion or intra-operative haematocrit between patients with ruptured and non-ruptured AAA, haemodilution cannot be responsible for the differences in coagulation and fibrinolysis between the groups.

Gertler *et al* (253) compared coagulation studies in 19 patients undergoing supracoeliac aortic clamping for thoraco-abdominal aortic aneurysm and four patients undergoing infrarenal AAA repair. After 30 minutes of ischaemia, coagulation factors and fibrinogen were significantly lower and D-dimer and PF 1+2 levels were significantly higher in the supracoeliac group compared with infrarenal group, and compared with pre-operative levels. Transfusion of blood products returned levels of coagulation factors and fibrinogen toward baseline levels by the end of surgery, but D-dimer and PF 1+2 levels did not return to normal levels. Changes in coagulation were similar in both groups of patients but more dramatic in the supracoeliac group, and the authors suggested that bacterial translocation due to hepatic and mesenteric ischaemia and/or the total ischaemic tissue burden were responsible. In 23 patients undergoing non-ruptured AAA repair, Holmberg et al (255) recently demonstrated a significant increase in levels of PF 1+2 and TAT during aortic clamping with PF 1+2 and TAT levels increasing further after aortic declamping. In control patients undergoing spinal surgery, TAT levels increased during operation and PF 1+2 levels were increased post-operatively. All three coagulation markers, however, were significantly higher in AAA patients before and during operation. Illig *et al* (252) recently examined coagulation and fibrinolysis in 10 patients who underwent elective supracoeliac aortic clamping for AAA (n=8) or occlusive disease (n=2) and eight patients who underwent infrarenal clamping for AAA. There was no significant difference in peri-operative thrombocytopenia, hypofibrinogenaemia, D-dimer levels, and coagulation factor consumption between the groups. During and early after supracoeliac clamping, however, there was evidence of deranged liver function, a significant increase in the levels of FDPs, a significant increase in t-PA antigen and a significant fall in α_2-antiplasmin levels compared with the infrarenal group and baseline

levels. PAI antigen levels were similar in both groups and did not increase until the post-operative period, but t-PA:PAI ratio was significantly higher during operation in the supracoeliac group. The authors speculated that increased t-PA production due to ischaemia and reduced hepatic degradation due to hepatocellular injury were responsible primary systemic fibrinolysis in supracoeliac clamping, and hepatic ischaemia may have contributed to the delay in PAI increase as in patients undergoing orthotopic liver transplantation. The authors further advocated factor replacement and antifibrinolytic therapy for bleeding in supracoeliac aortic clamping. At first sight, the findings of the present study appear to contradict the study by Illig's group (252). One reason for this apparent discrepancy may be due to the measurement of t-PA antigen as opposed to the measurement of t-PA activity. This failure to measure activity as well as antigen, as in the present study, might have led to the erroneous conclusion that ruptured AAA was also associated with enhanced fibrinolysis. It is increasingly apparent that t-PA antigen levels primarily reflect the level of inactive circulating t-PA/PAI complexes. This contention is demonstrated by the present study, where elevated t-PA antigen is, in fact, associated with markedly elevated PAI activity and markedly depressed t-PA activity, consistent with a procoagulant state (99,257).

Given that repair of ruptured aortic aneurysm, and a proportion of elective aneurysm operations, are associated with a procoagulant state (78,150), two important questions need to be addressed. Firstly, is the presence and the intensity of the procoagulant state associated with poor outcome as a result of thrombotic events ? Although the changes in coagulation and fibrinolysis observed in the present study have been associated with myocardial injury (68,95-100,102,103,187-189), MOF (36-38) and CVA (33) in other patient groups, it is not possible to answer this question directly from the present data for two reasons. Only data for patients who survived for 24 hours are included in the present

analysis and the number of patients and adverse clinical outcomes is small. These issues will be addressed in subsequent chapters. The second question is whether therapeutic intervention may ameliorate the adverse effects of this procoagulant state and thus improve outcome ? Apart from the fact that patients in this study with ruptured AAA sustained a period of pre-operative hypovolaemic shock, the most obvious difference between the groups relates to the use of systemic heparin during aneurysm repair. Reported effects of heparin include: minimal reduction in platelet count in a small proportion of patients; prolongation of the aPPT; marginal prolongation of the PTT in a small proportion of patients; elevation of the fibrinogen and a reduction in D-dimer levels due to a reduction in fibrin deposition; reduced PF 1+2 due to inhibition of thrombin generation by antithrombin III; and increased binding of thrombin to antithrombin III but no increase in TAT levels. Other than t-PA, no other fibrinolytic component appears to be affected by heparin: while the majority of clinical and volunteer studies have shown that repeated administration of unfractionated heparin over a number of days increases t-PA antigen, short-term studies have shown increases in t-PA activity similar in magnitude to what might be expected due to diurnal variation (258). Surgeons are naturally reluctant to systemically heparinise a patient with ruptured AAA, but these data suggest that following control of bleeding by aortic clamping judicious use of heparin may partly reverse the procoagulant state and, may, therefore, improve outcome from thrombotic complications (181). It is important to note, however, that patients with ruptured AAA exhibited very elevated levels of TAT which indicate that there is already a considerable, albeit ineffective, attempt by nature to inhibit thrombin activation. This may limit the efficacy of heparin in this situation as AT III is required for its action and the present study suggests that AT III levels may be low secondary to thrombin binding to form TAT. Other possible therapeutic interventions include angiotensin converting enzyme

(ACE) inhibitors (which may decrease PAI and increase t-PA levels) and specific inhibitors of PAI activity (324). There have been suggestions that patients with ruptured AAA might benefit from antifibrinolytic therapy, specifically with aprotonin. However, the routine administration of aprotinin has failed to demonstrate clinical benefit in elective and ruptured AAA repair (256,259), and may actually be associated with a hypercoagulable phase (175). The findings of the present study suggest that such therapy is contra-indicated in most patients undergoing operation for ruptured AAA.

The finding that the majority of patients undergoing elective repair of non-ruptured AAA also have elevated levels of TAT and PF 1+2 (indicating pathological levels of thrombin generation) even before operation may be related to the presence and volume of thrombus within the aneurysm sac (148,260). Indeed, others have reported increased levels of fibrinogen (261), D-dimer (136,148,260), soluble fibrin (255), TAT (136,148,255,269), PF 1+2 (255) and plasmin-antiplasmin (136,148) in patients with asymptomatic non-ruptured AAA. Milne *et al* (262) have also demonstrated significantly lower platelet counts with elevated plasma glycocalicin levels in patients with asymptomatic AAA. The authors speculated that their findings may represent increased platelet destruction and/or activation of platelets within the aneurysm sac leading to circulating dysfunctional platelets. These findings are in keeping with previous studies demonstrating increased platelet and fibrinogen deposition within the aneurysm sac (111,113,117,119,120).

Thus patients with AAA may have a low-grade coagulopathy which makes them particularly sensitive to the effects of operative trauma, hypotension and ischaemia (45,79,263). Increased duration of aortic cross-clamping, for example, may contribute to increased platelet and clotting factor consumption (124) and post-operative thrombocytopenia (263). The present study confirms the relationship between prolonged

aortic clamp time and increased operative blood loss, and a reduction in fibrinogen and platelet count and prolongation of clotting times. For the first time, prolonged aortic clamp time and increased operative blood loss were shown to be associated with increased inhibition of systemic fibrinolysis in non-ruptured AAA repair

Currently, the triggering mechanisms leading to the procoagulant state in ruptured AAA are unknown but, as it is present prior to operation, it is presumably related to haemorrhage and whole body hypoperfusion. There was no relationship between the degree of pre-operative hypotension and any of the haemostatic parameters studied, but there was a significant positive correlation between duration of symptoms and increased thrombin generation, and increased operative blood loss was associated with reduced intra-operative fibrinogen and platelet count, and prolonged intra-operative clotting times. Previous work from this group has demonstrated morphological evidence of endothelial cell activation before operation in patients with ruptured AAA, suggesting that this may be an early event in this group of patients (169). TF expression is the stimulus for thrombin generation and inhibition of fibrinolysis (264,265) and its role in haemostatic derangement in AAA repair requires further investigation (260,266).

In conclusion, these novel data demonstrate that ruptured AAA repair is associated with a procoagulant state. This may contribute to micro- and macrovascular thrombosis which, in turn, lead to the common causes of peri-operative morbidity and mortality, namely MI, MOF and thrombo-embolism.

6.6 Summary

- In hypotensive patients who survive to 24 hours after repair, ruptured AAA repair is associated with peri-operative intense thrombin generation and inhibition of systemic fibrinolysis.
- In patients undergoing elective repair of non-ruptured AAA, the majority exhibit increased peri-operative thrombin generation, and a proportion demonstrate increased systemic fibrinolysis during ischaemia and early reperfusion.
- In ruptured AAA, prolonged duration of symptoms of rupture was associated with increased thrombin generation; and increased operative blood loss was associated with reduced fibrinogen and platelet count, and prolonged clotting times.
- In non-ruptured AAA, prolonged aortic clamp time was associated with reduced fibrinogen and platelet count, prolonged clotting times and hypofibrinolysis; and increased operative blood loss was associated with reduced fibrinogen and platelet count, prolonged clotting times and hypofibrinolysis.
- This procoagulant state may contribute to micro- and macrovascular thrombosis which lead to the common causes of peri-operative morbidity and mortality.
- Antifibrinolytic therapy may be contra-indicated in most patients undergoing ruptured AAA repair.
- Further study is required to: a) confirm these findings in a larger cohort of patients, b) examine haemostasis in patients with peri-operative coagulopathy, c) assess whether the procoagulant state is associated with adverse clinical outcomes, and d) elucidate the triggering mechanisms for this procoagulant state.

Chapter 7

Pre-operative markers of coagulation and fibrinolysis in asymptomatic, acutely symptomatic and ruptured AAA

7.1 Introduction

The previous chapter demonstrated, for the first time, that ruptured AAA repair is associated with intense peri-operative thrombin generation and inhibition of systemic fibrinolysis. Elective repair of non-ruptured AAA was also associated with increased peri-operative thrombin generation, and a proportion of patients had increased intra-operative systemic fibrinolysis.

In the United Kingdom, over 50% of all AAA repairs are performed as an emergency because the surgeon believes, or is unable to exclude the possibility, that rupture has occurred. However, in approximately 20% of these patients the AAA is found to be intact at operation and sudden expansion or impending rupture are presumed to be responsible for the patient's symptoms (251). The operative mortality for this group of patients is twice that of symptomatic patients in whom rupture is not suspected and who are not operated upon as an emergency (251,267).

To date, there are no reports of haemostatic function in patients with acutely symptomatic non-ruptured AAA. We hypothesised that, as acutely symptomatic non-ruptured AAA is not associated with extra-mural haemorrhage, this group of patients would not manifest the same pattern of haemostatic derangement as patients with rupture. Further, haemostatic variables might therefore aid clinical decision-making regarding the timing of surgery in patients with acutely symptomatic non-ruptured AAA.

7.2 Aims

To corroborate the findings of the previous chapter in a larger cohort of patients undergoing repair of asymptomatic and ruptured AAA. To examine whether patients undergoing emergency repair of suspected ruptured but, in fact, intact AAA exhibit the same haemostatic derangement as patients operated upon for rupture. To determine the feasibility of using haemostatic markers as a diagnostic adjunct.

7.3 Methods

Patients

Sixty-six patients who underwent operation for infrarenal AAA were prospectively studied. Twenty two patients (19 men and 3 women of median age 69, range 56 - 81, years) underwent elective repair of asymptomatic AAA; 37 patients (33 men and 4 woman of median age 74, range 63 - 87, years) underwent attempted repair of ruptured AAA; and seven patients (7 men of median age 68, range 65 - 74, years) underwent emergency repair of acutely symptomatic non-ruptured AAA. Acutely symptomatic and ruptured AAA were defined as described above. Thirty-one patients with rupture had at least one documented episode of hypotension (systolic blood pressure less than 100mmHg) prior to surgery. Seven patients with acutely symptomatic and six patients with ruptured AAA were not hypotensive. Co-morbidity data are shown in Table 7.1.

Sample collection

Blood was sampled from an indwelling arterial line immediately prior to the induction of anaesthesia. Samples were prepared as described above.

Markers of thrombin generation and fibrinolysis

t-PA activity, PAI activity, PF 1+2 and D-dimer were assayed as described above.

Statistical analysis

The Mann-Whitney U test (MW) was used. In symptomatic and ruptured AAA, the sensitivity, specificity, and positive and negative predictive values of each assay (alone and in combination) for the diagnosis of rupture were compared with operative findings.

TABLE 7.1

Co-morbidity in patients with asymptomatic, symptomatic and ruptured AAA.

	Asymptomatic (n=22)	**Symptomatic** (n=7)	**Rupture** (n=37)
Co-morbidity			
None	3	3	6
Myocardial infarction	1	-	13
Angina pectoris	6	1	10
Coronary artery bypass graft	2	1	-
Hypertension	10	2	13
Congestive cardiac failure	-	-	2
Atrial fibrillation	1	-	2
Stroke	2	-	4
Peripheral arterial occlusive disease	4	-	2
Venous thrombo-embolism	1	1	-
Chronic obstructive airways disease	4	-	6
Non-insulin dependent diabetes mellitus	3	-	1
Cigarette smoking			
Non-smoker	3	3	17
Ex-smoker	10	1	2
Current smoker	9	3	12
Medications			
None	-	4	6
Aspirin	13	1	15
Diuretic	4	2	12
Nitrate	2	1	10
Angiotensin converting enzyme inhibitor	4	1	6
Calcium-channel blocker	5	1	4
Beta-adrenoceptor blocker	4	1	1
Bronchodilator	4	-	6

7.4 Results

Clinical data

There were no deaths after repair of asymptomatic or acutely symptomatic non-ruptured AAA. The 30-day in-hospital mortality rate for repair of ruptured AAA was 16 of 37 (43%) patients; seven patients died intra-operatively or within 24 hours of repair, and nine patients died in the late post-operative period. There were no deaths among the six patients with rupture who were normotensive prior to the operation.

Markers of fibrinolysis and thrombin generation

The median (range) values for pre-operative t-PA activity, PAI activity, PF 1+2 and D-dimer levels are shown in Table 7.2, and illustrated in Figures 7.1 to 7.4, respectively.

When compared with asymptomatic AAA, ruptured AAA was associated with significantly increased PAI activity ($p<0.001$), PF 1+2 ($p<0.001$) and D-dimer ($p<0.001$), but there was no significant difference in t-PA activity. When patients with ruptured AAA who survived to 24 hours after repair were compared with asymptomatic AAA (as in Chapter 6), PAI activity ($p<0.001$), PF 1+2 ($p<0.001$) and D-dimer ($p<0.001$) remained significantly higher in the rupture group, and again there was no significant difference in t-PA activity. Acutely symptomatic AAA was associated with significantly increased t-PA activity compared with asymptomatic AAA ($p=0.028$) but there was no difference in PAI activity, PF 1+2 and D-dimer.

When compared with ruptured AAA, acutely symptomatic non-ruptured AAA was associated with significantly increased t-PA activity ($p=0.023$), reduced PAI activity ($p=0.005$), reduced PF 1+2 ($p=0.001$) and reduced D-dimer ($p=0.005$) compared with ruptured AAA. When the seven normotensive patients with acutely symptomatic non-

ruptured AAA were compared with the six normotensive patients with rupture, the differences in t-PA activity (median 1.7, range 0.75-3.2 vs. rupture: median 0.22, range 0.11-1.0; p=0.01), PAI activity (median 6.3, range 3.2-15.4 vs. rupture: median 30.3, range 12.1-37.6; p=0.004) and PF 1+2 (median 2.1, range 1.1-5.2 vs. rupture: median 5.3, range 2.5-6.8; p=0.01) persisted, but there was no significant difference in D-dimer levels. When acutely symptomatic non-ruptured AAA was compared with patients with rupture who survived to 24 hours after repair, the statistical differences in all four markers persisted.

In ruptured AAA, there was no significant difference in any of the markers between hypotensive and normotensive patients, or between patients with retroperitoneal or intraperitoneal haemorrhage. There was no significant difference in pre-operative t-PA activity, PAI activity or PF 1+2 between survivors and non-survivors, but D-dimer was significantly higher in non-survivors (median 6329, range 1372-25947 vs. survivors: median 3582, range 155-9518; p=0.046).

When all symptomatic and ruptured AAA patients were examined, PF 1+2 was the most accurate assay for distinguishing non-ruptured from ruptured AAA. Using the upper limit of the normal laboratory range for PF 1+2 as a diagnostic cut-off (greater than 1.1 nmol/l), the test had a high sensitivity (36 of 37, 97%) and positive predictive value (36 of 41, 88%), but low specificity (1 of 7, 14%) and negative predictive value (1 of 2, 50%) for diagnosing ruptured AAA. When the cut-off for PF 1+2 was increased to greater than or equal to 2.5 nmol/l, the test had a high sensitivity (33 of 37, 89%), specificity (6 of 7, 86%) and positive predictive value (33 of 34, 97%), but low negative predictive value (6 of 10, 60%).

When only normotensive symptomatic and ruptured AAA patients were examined, PAI activity was the most accurate assay for distinguishing non-ruptured from ruptured AAA.

PAI activity above the normal range (greater than 15 AU/ml) had a high sensitivity (5 of 6, 83%) and specificity (6 of 7, 86%), and a high positive (5 of 6, 83%) and negative predictive value (6 of 7, 86%) for the diagnosis of rupture. When the cut-off for PAI activity was increased to greater than or equal to 16 AU/ml, the sensitivity and specificity was 5 of 6 (83%) and 7 of 7 (100%), and the positive and negative predictive values were 5 of 5 (100%) and 7 of 8 (88%), respectively.

TABLE 7.2

Pre-operative t-PA activity, PAI activity, PF 1+2 and D-dimer in asymptomatic, symptomatic and ruptured AAA.

		Median (range)		
Assay	**Normal range**	**Asymptomatic** (n=22)	**Symptomatic** (n=7)	**Rupture** (n=37)
t-PA activity	0.2-2.0 IU/ml	0.53 (0.1 - 7.2)	1.7 (0.8 - 3.2)	0.28 (0.1 - 9.6)
PAI activity	< 15 AU/ml	8.3 (0.9 - 34.6)	6.3 (3.2 - 15.4)	31 (0.1 - 39.4)
PF 1+2	0.4 - 1.1 nmol/l	1.9 (0.7 - 7.1)	2.1 (1.1 - 5.2)	6.4 (1.1 - 13.3)
D-dimer	630 - 850 ng/ml	1377 (190 - 5577)	1633 (753 - 3014)	4108 (155 - 25947)

FIGURE 7.1

Pre-operative t-PA activity in asymptomatic, symptomatic and ruptured AAA.

The upper limit of the normal range for t-PA activity (0.2-2.0 IU/ml) is shown by the horizontal line.

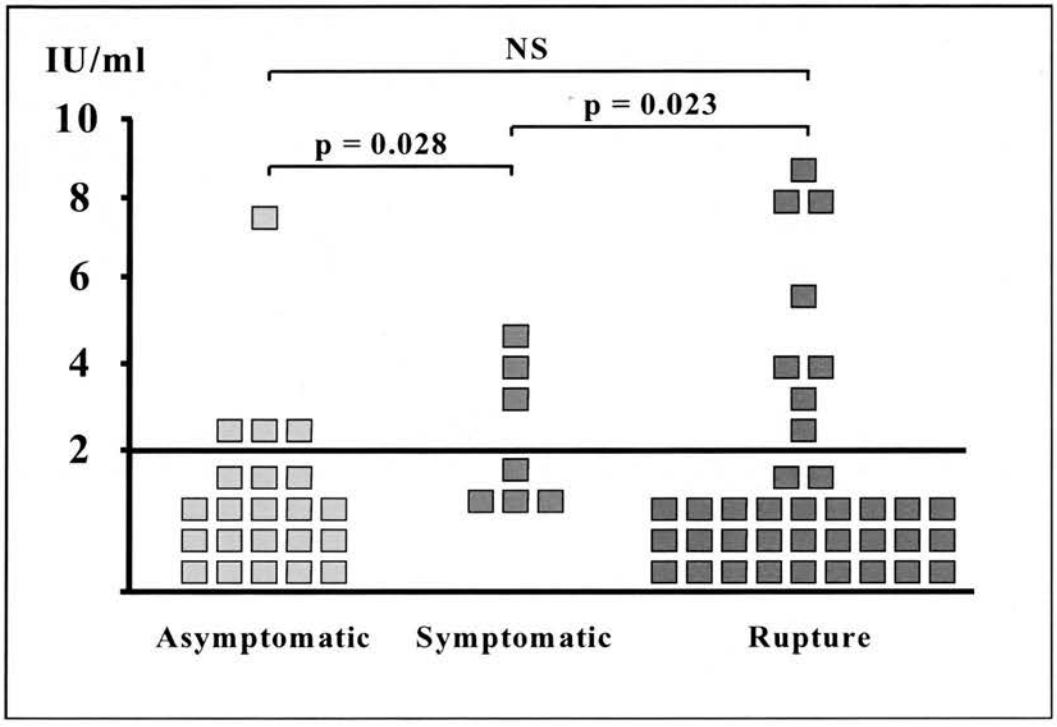


FIGURE 7.2

Pre-operative PAI activity in asymptomatic, symptomatic and ruptured AAA.

The upper limit of the normal range for PAI activity (<15 AU/ml) is shown by the horizontal line.

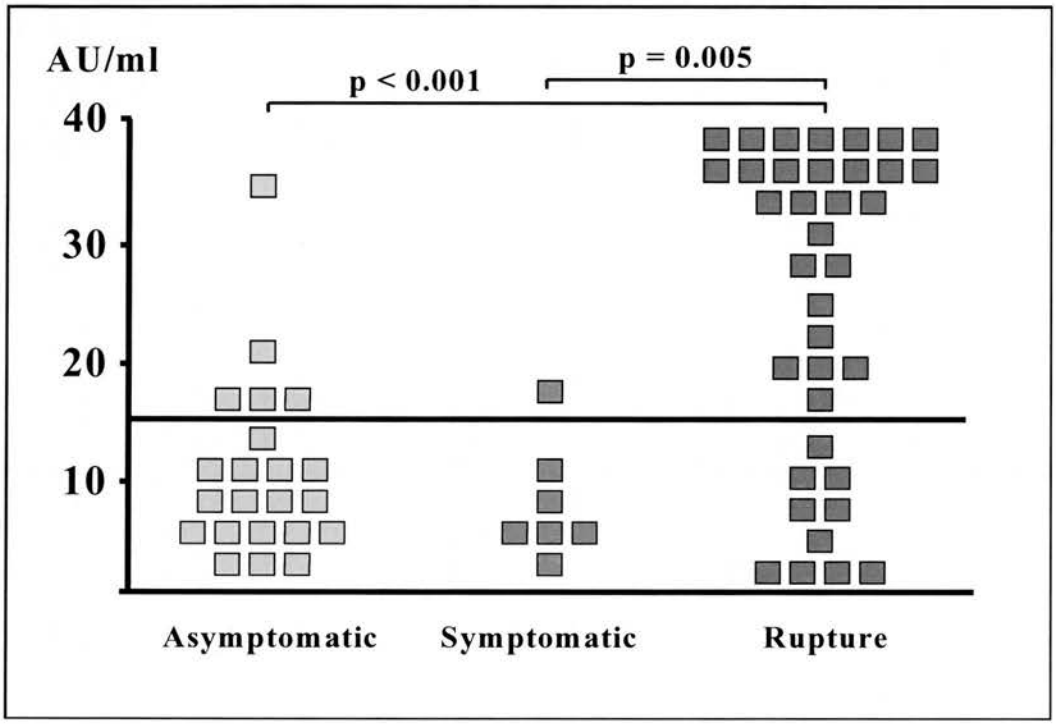


FIGURE 7.3

Pre-operative PF1+2 level in asymptomatic, symptomatic and ruptured AAA.

The upper limit of the normal range for PF 1+2 (0.4-1.1 nmol/l) is shown by the horizontal line.

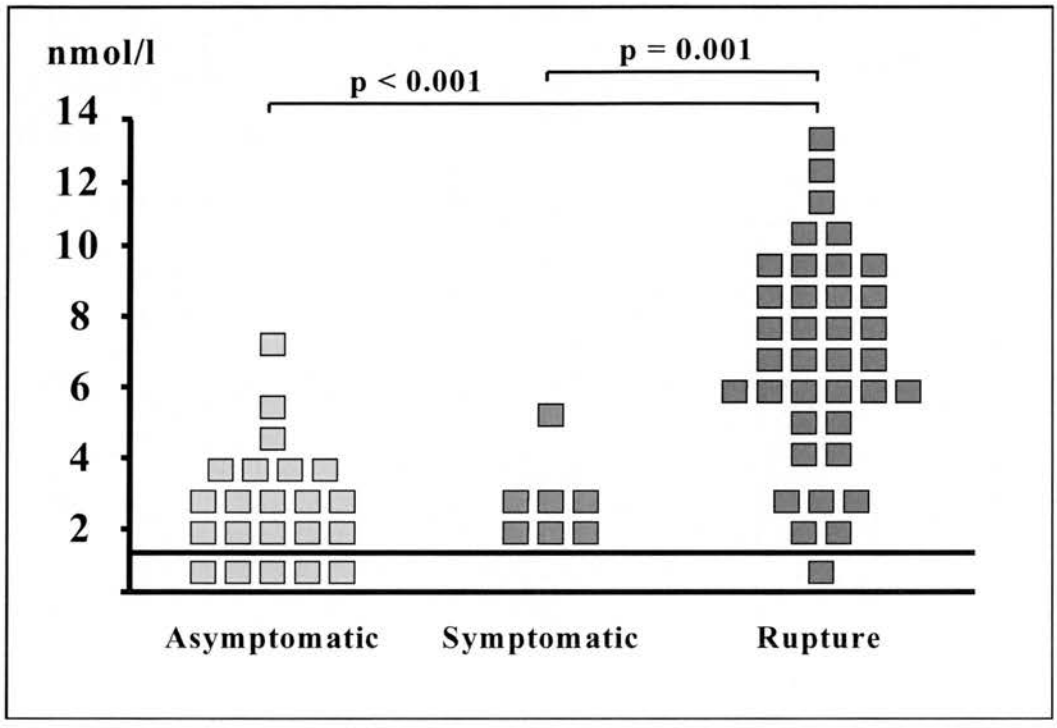
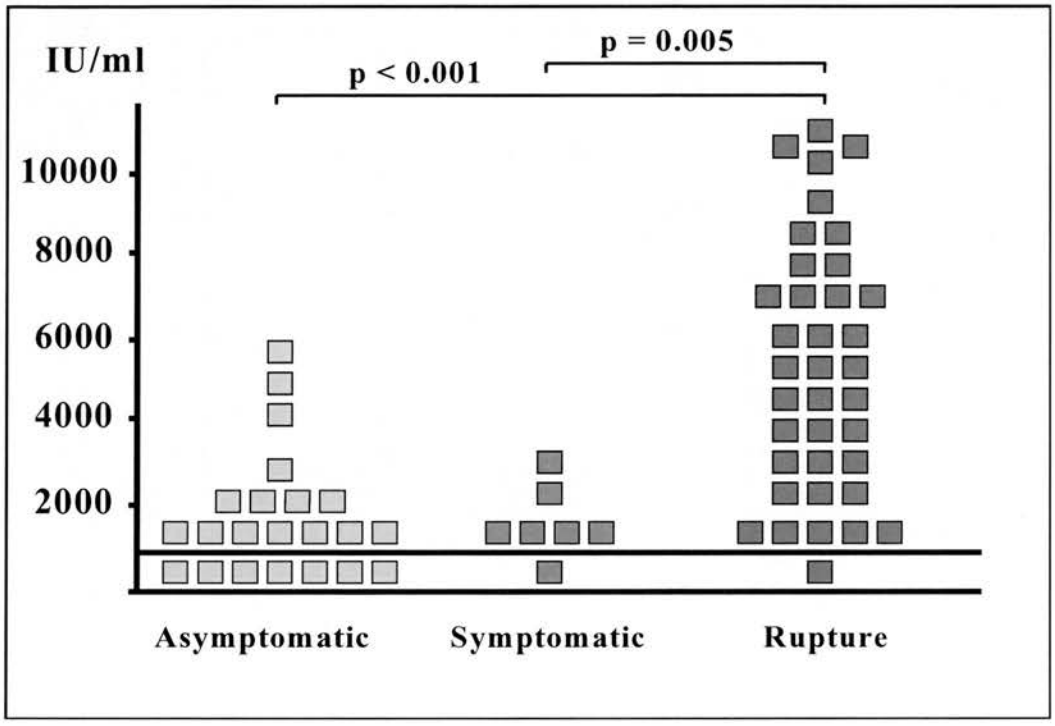


FIGURE 7.4

Pre-operative D-dimer level in asymptomatic, symptomatic and ruptured AAA.

The upper limit of the normal range for D-dimer (630-850 ng/ml) is shown by the horizontal line.



7.5 Discussion

The findings of this study corroborate the observations in the preceding chapter, provide further insight into the pathophysiology of haemostasis in ruptured AAA and reveal novel data regarding haemostasis in acutely symptomatic non-ruptured AAA.

Asymptomatic AAA was associated with increased thrombin generation in 17 of 21 (81%) patients, and increased systemic fibrinolysis in four (19%) patients, one of whom had considerably elevated t-PA activity. Previous studies have reported increased thrombin generation in asymptomatic AAA (148,254,255,260) in association with essentially normal systemic fibrinolytic activity (78,150,252).

Ruptured AAA was associated with inhibition of systemic fibrinolysis (elevated PAI activity), and intense thrombin generation. D-dimer is a marker of secondary fibrinolysis which occurs in response to thrombus formation and acts to restore microvascular patency. D-dimer levels were elevated in ruptured AAA, and this is secondary to increased thrombin generation. Markers of coagulation and fibrinolysis were not significantly different between normotensive and hypotensive patients with rupture, a finding which suggests that haemorrhage rather than hypotension may be one of the principal mechanisms which triggers the generalised procoagulant state in ruptured AAA. Unexpectedly, intraperitoneal haemorrhage from ruptured AAA was not associated with a significant difference in any of the haemostatic markers.

Acutely symptomatic non-ruptured AAA was associated with increased systemic fibrinolysis compared with asymptomatic AAA; and increased systemic fibrinolysis, reduced secondary fibrinolysis and reduced thrombin generation compared with ruptured AAA. t-PA activity was higher in patients with acutely symptomatic non-ruptured AAA compared with rupture but the median values were within the normal range in both

groups. The lower t-PA activity in ruptured AAA was secondary to increased inhibition of systemic fibrinolysis (as demonstrated by elevated PAI activity), a finding which was not present in the patients with acutely symptomatic non-ruptured AAA. D-dimer is a marker of secondary fibrinolysis which occurs in response to thrombus formation and acts to restore microvascular patency. D-dimer and PF 1+2 levels were elevated in both groups of patients but to a greater extent in ruptured AAA. These differences in haemostasis were also observed when normotensive patients with acutely symptomatic AAA were compared with normotensive patients with rupture.

The findings of the present study may be explained if one considers that sudden expansion or impending rupture of an aortic aneurysm is analogous to an aortic dissection. Fibrinolytic gene expression and focal fibrinolytic activity have been demonstrated within the aortic wall of patients with asymptomatic AAA (117,118), and aortic adventitia is associated with elevated fibrinolytic activator activity (114,121,122). Sudden aneurysm expansion may lead to bloodflow in the marginal thrombus adjacent to the diseased aortic wall and as the surface area of aortic adventitia exposed to blood flow increases there is increased local and systemic fibrinolysis (114). Such a process may account for the abdominal CT findings of a sickle-shaped thrombus or 'crescent sign' in aneurysms at risk of rupture. Aneurysm rupture and subsequent haemorrhage may trigger a procoagulant and hypofibrinolytic state which acts to minimise local blood loss but has the detrimental effect of contributing to micro- and macrovascular thrombosis.

At present, acutely symptomatic non-ruptured AAA are differentiated from ruptured AAA primarily on the basis of history and examination, but even the most experienced of vascular surgeons cannot always confidently exclude rupture on clinical assessment alone. Many clinicians have advocated the use of emergency computed tomography (CT) in this situation, but previous work from this group has shown that, in cases of true

clinical uncertainty, this has an unacceptably low sensitivity (79%) and specificity (77%) in haemodynamically stable patients (268). In the present study, there were significant differences in t-PA activity, PAI activity and PF 1+2 between normotensive patients with acutely symptomatic non-ruptured and ruptured AAA. Furthermore, pre-operative elevation of PAI activity appeared more accurate than emergency CT. While this may help to distinguish acutely symptomatic normotensive non-ruptured AAA from ruptured AAA, currently, the time taken to prepare the plasma and perform the assay (approximately 2 hours) precludes its use as diagnostic adjunct. The small numbers of patients studied with non-ruptured AAA does not allow the authors to reach strong conclusions or make firm clinical recommendations. However, should a rapid assay for PAI activity become available then it may be of value in distinguishing rupture from non-rupture in normotensive patients presenting with acute symptoms.

There were three reasons for measuring only pre-operative haemostatic markers in this study. Firstly, in the preceding chapter, there was little or no change in the levels of the haemostatic markers intra-operatively compared with pre-operatively. Secondly, if the findings were to have any value in distinguishing acutely symptomatic non-ruptured AAA from ruptured AAA, then only the pre-operative markers would be relevant. And finally, at the time, the assays were novel and consequently almost prohibitively expensive.

In conclusion, these data confirm that ruptured AAA is associated with thrombin generation and inhibition of systemic fibrinolysis, whereas asymptomatic AAA is associated with thrombin generation and increased fibrinolysis in a small proportion of patients. Patients with acute symptomatic non-ruptured AAA do not manifest the same pattern of haemostatic derangement evident in patients with rupture.

7.6 Summary

- Ruptured AAA is associated with thrombin generation and inhibition of systemic fibrinolysis, and asymptomatic AAA is associated with thrombin generation and increased fibrinolysis in a small proportion of patients.
- In ruptured AAA, there was no difference in markers of coagulation and fibrinolysis between normotensive and hypotensive patients, suggesting that haemorrhage rather than hypotension may trigger the procoagulant state.
- Acutely symptomatic non-ruptured AAA is associated with increased systemic fibrinolysis, and reduced thrombin generation compared with rupture. These differences were also observed when the normotensive patients with acutely symptomatic non-ruptured AAA were compared with normotensive patients with rupture.
- Pre-operative PAI activity above the normal range was more accurate than abdominal CT in a previous study of patients with suspected rupture.

Chapter 8

Peri-operative coagulopathy in ruptured AAA repair

8.1 Introduction

Approximately 40% of patients who fail to survive ruptured AAA repair die intra-operatively or in the immediate post-operative period (251). Uncontrollable haemorrhage is one of the principal causes of death in these patients and may occur due to technical problems and/or coagulopathy (13,39,43). In the ERVSU, haemorrhage and coagulopathy contributed to 30% and 17% of peri-operative deaths, respectively (Chapter 4). Wakefield et al (13) reported coagulopathy in 63% of patients who died during attempted repair of ruptured AAA. Peri-operative coagulopathy in patients with ruptured AAA is associated with a poor prognosis (3,10,30,44) and is as significant a predictor of poor outcome as major cardiac events, respiratory failure, ARF, CVA and distal embolisation (7).

In patients undergoing early re-operation for haemorrhage after rupture, an abnormal coagulation screen at the end of the primary operation is universal (43). Peri-operative haemostatic derangement, as demonstrated by low pre-operative platelet count (44,45) and prolonged clotting times (44), has been shown to be associated with major peri-operative morbidity and mortality mainly due to haemorrhage, and thrombotic events including MI, MOF and CVA (43,44,45). Previous investigations of haemostasis in ruptured AAA repair are few and have largely consisted of the measurement of the standard laboratory markers of haemostatic function (44,45,114,137,138). As shown in chapter 6, in patients who survive to 24 hours post-operatively, ruptured AAA repair is associated with intense thrombin generation and inhibition of systemic fibrinolysis. The pathophysiology of the haemostatic derangement which occurs in patients with peri-operative coagulopathy has not previously been studied.

8.2 Aims

To examine serial peri-operative changes in markers of coagulation and fibrinolysis in patients undergoing ruptured AAA repair complicated by peri-operative coagulopathic haemorrhage.

8.3 Methods

Patients

Eight patients (8 men of median age 74, range 69-87, years) operated upon for ruptured infrarenal AAA were studied. All of these patients had clinical and laboratory evidence of coagulopathy and haemorrhage and all had an aortic graft inserted in an apparently technically adequate manner and the circulation restored to the lower extremities.

The median (range) delay between the onset of symptoms of rupture and hospital admission was 7 (2-14) hours. All patients had at least one documented episode of hypotension (systolic blood pressure less than 100mmHg) prior to surgery. Two patients with retroperitoneal and intraperitoneal rupture had a documented episode of loss of consciousness prior to admission, and one patient was transferred from another hospital wearing a pneumatic anti-shock garment. No patient had a history of liver disease. Co-morbidity data are shown in Table 8.1.

Operative methods

Six patients had retroperitoneal, and two intraperitoneal rupture. The operations were performed as previously described. A dacron aorto-aortic graft was inserted in five patients, an aorto-bi-iliac graft in two patients and an aorto-bifemoral graft in one patient. Clinical and operative data are shown in Table 8.2.

Sample collection

Blood was sampled from an indwelling arterial line immediately prior to the induction of anaesthesia (sample A); immediately before release of the aortic clamp (sample B); and five minutes (sample C) and 24 hours (sample D) after aortic clamp release in those patients who survived repair. The samples were prepared as described above.

Assays of haemostatic function

Haematocrit, platelet count, fibrinogen, PT and aPTT were measured in the routine haematology laboratory. Thrombocytopenia was defined as a platelet count less than 150 x 10^9 /l. Hypofibrinogenaemia was defined as a fibrinogen level less than 1.5 g/l. PF 1+2 was assayed as a marker of thrombin generation, and t-PA and PAI activities as markers of primary systemic fibrinolysis. Assays were performed as described above.

TABLE 8.1

Co-morbidity in patients with peri-operative coagulopathy.

	No. of patients (n=8)
Co-morbidity	
None	2
Myocardial infarction	3
Angina pectoris	2
Hypertension	2
Atrial fibrillation	1
Chronic obstructive airways disease	1
Cigarette smoking	
Non-smoker	3
Ex-smoker	1
Current smoker	4
Medications	
None	3
Aspirin	2
Diuretic	2
Nitrate	2
Bronchodilator	1

TABLE 8.2

Clinical and operative data in patients with peri-operative coagulopathy.

	Median (range)
Pre-operative	
Crystalloid administration (l)	1.5 (0.3 - 2.6)
Colloid administration (l)	0.2 (0 - 2.0)
Intra-operative	
Operation time (mins)	145 (110 - 250)
Aortic clamp time (mins)	80 (45 - 135)
Measured blood loss (l)	6.4 (1.7 - 11.0)
Crystalloid administration (l)	3.5 (2.0 - 7.5)
Colloid administration (l)	2.3 (0 - 5.0)
RCC administration (units) [1]	14 (8 - 22)
FFP administration (units) [2]	3 (2 - 12)
Platelet administration (bags) [3]	1 (1 - 2)

KEY: [1] RCC = 300 ml, [2] FFP = 300ml, [3] one bag of platelet transfusion = 4 pooled units (250 ml)

8.4 Results

Clinical data

Three patients died intra-operatively from uncontrollable coagulopathic haemorrhage after an aortic graft had been inserted. The remaining five patients had clinical and laboratory evidence of on-going coagulopathy during the first 24 hours post-operatively. One of these patients died in the early post-operative period from continuing haemorrhage, and two patients died in the late post-operative period. Clinicopathological and outcome data are shown in Table 8.3.

TABLE 8.3

Clinicopathological and outcome data in patients with peri-operative coagulopathy.

	Age	Complications		Outcome
		Intra-operative	Post-operative	
1	71	Coagulopathy	Coagulopathy (2 FFP, 10 cryo), inotrope, respiratory failure, IPPV 114 hours	Survived
2	69	Coagulopathy, inotrope	-	Intra-op. death
3	74	Coagulopathy, inotrope	Coagulopathy (2 RCC, 4 FFP), inotrope, IPPV 14 hours	Survived
4	73	Coagulopathy, inotrope, anuria	-	Intra-op. death
5	74	Coagulopathy	Coagulopathy (8 RCC, 6 FFP, 2 PC, 20 cryo), inotrope, DVT, respiratory failure, IPPV 261 hours, bilateral BKA	Death post-op. day 40
6	75	Coagulopathy	Coagulopathy (6 RCC, 1 PC), CCF, inotrope, respiratory failure, IPPV 451 hours, RRT for ARF	Death post-op. day 25
7	73	Coagulopathy, inotrope, anuria	-	Intra-op. death
8	87	Coagulopathy	Coagulopathy (18 RCC, 9 FFP, 3 PC), inotrope, IPPV 18 hours	Death 18 hours post-op.

KEY: Cryo = cryoprecipitate, PC = platelet concentrate,

Haematocrit, platelet count, fibrinogen, PT and aPTT

The median (range) values for haematocrit, platelet count, fibrinogen, PT and aPTT are shown in Table 8.4. Fibrinogen levels are shown in Figure 8.1. Pre-operative standard haemostatic markers were within the normal range in three patients, two of whom survived. Of the remaining patients, all five had thrombocytopenia, three had prolonged clotting times, and one had hypofibrinogenaemia. Intra-operatively, all of the patients had thrombocytopenia and prolonged clotting times, six had hypofibrinogenaemia and two had fibrinogen levels near the lower limit of the normal range. At 24 hours, all patients had thrombocytopenia, and only the two survivors had normal clotting times and fibrinogen levels.

TABLE 8.4

Haematocrit, platelet count, fibrinogen, PT and aPTT.

Assay	Normal range	Sample	Median (range)
Haematocrit	0.37 - 0.54	A	0.276 (0.140 - 0.342)
		B	0.235 (0.194 - 0.439)
		C	0.236 (0.183 - 0.420)
		D	0.329 (0.329 - 0.357)
Platelet count	150 - 350 x 10^9/l	A	131 (40 - 321)
		B	91 (48 - 127)
		C	79 (44 - 114)
		D	66 (23 - 102)
Fibrinogen	1.5 - 4.0 g/l	A	2.37 (0.74 - 6.31)
		B	1.29 (0.1 - 1.64)
		C	1.03 (0.24 - 1.64)
		D	4.0 (2.96 - 5.64)
PT	10.5 - 14.5 s	A	13.7 (11.0 - 28.8)
		B	22.9 (12.0 - 123.0)
		C	22.4 (13.0 - 110.0)
		D	13.6 (13.0 - 15.8)
aPTT	28 - 40 s	A	37.1 (27.8 - 75.0)
		B	104.1 (44.4 - > 210.0)
		C	86 (44.4 - >210.0)
		D	41.3 (32.1 - 55.9)

Markers of thrombin generation and fibrinolysis

The median (range) values for PF 1+2, t-PA activity and PAI activity are shown in Table 8.5. All of the patients had evidence of intense thrombin generation (elevated PF 1+2 levels) before, during and after operation (Figure 8.2). Pre-operatively, three patients had evidence of increased systemic fibrinolysis (elevated t-PA activity and reduced or normal PAI activity) which persisted intra-operatively. All of these patients died, one intra-operatively, one within 24 hours and one in the late post-operative period. In two further patients, t-PA activity was normal pre-operatively but became elevated before aortic declamping, and these patients also died intra-operatively (Figure 8.3). Pre-operatively, five patients had evidence of inhibition of systemic fibrinolysis (reduced t-PA activity and elevated PAI activity). PAI activity remained elevated intra-operatively and at 24 hours post-operatively in three patients, two of whom survived (Figure 8.4).

TABLE 8.5

PF 1+2, t-PA activity and PAI activity.

Assay	Normal range	Sample	Median (range)
PF 1+2	0.4 - 1.1 nmol/l	A	6.7 (2.4 - 11.6)
		B	5.1 (2.0 - 8.4)
		C	6.1 (2.7 - 7.9)
		D	3.5 (1.6 - 5.6)
t-PA activity	0.2 - 2.0 IU/ml	A	0.86 (0.09 - 9.6)
		B	7.0 (0.19 - 13.7)
		C	5.8 (0.16 - 14.1)
		D	0.72 (0.73 - 2.3)
PAI activity	< 15 AU/ml	A	28.7 (0.1 - 38.9)
		B	7.9 (0.1 - 39.4)
		C	18.3 (0.1 - 39.3)
		D	13.6 (8.9 - 22.2)

FIGURE 8.1

Individual data points for fibrinogen level immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 24 hours (sample D) after aortic clamp release.

Non-survivors of ruptured AAA are represented by the red squares and survivors by the yellow circles.
The upper limit of the normal range for fibrinogen (1.5-4.0 g/l) is shown by the horizontal line.

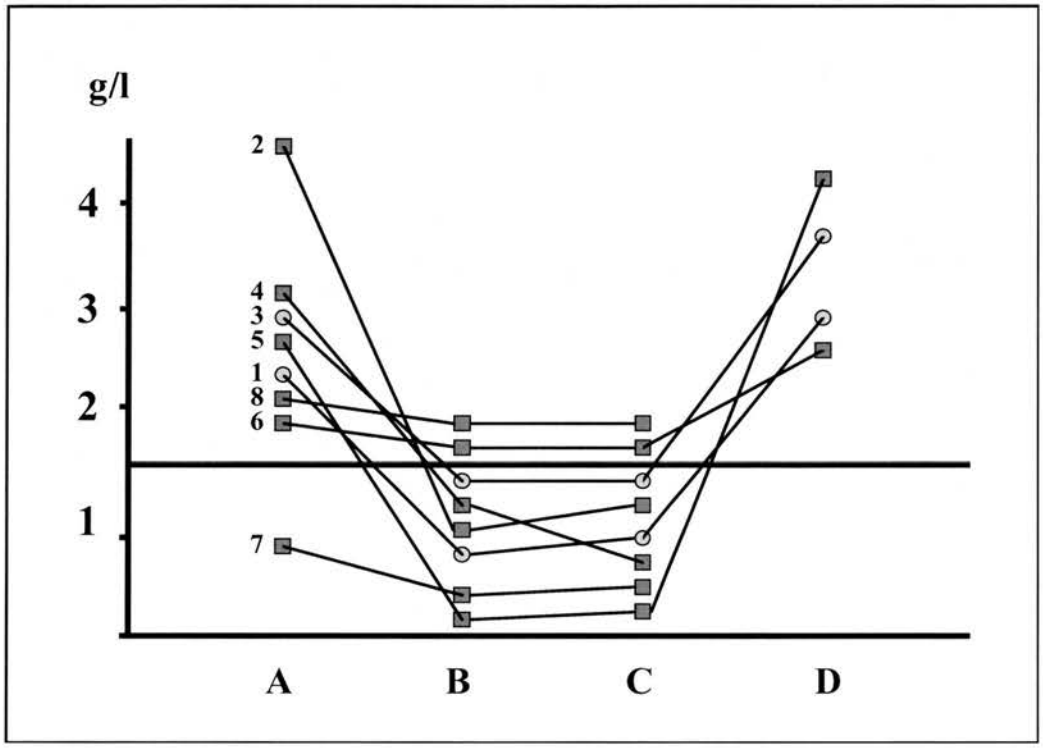


FIGURE 8.2

Individual data points for PF 1+2 immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 24 hours (sample D) after aortic clamp release.

Non-survivors of ruptured AAA are represented by the red squares and survivors by the yellow circles.
The upper limit of the normal range for PF 1+2 (0.4-1.1 nmol/l) is shown by the horizontal line.

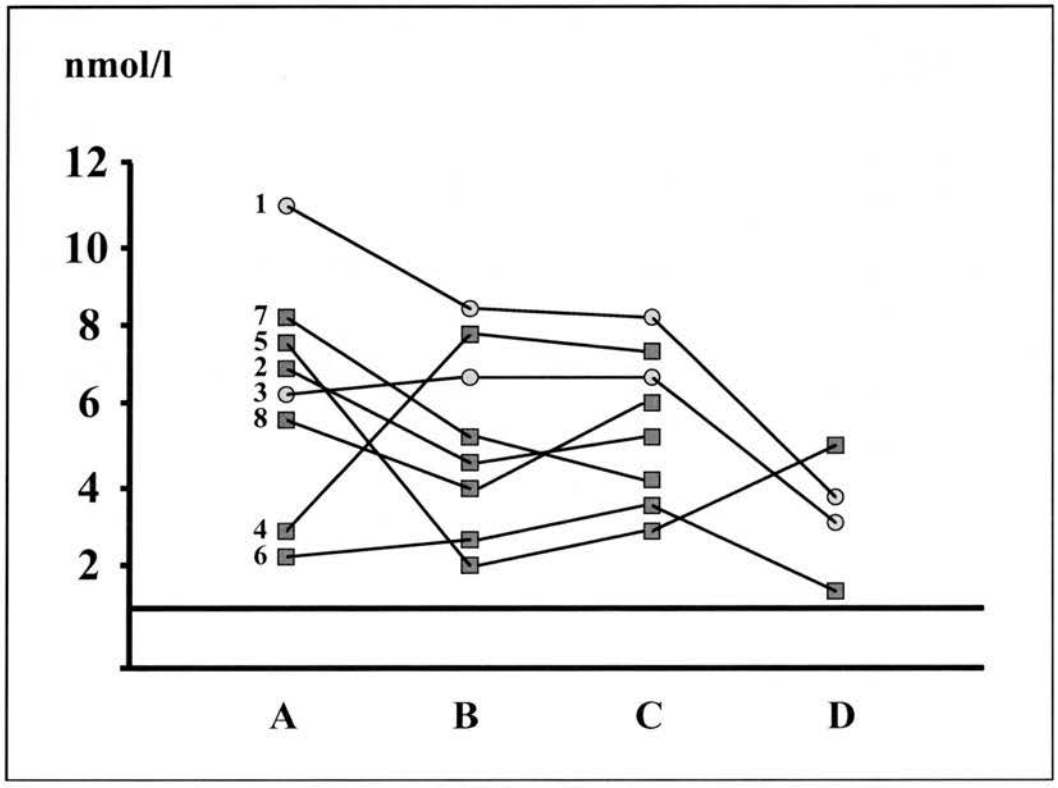


FIGURE 8.3

Individual data points for t-PA activity immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 24 hours (sample D) after aortic clamp release.

Non-survivors of ruptured AAA are represented by the red squares and survivors by the yellow circles.
The upper limit of the normal range for t-PA activity (0.2-2.0 IU/ml) is shown by the horizontal line.

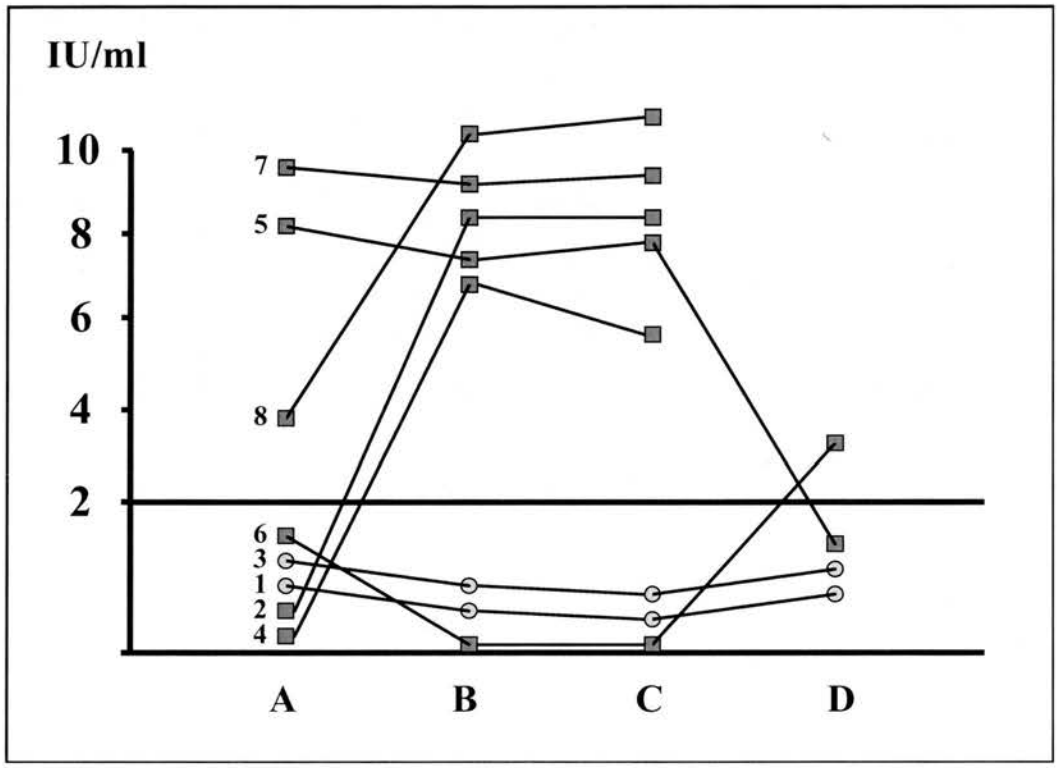
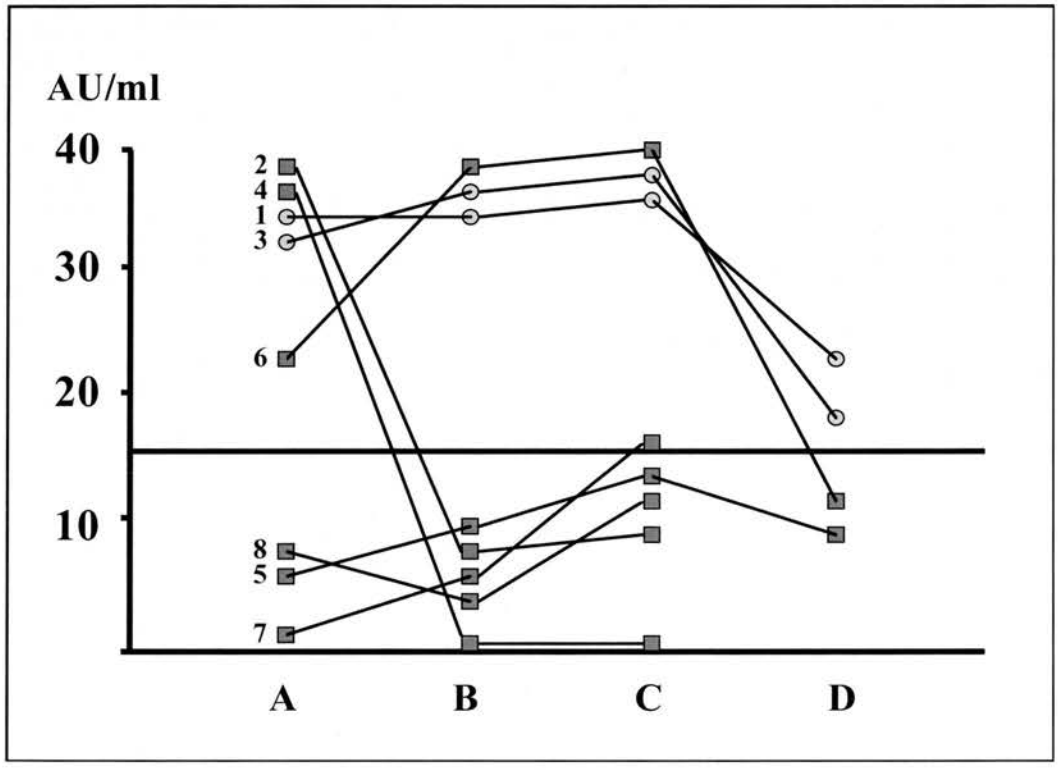


FIGURE 8.4

Individual data points for PAI activity immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 24 hours (sample D) after aortic clamp release.

Non-survivors of ruptured AAA are represented by the red squares and survivors by the yellow circles.
The upper limit of the normal range for PAI activity (< 15 AU/ml) is shown by the horizontal line.



8.5 Discussion

The present study is the first to examine serial peri-operative changes in coagulation and fibrinolysis in patients with ruptured AAA complicated by peri-operative coagulopathic haemorrhage. In Chapter 6, it was demonstrated that, in patients who survive to 24 hours, ruptured AAA repair is associated with intense thrombin generation and inhibition of systemic fibrinolysis. All of the patients in the present study were shocked pre-operatively, two had intraperitoneal rupture in association with loss of consciousness and cardiac arrest, and all received massive volumes of intravenous fluid, RCC, and clotting factors intra-operatively. All of the patients had increased thrombin generation but, unlike those who survived their operation, there was evidence of increased systemic fibrinolysis in five patients, all of whom died, four within the first 24 hours after surgery. Mulcare *et al* (137) have previously reported increased post-operative fibrinolysis in four patients with rupture who developed excessive bleeding.

It has been suggested that, in patients with non-ruptured AAA, there exists a compensated and subclinical form of coagulopathy which may render the patient particularly sensitive to the pathophysiological effects of aneurysm rupture and repair such that decompensation occurs and clinically apparent coagulopathy develops (45,179,137,139). Several mechanisms may contribute to the development of coagulopathy. Patients with vascular disease frequently have a number of concommitant coagulation disorders such as pre-existing liver and renal impairment, and regular antiplatelet and anticoagulant therapy. Increased levels of elastase have been demonstrated within the aortic wall of patients with aortic aneurysms (156,157) and this may lead to activation of the coagulation and fibrinolytic systems if released into the circulation due to aortic wall disruption. Fibrinogen, fibrin, and platelet consumption

occurs within the aneurysm thrombus (111), surgical wounds, the aortic graft (142,144), and within the ruptured aneurysm haematoma (39). Blood loss, fluid resuscitation and blood transfusion have a dilutional effect on the levels of clotting factors and platelets, and hypothermia due to shock and prolonged and complicated surgery is associated with adverse platelet effects (172). Intestinal ischaemia and endotoxaemia (72) may contribute to the increased incidence of coagulopathy associated with supracoeliac aortic clamping (124,125). Finally, the relationship between prolonged aortic cross-clamp times and increased incidence of coagulopathy suggests that as the magnitude of the ischaemic insult increases, then the risk of coagulopathy increases (42,124).

Based upon these data and the findings of Chapter 6, patients with ruptured AAA may be classified into two categories. Those who have 'compensated' haemorrhagic shock exhibit thrombin generation and inhibition of systemic fibrinolysis and appear likely to survive to at least 24 hours post-operatively. By contrast, those with 'decompensated' shock exhibit thrombin generation and increased systemic fibrinolysis and appear likely to die intra-operatively or in the immediate post-operative period from coagulopathy despite the apparently technically adequate insertion of a graft. Of practical importance is the fact that those patients who die from peri-operative haemorrhage have a hyperfibrinolytic state on admission to the resuscitation room and/or before aortic declamping. There was no difference in the duration between the onset of symptoms of rupture and hospital admission in these patients compared with those who survived to 24 hours. PAI is presumably consumed during the period of haemorrhagic shock resulting in high levels of circulating free t-PA. This leads to high levels of circulating free plasmin which leads to consumption of fibrinogen and fibrin. t-PA and PAI activities are of value as a research tool, but it takes several hours to prepare the plasma and perform the assays and this precludes their clinical use in identifying this hyperfibrinolytic state.

There are two clinical methods which may identify those patients with coagulopathy secondary to hyperfibrinolysis. Euglobulin clot lysis time (ECLT) measures the time for a clot to lyse in a test tube. If fibrinolysis predominates there is early clot lysis, and if coagulation factor consumption predominates then there is little or no clot formation in the first place. Thromboelastography (TEG) measures the strength of the blood clot as it forms and can determine the whole blood clotting time, coagulation factor and platelet function, fibrinolysis and hypercoagulability. Using a whole blood sample, a result can be obtained within 15-20 minutes. Given that the hyperfibrinolytic state may be identified early during aneurysm repair, can therapeutic intervention improve outcome ? Peri-operative administration of FFP and platelets have been advocated in patients with ruptured AAA (45,138,179). The routine administration of the antifibrinolytic agent, aprotinin, in elective and ruptured AAA repair has failed to demonstrate clinical benefit (256,259). However, the present study suggests that the use of antifibrinolytic agents as well as the administration of FFP and cryoprecipitate may prove more beneficial if targeted at the selected group of patients with ruptured AAA who demonstrate hyperfibrinolysis.

In conclusion, these data demonstrate that patients with ruptured AAA who develop peri-operative coagulopathy secondary to hyperfibrinolysis have a poor prognosis. The measurement of peri-operative fibrinolytic function may help to identify the select group of patients with rupture who are at increased risk of developing life-threatening coagulopathic haemorrhage, and thereby allow early aggressive therapeutic intervention. Due to the limitations of such an observational study of a small and heterogenous group of patients, it is not possible to conclude whether the hyperfibrinolytic state is a contributory factor in the development of intra-operative coagulopathy or it is simply a manifestation of severe and irreversible whole body hypoperfusion.

8.6 Summary

- Patients with peri-operative coagulopathic haemorrhage have evidence of increased thrombin generation but, unlike those who survive to 24 hours, a proportion demonstrate hyperfibrinolysis on admission and/or before aortic declamping which is associated with an extremely poor prognosis.
- Ruptured AAA may be classified into two categories, according to the pattern of haemostatic derangement: 'compensated' shock leads to thrombin generation and inhibition of systemic fibrinolysis and is associated with survival to at least 24 hours post-operatively; 'decompensated' shock leads to thrombin generation and hyperfibrinolysis and is associated with intra- or early post-operative death from coagulopathy.
- Measurement of the fibrinolytic function may help to identify the patients who are at increased risk of developing life-threatening coagulopathy, thereby allowing early intervention with antifibrinolytic agents, FFP and cryoprecipitate.

Chapter 9

von Willebrand factor and platelet count in ruptured and non-ruptured AAA repair

9.1 Introduction

Ruptured and non-ruptured AAA repair are usually associated with a generalised procoagulant state with evidence of increased thrombin generation. This may be because endothelial cell injury leads to the exposure of subendothelial collagen and elastin that triggers the extrinsic coagulation cascade.

Endothelial cell activation and injury is also associated with the rapid release of vWF into the circulation. vWF binds to exposed subendothelial collagen at sites of vessel injury; platelets then irreversibly bind to vWF. Plasma vWF accounts for 60% of total platelet adhesion with the remaining 40% provided by endothelial cell vWF (59). vWF is essential for the formation of occlusive platelet thrombi at sites of arterial injury (56).

Previous work from this department has demonstrated that AAA repair is associated with early peri-operative thrombocytopenia and late post-operative thrombocytosis (45,263). Furthermore, in ruptured AAA, a strong association exists between platelet count and mortality, MOF and thrombo-embolic events (45). Although the underlying mechanisms remain ill-defined, vWF and its effect on platelets may have an important role in the procoagulant state which occurs in patients undergoing AAA repair. To date, however, there have been few studies examining endothelial cell activation in elective aortic surgery (145,260) and none in patients with ruptured AAA.

9.2 Aims

To examine peri-operative vWF, sTM and platelet count in patients operated for ruptured and non-ruptured AAA, and to determine the relationship between these markers of endothelial cell activation and platelet count.

9.3 Methods

Patients

Twenty patients (18 men and 2 women of median age 74, range 63-86, years) operated for ruptured and 10 patients (8 men and 2 woman of median age 69, range 58-80, years) operated for asymptomatic non-ruptured infrarenal AAA were prospectively studied. Co-morbidity data are shown in Table 9.1.

In patients operated for ruptured AAA, the median (range) delay between the onset of symptoms of rupture and hospital admission was 5 (2-14) hours. All patients with rupture had at least one documented episode of hypotension (systolic blood pressure less than 100mmHg) prior to surgery. On admission to the resuscitation room or immediately before anaesthetic induction, the median (range) peripheral body temperature of patients with ruptured AAA was 35.4 (32.2-37.5) C. In patients undergoing operation for non-ruptured AAA, the median (range) antero-posterior diameter of the aneurysm measured by abdominal ultrasonography was 6.5 (5.5-8.0) cm.

Operative methods

The operations were performed as previously described. Fourteen patients had retroperitoneal, and six intraperitoneal rupture. A dacron tube graft was inserted in 17 patients (13 rupture, 4 non-rupture), an aorto-bi-iliac graft in six (2 rupture, 4 non-rupture) and an aorto-bifemoral graft in five (3 rupture, 2 non-rupture). Two patients with rupture had no graft inserted and died intra-operatively.

Definition of peri-operative complications

These were defined as described above.

Sample collection

Blood was sampled from an indwelling arterial line immediately prior to the induction of anaesthesia (sample A); immediately before release of the aortic clamp (sample B); five minutes (sample C) and 24 (sample D) and 48 hours (sample E) after aortic clamp release. Samples were prepared as described above.

Haemostatic and endothelial markers and C-reactive protein

Haematocrit, platelet count, fibrinogen, PT, aPTT, D-dimer, vWF antigen, soluble thrombomodulin (sTM) and CRP were assayed as described above. D-dimer was determined in all patients pre-operatively, and at all sample points in those who developed post-operative coagulopathy.

Statistical analysis

The Mann-Whitney U test (MW) and Spearman rank test were used.

TABLE 9.1

Co-morbidity in patients operated for ruptured and non-ruptured AAA.

	Ruptured AAA (n=20)	**Non-ruptured AAA** (n=10)
Co-morbidity		
None	5	1
Myocardial infarction	3	-
Angina pectoris	4	2
Coronary artery bypass graft	-	1
Hypertension	7	2
Congestive cardiac failure	1	-
Atrial fibrillation	1	-
Stroke	2	1
Peripheral arterial occlusive disease	1	2
Venous thrombo-embolism	-	1
Chronic obstructive airways disease	2	2
Non-insulin dependent diabetes mellitus	-	1
Cigarette smoking		
Non-smoker	11	2
Ex-smoker	4	4
Current smoker	5	4
Medications		
None	7	3
Aspirin	7	5
Diuretic	6	1
Nitrate	2	1
Angiotensin converting enzyme inhibitor	1	-
Calcium-channel blocker	1	-
Beta-adrenoceptor blocker	-	2
Bronchodilator	2	2

9.4 Results

Clinical data

Clinical and operative data for both groups of patients are summarized in Table 9.2. Three patients with ruptured AAA died during attempted repair, and one died in the recovery room immediately after graft insertion. Fifteen of 16 patients with rupture who survived to leave the operating room subsequently developed major post-operative complications, and five of these patients died in the post-operative period. There were no deaths after repair of non-ruptured AAA and four patients developed major post-operative complications (Table 9.3). No patients with non-ruptured AAA required admission to the ITU or assisted ventilation.

TABLE 9.2

Clinical and operative data in patients with ruptured and non-ruptured AAA.

	Ruptured AAA median (range) (n=20)	**Non-ruptured AAA** median (range) (n=10)	**p value** *
Pre-operative			
Serum creatinine (μmol/l)	129 (77 - 233)	92 (63 - 123)	0.008
Crystalloid and colloid administration (l)	0.7 (0.1 - 5.5)	-	< 0.001
Intra-operative			
Aortic clamp time (minutes)	70 (30 - 150)	75 (25 - 150)	NS
Measured blood loss (l)	4.0 (1.0 - 11.0)	2.4 (0.4 - 6.0)	NS
Crystalloid and colloid administration (l)	3.5 (1.5 - 12.5)	3.8 (2.0 - 7.1)	NS
RCC administration (units) [1]	8 (5 - 22)	4 (0 - 10)	0.001
FFP administration (units) [2]	2 (0 - 12)	0 (0 - 2)	< 0.001
Platelet administration (bags) [3]	1 (0 - 2)	0 (0 - 1)	0.001
Post-operative			
Duration of ITU stay (hours)	73 (13 - 579)	-	
Duration of IPPV (hours)	22 (9 - 451)	-	
Duration of hospital stay (days)	19 (9 - 50)	9 (7 - 40)	

KEY: * Mann-Whitney U test, [1] RCC = 300 ml, [2] FFP = 300ml, [3] one bag of platelet transfusion = 4 pooled units (250 ml)

TABLE 9.3

Post-operative complications and procedures in ruptured and non-ruptured AAA.

	Ruptured AAA (n= 15 / 20)	**Non-ruptured AAA** (n= 4 / 10)
Cardiovascular		
AF	6	1
CCF	7	3
MI	2	0
CVA	3	0
CLI	3	1
DVT	2	1
Respiratory		
Chest infection	11	2
Respiratory failure	7	0
ARDS	1	0
Acute Renal Failure (receiving RRT)	8 (3)	1
Coagulopathy	3	0
Sepsis syndrome	3	0
Colon ischaemia	1	0
Inotropic support		
Adrenaline	5	0
Renal dose dopamine	11	0

Haemostatic and endothelial cell markers

The values of haemostatic markers are shown in Table 9.4. In ruptured AAA, platelet count was significantly lower in non-survivors compared with survivors pre-operatively (median 119, range 40-321 vs. survivors: median 224, range 144-303; p= 0.007), immediately before (median 81, range 48-116 vs. survivors: median 123, range 86-189; p= 0.009) and 5 minutes (median 74, range 44-93 vs. survivors: median 114, range 59-146; p= 0.009) and 24 hours (median 72, range 23-86 vs. survivors: median 102, range 50-133; p= 0.02) after aortic clamp release. There was also a significant negative correlation between platelet count at 48 hours post-operatively and duration of ITU stay (r= -0.536, p= 0.039) but no significant relationship existed between platelet count and duration of assisted ventilation, hospital stay or the number of major complications.

The values of vWF, sTM and CRP are shown in Table 9.5. Pre-operative vWF was elevated above the normal range in 6 of 10 patients with non-ruptured AAA, and 13 of 20 with ruptured AAA. Pre-operative levels of sTM were elevated above the normal range in all patients in both groups, but there was no significant difference between the groups. There was a significant positive relationship between pre-operative vWF and sTM in all patients (r= +0.574, p= 0.001) and in patients with ruptured AAA (r= +0.492, p= 0.032). At 24 hours, vWF was above the normal range in all patients operated upon for non-ruptured AAA and 14 of 16 survivors of ruptured AAA.

There was no relationship between duration of symptoms of rupture and vWF, platelet count or CRP. In ruptured AAA, there was a significant negative correlation between CRP at 24 hours post-operatively and vWF pre-operatively (r= -0.593, p= 0.015), and platelet count pre-operatively (r= -0.552, p= 0.027), immediately before (r= -0.657, p= 0.011) and 5 minutes after aortic clamp release (r= -0.552, p= 0.041).

In non-ruptured AAA, there was a significant positive correlation between pre-operative vWF and haematocrit (r= +0.721, p= 0.019). There was no correlation between vWF and haematocrit or platelet count in ruptured AAA. There was no correlation between pre-operative body temperature and vWF or platelet count in patients with ruptured AAA. In ruptured AAA, there was a significant positive correlation between vWF and platelet count pre-operatively (r= +0.477, p=0.033), immediately before (r = +0.467, p= 0.044) and 5 minutes after clamp release (r = +0.495, p=0.043). In non-ruptured AAA, there was a significant positive correlation between vWF and platelet count pre-operatively (r = +0.794, p= 0.006), immediately before (r = +0.648, p= 0.043) and 5 minutes after clamp release (r= +0.634, p= 0.048).

There was significant negative correlation between operative blood loss and vWF 5 minutes after aortic clamp release in all patients with ruptured AAA (r= -0.526, p= 0.03) which became more significant when only patients who survived the repair were examined (r= -0.662, p= 0.007). There was no significant relationship between aortic clamp time and vWF. There was a significant negative correlation between operative blood loss (r= -0.569, p= 0.027) and aortic clamp time (r= -0.574, p= 0.02) and platelet count 5 minutes after aortic clamp release in all patients with ruptured AAA. The relationship between blood loss and platelet count 5 minutes after clamp release became more significant when only patients who survived the repair were examined (r= -0.622, p= 0.023). In non-ruptured AAA, there was significant negative correlation between aortic clamp time and vWF 5 minutes after aortic clamp release (r= -0.632, p= 0.05). There was no relationship between blood loss and vWF, or blood loss or aortic clamp time and platelet count.

Two patients with rupture had a post-operative MI. Both patients had significant cardiac disease and were hypotensive before and during the operation. vWF levels at 48 hours

(3.0 and 3.07 IU/ml) were higher than those patients who did not have an MI (median 2.12, range 0.98 - 2.86 IU/ml; p=0.026) and were associated with low platelet count (124 and 57 x 10^9/l) and elevated fibrinogen levels (7.32 and 4.28 g/l). There was no significant difference in vWF levels between patients who did and did not have other complications.

TABLE 9.4

Haematocrit, platelet count, fibrinogen, PT, aPTT and D-dimer.

Assay (normal range)	**Sample point**	**Ruptured AAA** median (range) (n=20)	**Non-ruptured AAA** median (range) (n=10)	**p value** *
Hct	A	0.41 (0.33 - 0.47)	0.31 (0.13 - 0.45)	< 0.001
(0.37 - 0.54)	B	0.3 (0.25 - 0.37)	0.29 (0.18 - 0.44)	NS
	C	0.3 (0.23 - 0.35)	0.29 (0.18 - 0.42)	NS
	D	0.33 (0.25 - 0.39)	0.33 (0.26 - 0.42)	NS
	E	0.33 (0.27 - 0.37)	0.31 (0.24 - 0.41)	NS
Platelet count	A	182 (40 - 321)	193 (75 - 744)	NS
(150-350 x 10^9/l)	B	110 (48 - 189)	140 (103 - 541)	0.007
	C	93 (44 - 164)	140 (91 - 577)	0.006
	D	97 (23 - 133)	136 (85 - 604)	0.007
	E	94 (43 - 124)	136 (74 - 435)	0.017
Fibrinogen	A	2.8 (1.59 - 6.02)	2.36 (0.46 - 6.31)	NS
(1.5 - 4.0 g/l)	B	1.74 (0.72 - 5.39)	1.2 (0.36 - 2.51)	0.02
	C	1.56 (0.36 - 5.44)	1.08 (0.46 - 1.82)	NS
	D	3.7 (2.5 - 5.44)	2.96 (1.13 - 4.63)	0.018
	E	6.03 (4.79 - 7.47)	5.57 (2.56 - 7.32)	NS
PT	A	12.5 (10.9 - 14.5)	14 (9.7 - 120)	NS
(10.5 - 14.5 s)	B	18.1 (14.5 - 25.5)	20.8 (12 - 120)	NS
	C	19.9 (14.4 - 120)	20.1 (10 - 31.2)	NS
	D	14.2 (12.4 - 21.4)	15.6 (12 - 18.6)	NS
	E	13.1 (11.5 - 18.8)	14.6 (12.4 - 19.3)	NS
aPTT	A	31.3 (25.4 - 49.3)	36.8 (27.6 - 210)	NS
(28 - 40 s)	B	210 (55.9 - 210)	59.5 (33.8 - 210)	0.021
	C	210 (56.8 - 210)	57.3 (41.7 - 210)	0.016
	D	37.1 (28.6 - 210)	47.4 (32.1 - 76)	NS
	E	34.7 (27.9 - 51)	37.3 (29.8 - 52.5)	NS
D-dimer	A	3886 (1236 - 17552)	1547 (550 - 5334)	0.004
(630 - 850 ng/ml)				

KEY: * = Mann-Whitney U test

TABLE 9.5

Endothelial cell markers and C-reactive protein.

Assay (normal range)	**Sample point**	**Ruptured AAA** median (range) (n=20)	**Non-ruptured AAA** median (range) (n=10)	**p value** *
vWF (0.42 - 1.22 IU/ml)	A	1.9 (0.47 - 3.64)	1.4 (0.44 - 3.6)	NS
	B	0.81 (0.14 - 2.12)	0.96 (0.3 - 3.06)	NS
	C	0.91 (0.21 - 1.7)	0.86 (0.29 - 2.75)	NS
	D	1.68 (0.8 - 3.12)	1.89 (1.5 - 3.99)	NS
	E	2.13 (0.98 - 3.07)	2.62 (1.41 - 4.02)	NS
sTM (< 25ng/ml)	A	108 (40 - 381)	88 (66 - 555)	NS
CRP (< 10 mg/l)	A	7.7 (0.3 - 178.3)	5 (0.3 - 18.6)	NS
	B	4 (0.3 - 116)	2.4 (0.3 - 13.4)	NS
	C	4 (0.3 - 114.2)	1.8 (0.3 - 8.2)	NS
	D	127 (41.8 - 400)	101.7 (41.5 - 180.8)	NS
	E	165.1 (57.5 - 400)	111.2 (90.2 - 257.6)	NS

KEY: * = Mann-Whitney U test

9.5 Discussion

The present study is the first to examine the relationship between plasma vWF, sTM and platelets in patients undergoing ruptured and non-ruptured AAA repair. The principal findings are that elevated vWF levels are present in approximately 60% of patients with intact or ruptured AAA pre-operatively, and almost 100% post-operatively. Furthermore, there is a significant positive association between peri-operative vWF levels and platelet count in both groups, and increased operative blood loss and aortic clamp times exacerbate the intra-operative fall in vWF and platelet count. However, there is no significant difference in peri-operative vWF levels between patients with ruptured and non-ruptured AAA. This finding significantly reduces the degree of certainty ascribed to the conclusions of this study.

Elevated plasma vWF levels are associated with an increased thrombotic tendency and have been reported in patients with peripheral (64,65) and coronary arterial occlusive disease (66-68). In patients undergoing elective repair of non-ruptured AAA, Yamazumi *et al* (260) showed no difference in pre-operative vWF levels compared with control patients. Gibbs *et al* (145) demonstrated an early post-operative increase in vWF levels in association with a procoagulant state in 19 patients undergoing elective aortic surgery for AAA or occlusive disease. A previous study from this department demonstrated ultrastructural changes in endothelial cell morphology consistent with endothelial cell activation in patients with ruptured AAA, but no such changes were observed in non-ruptured AAA (169). The present study, however, suggests that the endothelium is activated in the majority of patients with non-ruptured AAA, and that this is of similar magnitude to that which occurs in patients presenting with ruptured AAA. In keeping

with the findings of Gibbs *et al* (145), vWF levels were almost universally elevated at 24 hours in both groups of patients.

Previous work from this department has demonstrated a strong association between pre-operative thrombocytopenia and mortality, and immediate post-operative platelet count and the development of MOF in patients with ruptured AAA (45). Similar but less dramatic changes in platelet count are observed after elective aortic reconstruction for AAA and occlusive disease (263). The findings of the present study concur with our previous findings in that peri-operative PC was significantly lower in non-survivors of rupture, and low platelet count at 48 hours post-operatively was associated with prolonged ITU stay. The relationship between early peri-operative thrombocytopenia and mortality suggests that the changes in platelet count in ruptured AAA may contribute to, rather than occur as a consequence of, major morbidity and mortality. The finding that two patients with post-operative MI had high post-operative vWF levels is support for the relationship between vWF and thrombotic events. In ruptured AAA, there was no evidence that haemodilution, duration of symptoms or pre-operative hypothermia had an effect on vWF or platelet count. However, an elevated acute phase response and increased operative blood loss were associated with reduced peri-operative vWF and platelet count. These data, combined with the fact that there no significant difference in vWF levels between patients with ruptured and non-ruptured AAA, suggest that the stress response to surgery may have contributed to the changes in vWF and platelet count. As a control group of patients were not included, it is not possible to clarify this association from the present study.

Patients with non-ruptured AAA had epidural in addition to general anaesthesia and received intra-operative heparin. Epidural anaesthesia is associated with a reduction in adreno-cortical system function compared with general anaesthesia, but the effects on

vWF and platelets are unclear: factor VIII antigen or complex may be reduced (269,270) or unaffected (271) and platelet function may be reduced (272) or increased (273) compared with general anaesthesia. In elective aortic surgery, epidural and general anaesthesia have been shown to have no beneficial effect on peri-operative coagulation compared with general anaesthesia alone (147). Standard heparin has no effect on platelet count (274) but inhibits interactions between vWF and platelets (275) and stimulates vWF-independent platelet aggregation and activation (276,277).

One can speculate that EC activation and injury leads to vWF release. The vWF binds platelets and initiates the formation of occlusive thrombi at sites of endothelial injury. The resultant consumption of vWF and platelets secondary to macro- and microvascular thrombus formation leads to a fall in their circulating levels. This may partly explain the relationship between thrombocytopenia and poor outcome associated with AAA repair. Intravenous DDAVP leads to an acute increase in vWF levels with increased platelet adhesiveness. A randomised trial in patients undergoing elective aortic surgery failed to demonstrate a significant difference in operative blood loss or transfusion requirement, or pre- and post-operative bleeding time, platelet count or haematocrit (180). The limited efficacy of DDAVP in AAA patients may be explained by the finding of naturally elevated pre- and post-operative vWF levels, and because the proposed mechanism for the intra-operative fall in vWF is increased consumption secondary to increased platelet thrombi formation, rather than an actual deficiency of endogenous vWF.

In conclusion, these novel data demonstrate that ruptured and non-ruptured AAA are associated with peri-operative EC activation. vWF may initiate peri-operative formation of occlusive platelet thrombi at sites of endothelial injury, thereby contributing to the poor outcome associated with thrombocytopenia in patients undergoing AAA repair.

9.6 Summary

- Endothelial cell activation, as demonstrated by elevated vWF levels, is present in approximately 60% patients with ruptured and non-ruptured AAA pre-operatively, and almost 100% of patients post-operatively
- There is a significant positive association between peri-operative vWF levels and platelet count in ruptured and non-ruptured AAA.
- There is a negative correlation between operative blood loss and aortic clamp time and intra-operative vWF levels and platelet count.
- vWF may contribute to the procoagulant state in patients undergoing AAA repair by binding platelets initiating the formation of occlusive thrombi at sites of endothelial injury. The resultant consumption of vWF and platelets secondary to macro- and microvascular thrombus formation may lead to a fall in their circulating levels, thus partly explaining the relationship between thrombocytopenia and poor outcome associated with AAA repair.

Chapter 10

Haemostasis and myocardial injury in ruptured and non-ruptured AAA repair

10.1 Introduction

Cardiac events are a major cause of peri-operative morbidity and mortality in patients undergoing repair of ruptured and non-ruptured AAA (7,30,44,182). A hypofibrinolytic state has been demonstrated in patients with peripheral vascular disease (64,104,105) and may predict cardiovascular events in symptomatic patients (105). There is also considerable evidence to support a hypofibrinolytic state as a causative factor in the development of coronary artery disease and acute coronary events (95-100, 102,103,188,189).

We hypothesised that the procoagulant and hypofibrinolytic state which occurs during ruptured AAA repair may predispose to the development of peri-operative clinical and sub-clinical myocardial injury.

10.2 Aims

To examine the relationship between peri-operative changes in coagulation and fibrinolysis and myocardial injury in patients undergoing ruptured AAA repair, and to compare the findings with patients undergoing elective repair of non-ruptured AAA.

10.3 Methods

Patients

Ten patients (8 men and 2 women of median age 76, range 71-86, years) who underwent repair of ruptured infrarenal AAA and nine patients (8 men and 1 woman of median age 69, range 58-80, years) operated for asymptomatic non-ruptured infrarenal AAA were prospectively studied. These patients were described in Chapter 6. All patients with rupture had at least one documented episode of hypotension (systolic blood pressure less than 100mmHg) prior to surgery. Six patients with rupture had cardiac co-morbidity including hypertension (n=3), MI (n=2), angina pectoris (n=1) and CCF (n=1). Four patients with non-ruptured AAA had cardiac co-morbidity including hypertension (n=2), exertional angina pectoris (n=2) and previous coronary artery bypass grafting (n=1). Three patients with rupture and four with non-ruptured AAA were taking regular aspirin.

Operative methods

The operations were performed as previously described. Eight patients operated for rupture had at least one episode of intra-operative hypotension: on induction of anaesthesia (n=2), before aortic clamping (n=2), during lower torso ischaemia (n=3), and after aortic declamping (n=5). Four patients received low dose dopamine infusion (2-5 μg/kg/min), one received adrenaline infusion and one received both dopamine and adrenaline intra-operatively. Five patients operated for non-ruptured AAA had at least one episode of intra-operative hypotension: on induction of anaesthesia (n=4), before aortic clamping (n=2), during lower torso ischaemia (n=2), and after aortic declamping (n=2). One patient received intra-operative adrenaline infusion.

Assays of haemostatic function and myocardial injury

Haematocrit, platelet count, fibrinogen, PT, aPTT, PAI activity, t-PA activity and PF 1+2 were assayed. cTn I greater than or equal to 0.5 ng/ml was positive for myocardial injury. Serum CK was measured if cTn I was positive. CK-MB was measured if total CK was elevated above the normal range. CK-MB greater than 16U/l and greater than or equal to 6% of total CK is positive for MI. Samples were analysed as described above.

Sample collection

Blood for haemostatic markers and haematocrit was sampled before induction of anaesthesia (sample A), immediately before (sample B) and five minutes (sample C) and 24 hours (sample D) after aortic clamp release. cTn I is detectable 2-6 hours after myocardial cell injury. Therefore, blood was sampled for markers of myocardial injury immediately prior to the induction of anaesthesia (sample E), and 6 hours (sample F), 24 hours (sample G) and 48 hours (sample H) after aortic clamp release (Figure 10.1). Samples were prepared as described above.

Definition of major peri-operative cardiac complications

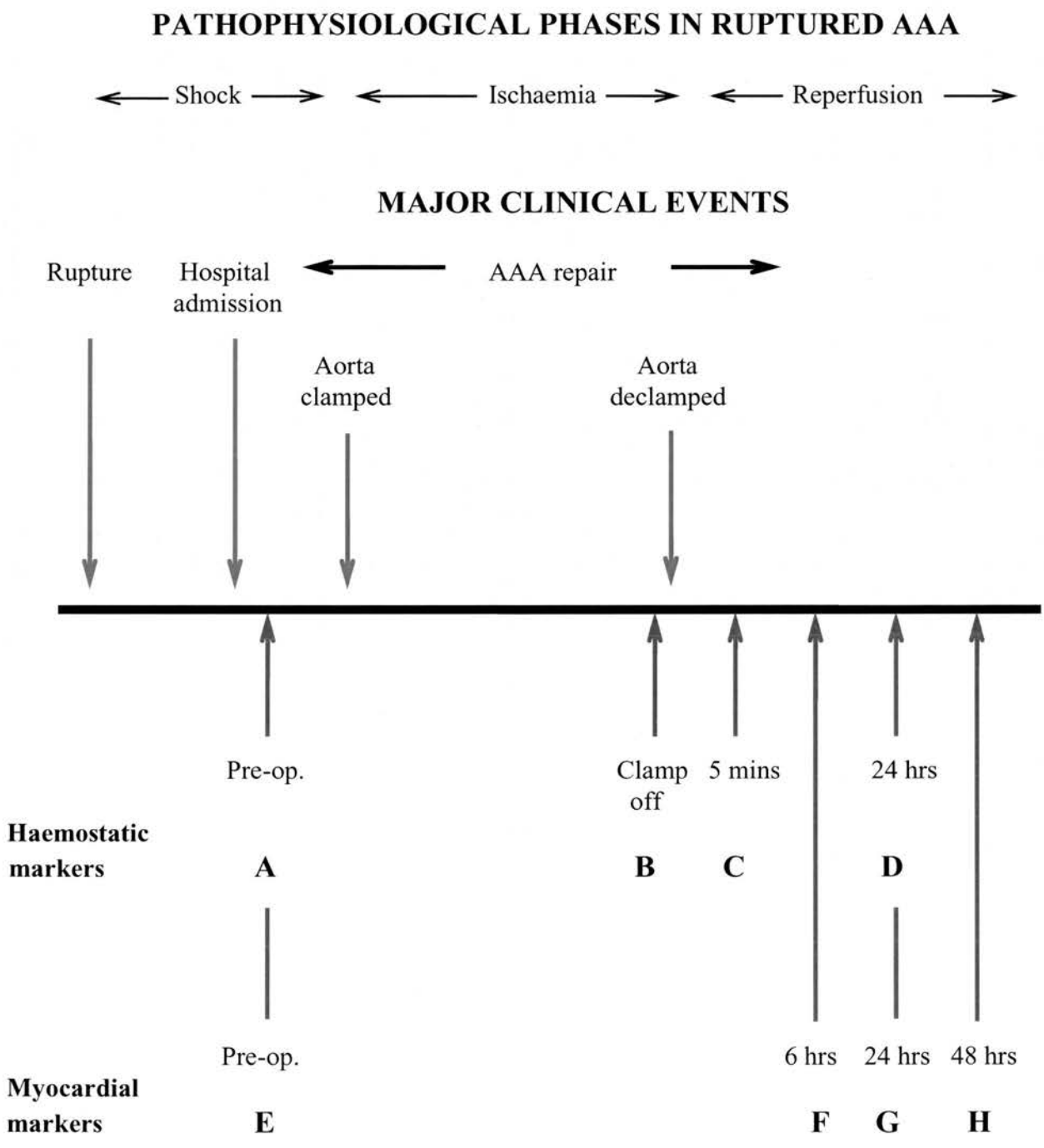
These were defined as described above, and consisted of acute MI, cardiac failure, and significant cardiac arrhythmia.

Statistical analysis

The Spearman rank test was used to correlate the levels of haemostatic markers with cTn I. Where levels of cTn I were below the limit of detection of the assay, no value was assigned to the sample and statistical analysis was performed using this convention.

FIGURE 10.1

Sampling points.



10.4 Results

Clinical data

All patients survived to 24 hours after repair and two patients with rupture died in hospital from pneumonia and CLI on post-operative day 21 (patient 3), and from ARDS and ARF on post-operative day 10 (patient 9) (Table 10.2). Three patients with non-rupture and six with ruptured AAA had clinically apparent major post-operative cardiac complications (Tables 10.1 and 10.2).

TABLE 10.1

Peri-operative cardiac events, cTn I and CK-MB in patients with non-ruptured AAA.

Patient	Cardiac co-morbidity	Hypotension Intra-op.	Post-op. cardiac complications	cTn I (ng/ml) / CK-MB (%) Sample E	F	G	H
1	N	N	AF, CCF	ND --	ND --	ND --	ND --
2	N	N	None	ND --	ND --	1.7 3	1.0 4
3	Y	Y	None	ND --	ND --	ND --	ND --
4	N	N	None	ND --	0.8 ND	ND --	ND --
5	Y	Y	None	ND --	ND --	ND --	ND --
6	Y	Y	Angina, CCF	ND --	ND --	1.7 3	20 4
7	N	Y	CCF	ND --	ND --	0.7 2	ND --
8	N	Y	None	ND --	ND --	ND --	ND --
9	N	N	None	ND --	ND --	ND --	ND --

KEY: ND = not detected

TABLE 10.2

Peri-operative cardiac events, cTn I and CK-MB in patients with ruptured AAA.

Patient	Cardiac co-morbidity	Hypotension Pre-op.	Hypotension Intra-op.	Post-op. cardiac complications	cTn I (ng/ml) / CK-MB (%) Sample E	F	G	H
1	Y	Y	Y	MI, CCF, AF, cardiac arrest	ND --	25 20	106 8	123 6
2	N	Y	N	CCF	ND --	ND --	0.6 ND	ND --
3	Y	Y	Y	AF *	ND --	ND --	ND --	ND --
4	N	Y	Y	CCF	1.1 ND	7.4 11	17 3	6.3 3
5	Y	Y	Y	MI, CCF, AF	1.3 ND	71 13	110 5	70 4
6	Y	Y	Y	None	3.6 ND	0.9 4	0.6 3	ND --
7	N	Y	Y	AF	ND --	ND --	2.6 3	1.0 2
8	Y	Y	N	None	ND --	28 14	51 5	22 5
9	N	Y	Y	None *	ND --	ND --	ND --	ND --
10	Y	Y	Y	None	6.8 ND	5.5 ND	2.9 4	1.6 ND

KEY: ND = not detected, * = post-operative death

Assays of haemostatic function and myocardial injury

The median (range) values for platelet count, fibrinogen, PT, aPTT and haematocrit are shown in Table 6.3. The median (range) values for t-PA activity, PAI activity and PF 1+2 are shown in Table 6.4.

In non-ruptured AAA, serum cTn I was detectable in 6 of 36 samples (Figure 10.2). Serum CK was elevated in 5 of these 6 samples, and CK-MB was greater than 6% of the total CK in none of these 5 samples (Table 10.1). CTn I was positive for myocardial injury at one or more sample point in three patients, but CK-MB was positive for MI in none of the patients. Two of three patients with elevated cTn I had clinically apparent cardiac events.

In ruptured AAA, serum cTn I was detectable in 24 of 40 samples (Figure 10.3). Serum CK was elevated in 17 of these 24 samples, and CK-MB was greater than 6% of the total CK in 5 of these 17 samples (Table 10.2). Four patients had elevated cTn I on admission to the hospital. These patients were not clinically distinct from patients who did not have elevated cTn I on admission. CTn I was positive for myocardial injury at one or more sample point in eight patients, and CK-MB was positive for MI at one or more sampling point in four. Three of four patients with elevated cTn I and CK-MB had clinically apparent cardiac events.

In ruptured AAA, cardiac co-morbidity and intra-operative hypotension were not associated with a significant difference in cTn I levels. There was also no correlation between cTn I levels and duration of symptoms, operative blood loss or aortic clamp time. The number of patients with non-ruptured AAA and elevated cTn I were too small to make valid statistical conclusions.

FIGURE 10.2

Individual data points for cTn I in non-ruptured AAA repair immediately before induction of anaesthesia (sample E), and 6 hours (sample F), 24 hours (sample G) and 48 hours (sample H) after aortic clamp release.

The normal range for cTn I (< 0.5 ng/ml) is shown by the horizontal line.

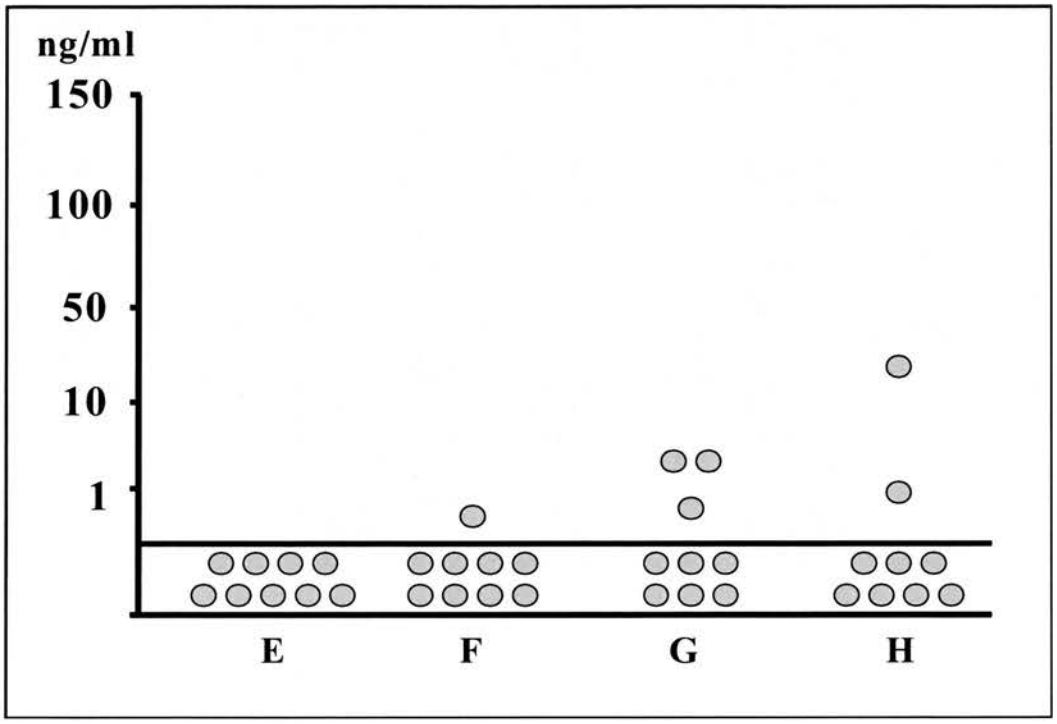
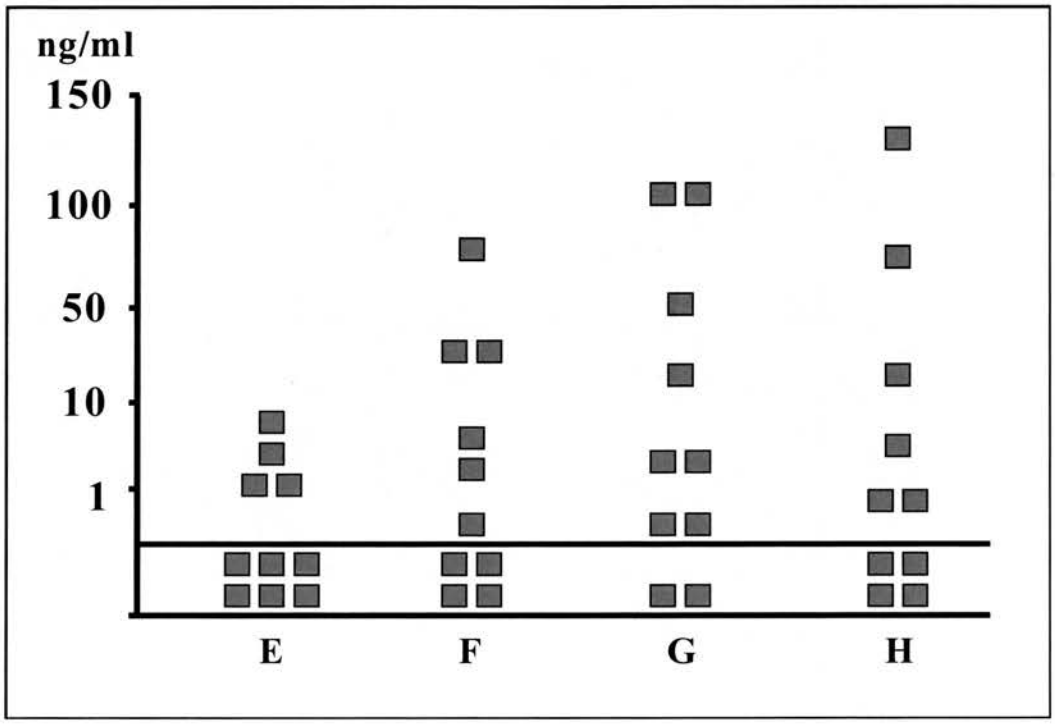


FIGURE 10.3

Individual data points for cTn I in ruptured AAA repair immediately before induction of anaesthesia (sample E), and 6 hours (sample F), 24 hours (sample G) and 48 hours (sample H) after aortic clamp release.

The normal range for cTn I (< 0.5 ng/ml) is shown by the horizontal line.



Relationship between haemostatic markers and cardiac troponin I

There was no significant relationship between platelet count, fibrinogen, PT and aPTT, and cTn I at any sampling point. There was no significant relationship between t-PA activity and PF 1+2, and cTn I at any sampling point. There was also no significant relationship between PAI activity before operation and cTn I at any sampling point. There was, however, a significant positive correlation between PAI activity immediately before aortic clamp release and cTn I at 6 hours ($r= +0.829$, $p= 0.042$), 24 hours ($r= +0.762$, $p= 0.028$) and 48 hours ($r= +0.829$, $p= 0.042$) after aortic clamp release; and PAI activity 5 minutes after aortic clamp release and cTn I at 6 hours ($r= +0.943$, $p= 0.005$) and 24 hours ($r= +0.881$, $p= 0.004$) after aortic clamp release.

10.5 Discussion

The principal finding of the present study is that the hypofibrinolytic state which occurs during ruptured AAA repair is associated with peri-operative myocardial injury as demonstrated by elevated levels of cTn I.

cTn I is currently the most specific and sensitive marker for myocardial injury (193-197) and has major advantages over CK-MB and cTnT in patients undergoing major peripheral vascular surgery: it is entirely cardiospecific, does not accumulate in patients with renal failure, has not been shown to be released from skeletal muscle, and is detectable after myocardial ischaemia and minor myocardial injury. Elevated levels may also be detected in conditions where myocardial necrosis is uncommon such as myocarditis (278) and myocardial strain which accompanies cardiac dilation and hypertrophy (279).

Adams and colleagues (194) studied 96 patients undergoing vascular surgery and 12 patients undergoing spinal surgery. Eight of 96 (8.3%) patients undergoing vascular surgery had abnormal post-operative echocardiographic examinations suggestive of MI, and of these all eight had elevated cTn I and six had elevated CK-MB. Of the 100 patients who had normal post-operative echocardiography, one patient had a slightly elevated cTn I whereas 19 had elevated CK-MB. The authors concluded that cTn I measurement was very sensitive and specific for peri-operative MI and CK-MB was associated with a high incidence of false positive results. Of 291 patients who underwent major vascular surgical procedures (including 61 patients operated for AAA), Lee and colleagues (280) reported elevated post-operative cTn T in approximately 10% and clinically apparent cardiac complications in approximately 6%. Metzler and colleagues (281) measured peri-operative cTn T and cTn I in 67 patients undergoing elective non-

cardiac surgery, of whom 38 had underwent (undefined) vascular surgical procedures. Of 13 patients with elevated cTn T, 10 were in the vascular surgery group, and eight had major post-operative cardiac complications (unstable angina, CCF, arrhythmia, MI). In the present study, cTn I was positive for myocardial injury in 3 of 9 patients with non-ruptured AAA, but no patient had a CK-MB positive for MI. Two of the patients with elevated cTn I had cardiac events. In ruptured AAA, 2 of 10 patients had a clinically apparent MI, four had a positive CK-MB. Eight patients had elevated cTn I, of whom three had no clinically apparent cardiac complications. Three of four patients with elevated cTn I and CK-MB had cardiac events. CTn I is a very sensitive marker of myocardial injury, and, it possible that the elevated levels in the patients with no cardiac complications may have been due to ischaemia and 'minor' necrosis (280) affecting areas of the heart which are not crucial for normal cardiac function.

Between 75% and 95% of patients undergoing elective aortic reconstruction have double or triple vessel coronary artery disease with stenoses greater than 70% (184,185). Myocardial injury is also associated with a procoagulant state but there is evidence that peri-operative MI is most often of the non-Q-wave type (190,191) which is secondary to sustained subendocardial ischaemia. Atherosclerotic plaque rupture with subsequent occlusive thrombus formation generally results in Q-wave infarction on electrocardiography (ECG) (186). Gibbs *et al* (145) demonstrated an early post-operative hypercoagulable state in patients undergoing elective aortic surgery for aneurysmal and occlusive disease, and were the first to speculate that this may contribute to coronary artery thrombosis and MI. The present study has confirmed an association between derangement of intra-operative haemostatic function and myocardial injury. There was a positive association between PAI activity and cTn I in those patients who had detectable levels of cTn I, with PAI activity being elevated at least 6 hours before

cTn I was detected. Some patients had elevated PAI activity with no evidence of myocardial injury, indicating that the hypofibrinolytic state may be only one of many contributory factors in the pathophysiology of myocardial injury. Possible mechanisms contributing to the development of peri-operative myocardial injury and MI in patients with ruptured AAA include; a hypofibrinolytic state leading to thrombosis in the presence of a critical coronary artery stenosis or on an unstable coronary artery plaque, both of which cause mechanical disruption to blood flow; dilation of the left ventricle on aortic clamping which leads to distortion of an atherosclerotic plaque in the coronary arteries with subsequent plaque fissure and exposure of procoagulant media (181); and coronary artery hypoperfusion secondary to hypotension. The risk of cardiac complications associated with this hypercoagulable state may, therefore, be potentiated by periods of haemodynamic instability and pre-existing coronary artery disease. In the present study, however, pre-existing cardiac disease, intra-operative hypotension, operative blood loss and aortic clamp time had no demonstrable effect on cTn I levels

In conclusion, these data support the hypothesis that the procoagulant state which occurs during ruptured AAA repair contributes to the development of post-operative myocardial injury. It is interesting to speculate that the procoagulant and hypofibrinolytic state may represent both a homeostatic and pathological response; it allows the patient to survive the initial rupture event and operative repair, but contributes to the development of major post-operative cardiac complications. By contrast, increased systemic fibrinolysis as demonstrated in Chapter 8 may be a pathological response to rupture and is associated with early mortality, usually secondary to coagulopathy and haemorrhage.

In patients undergoing AAA repair, screening for myocardial injury using cTn I may be of value if a) it predicts post-operative cardiac problems such as cardiac failure or arrhythmias and allows these to be treated prophylactically, and b) allows for therapeutic

intervention peri-operatively to improve the myocardial perfusion either directly by emergency coronary angiography and percutaneous transluminal coronary angioplasty or indirectly by correcting the procoagulant and hypofibrinolytic state. A recent randomised trial (181) of systemic heparinisation versus no heparinisation during elective AAA repair demonstrated a significant reduction in the incidence of fatal and non-fatal MI in those patients who received heparin. The effect of intra-operative heparinisation on haemostasis and myocardial injury in patients with ruptured AAA requires further investigation.

10.6 Summary

- cTn I demonstrated peri-operative myocardial injury in 33% of patients with non-ruptured AAA and 80% of patients with ruptured AAA.
- CK-MB was elevated in no patient with non-ruptured AAA, and 40% of patients with ruptured AAA.
- There was a significant relationship between intra-operative PAI activity and post-operative cTn I levels, indicating that there is an association between hypofibrinolytic state which occurs during ruptured AAA repair and the development of peri-operative myocardial injury.

Chapter 11

The endothelins and haemostasis in ruptured AAA repair

11.1 Introduction

Vasoconstriction is the first event in the haemostatic response to vascular injury. Endothelin (ET)-1 is the most potent known vasoconstrictor. Furthermore, there is evidence that ET stimulates thrombin generation and a local inflammatory response, which also contribute to intravascular thrombus formation (205-208). ET release may be pathological (217-222) or homeostatic (222,223). Elevated plasma ET levels have been demonstrated in number of critical illnesses which may complicate ruptured AAA repair (208-216). Increased plasma ET levels have been demonstrated in patients undergoing non-ruptured AAA repair with infrarenal (224,225) and supracoeliac aortic cross-clamping (226), as well as in one animal model of infrarenal aortic clamping and subsequent exsanguination (227). To date, however, there are no reports of the ET response in patients undergoing repair of ruptured AAA.

We hypothesised that haemorrhagic shock, ischaemia and reperfusion would lead to increased synthesis and secretion of ET which would predispose to the development of organ failure, one of the principal causes of death in this group of patients.

11.2 Aims

To examine peri-operative changes in plasma levels of big ET-1 and ET-1 in patients undergoing repair of ruptured AAA, and to examine the relationship between ET levels and markers of coagulation and fibrinolysis.

11.3 Methods

Patients

Fourteen consecutive patients (13 men and 1 woman of median age 74, range 65-86, years) who underwent repair of ruptured infrarenal AAA and survived to at least 24 hours after surgery, were prospectively studied. The median (range) delay between the onset of symptoms of rupture and hospital admission was 5 (3-14) hours. All patients had at least one documented episode of hypotension (systolic blood pressure less than 100mmHg) prior to surgery. Co-morbidity data are shown in Table 11.1.

Operative methods

All patients had retroperitoneal ruptured AAA and none had intraperitoneal rupture. The operations were performed as previously described. An aorto-aortic graft was inserted in 10 patients, an aorto-bifemoral graft in three, and an aorto-bi-iliac graft in one patient.

Sample collection and assay methods

For estimation of ET-1 and big ET-1, blood was sampled from an indwelling arterial line before induction of anaesthesia (sample A), immediately before aortic clamp release (sample B), and five minutes (sample C) and 6 hours (sample D) after aortic clamp release. For estimation of haematocrit, t-PA and PAI activities and PF 1+2, blood was sampled before induction of anaesthesia (sample A); immediately before release of the aortic clamp (sample B); five minutes (sample C) and 24 hours (sample D) after aortic clamp release. All samples were prepared and assays performed as described above.

Definitions of post-operative organ failure

These were defined as described above.

Statistical analysis

The Mann-Whitney U test, Kruskal-Wallis one-way analysis of variance and Spearman rank test were used.

TABLE 11.1

Co-morbidity in survivors and non-survivors of ruptured AAA repair.

	Survivors (n=9)	Non-survivors (n=5)
Co-morbidity		
None	2	3
Myocardial infarction	2	-
Angina pectoris	2	1
Hypertension	3	-
Congestive cardiac failure	1	-
Atrial fibrillation	-	1
Stroke	1	-
Peripheral arterial occlusive disease	1	-
Chronic obstructive airways disease	2	-
Cigarette smoking		
Non-smoker	5	3
Ex-smoker	2	-
Current smoker	2	2
Medications		
None	3	4
Aspirin	3	1
Diuretic	5	-
Nitrate	1	-
Calcium-channel blocker	1	-
Bronchodilator	2	-

11.4 Results

Clinical data

All patients were admitted to the ITU post-operatively. All patients survived for at least 24 hours post-operatively, but five (36%) died in the post-operative period. Clinical and operative data for survivors and non-survivors are summarized in Table 11.2 and post-operative complications are shown in Table 11.3.

TABLE 11.2

Clinical and operative data in survivors and non-survivors of ruptured AAA repair.

	Survivors median (range) (n=9)	**Non-survivors** median (range) (n=5)	**p value** *
Pre-operative			
Duration of symptoms (hr)	4 (3 - 14)	6 (5 - 12)	NS
Serum creatinine (μmol/l)	138 (82 - 176)	115 (77 - 183)	NS
Crystalloid and colloid administration (l)	0.5 (0.2 - 5.5)	0.7 (0.1 - 1.0)	NS
Intra-operative			
Total operation time (minutes)	140 (75 - 240)	105 (75 - 200)	NS
Aortic clamp time (minutes)	90 (40 - 185)	75 (55 - 135)	NS
Measured blood loss (l)	4.0 (1.0 - 6.4)	3.3 (1.5 - 11.0)	NS
Crystalloid and colloid administration (l)	3.4 (1.5 - 8.0)	3.8 (3.5 - 5.0)	NS
RCC administration (units) [2]	8 (5 - 11)	8 (6 - 22)	NS
FFP administration (units) [3]	2 (2 - 6)	2 (2 - 12)	NS
Platelet administration (bags) [4]	1 (1)	1 (0 - 1)	NS

KEY: * Mann-Whitney U test, [2] RCC = 300 ml, [3] FFP = 300ml, [4] one bag of platelet transfusion = 4 pooled units (250 ml)

TABLE 11.3

Post-operative complications and procedures in ruptured AAA repair.

	Survivors (n=9)	**Non-survivors** (n=5)
Cardiovascular		
CCF	4	3
MI	1	-
CVA	2	1
CLI	1	2
Respiratory		
Respiratory failure	3	4
ARDS	-	1
Pneumonia	6	5
Acute Renal Failure	3	5
Coagulopathy	1	2
Sepsis syndrome	1	2
Colon ischaemia	1	-
Inotropic support		
Adrenaline	2	3
Dopamine	7	4

Plasma levels of big ET-1, ET-1 and haemostatic markers.

The values of big ET-1 and ET-1 in survivors and non-survivors are shown in Figures 11.1 and 11.2, respectively. The values for haematocrit, t-PA activity, PAI activity and PF 1+2 are shown in Table 11.4. There was no significant difference in haematocrit or the levels of haemostatic markers between survivors and non-survivors.

Big ET-1 was above the normal laboratory range at one or more sampling points in all patients, and ET-1 was above the normal range in all survivors and 4 of 5 non-survivors. When compared with non-survivors, survivors had significantly higher levels of big ET-1 at all four sampling points, and significantly higher levels of ET-1 after 5 minutes reperfusion. When compared with pre-operative levels, there was a significant increase in big ET-1 levels after 6 hours of reperfusion in survivors. In non-survivors, there was a significant increase in ET-1 levels between 5 minutes and 6 hours after reperfusion.

There was no relationship between duration of symptoms, operative blood loss or aortic clamp time and plasma ET-1 and big ET-1 levels at any sampling point. Pre-operative ET-1 levels were significantly lower in eight patients who subsequently developed ARF (median 3.72, range 2.76-6.0 pg/ml) than in six patients who did not (median 5.89, range 3.86-7.23 pg/ml, $p=0.02$). There was no significant difference in big ET-1 or ET-1 levels between patients who did and did not have cardiac failure, respiratory failure, or coagulopathy.

FIGURE 11.1

Individual data points for big ET-1 immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 6 hours (sample D) after aortic clamp release.

Non-survivors are represented by the red squares and survivors by the yellow circles.

The upper limit of the normal range for big ET-1 (10 - 60 pg/ml) is shown by the horizontal line.

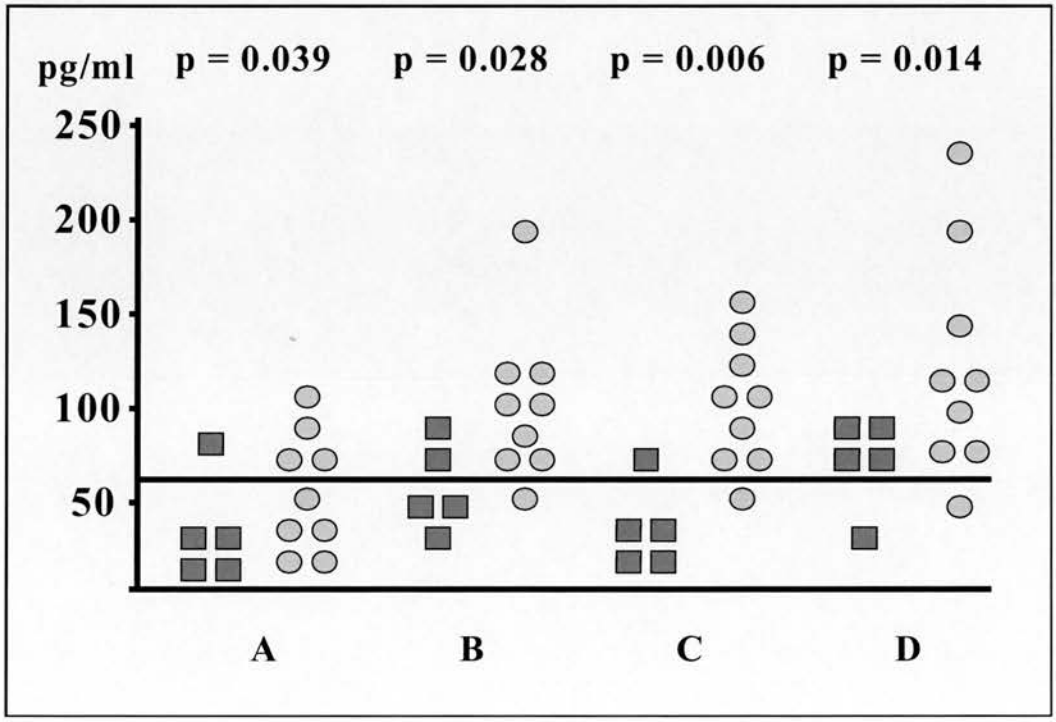


FIGURE 11.2

Individual data points for ET-1 immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C) and 6 hours (sample D) after aortic clamp release.

Non-survivors are represented by the red squares and survivors by the yellow circles.

The upper limit of the normal for ET-1 (1.5 - 4.5 pg/ml) is shown by the horizontal line. NS = not significant.

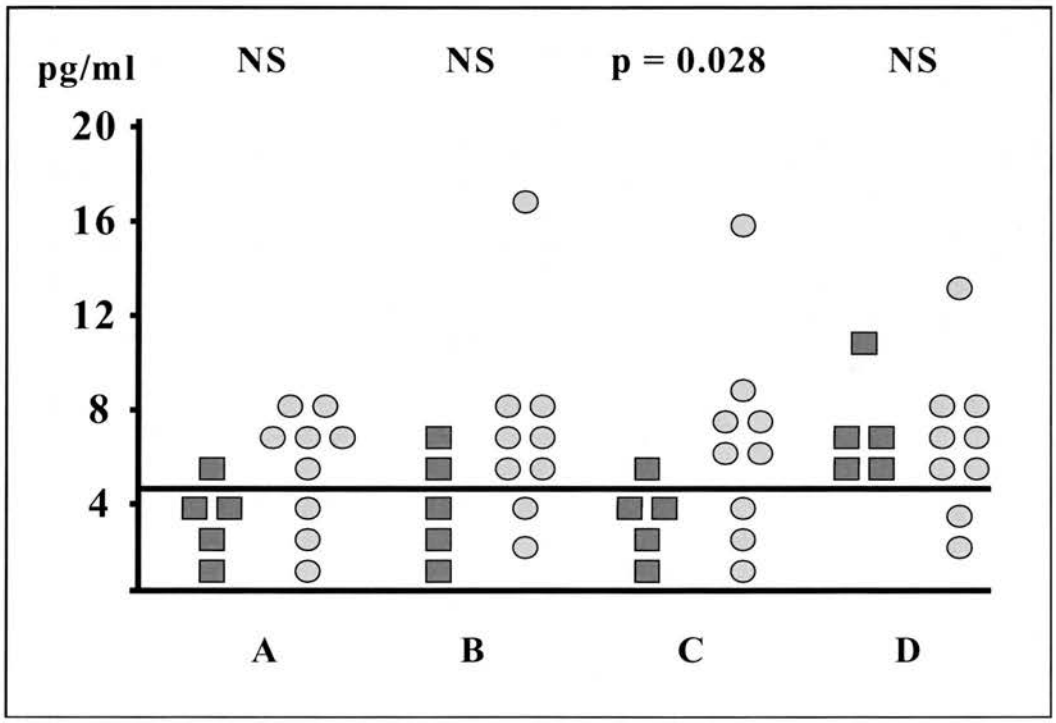


TABLE 11.4

Haematocrit, t-PA activity, PAI activity and PF 1+2 levels.

Assay	Normal range	Sample	Median (range)
Haematocrit	0.37 - 0.54	A	0.316 (0.129 - 0.367)
		B	0.288 (0.194 - 0.439)
		C	0.295 (0.183 - 0.42)
		D	0.329 (0.257 - 0.357)
t-PA activity	0.2 - 2.0 IU/ml	A	0.24 (0.06 - 7.9)
		B	0.28 (0.08 - 6.4)
		C	0.32 (0.09 - 7.3)
		D	0.68 (0.15 - 2.3)
PAI activity	< 15 AU/ml	A	35.2 (2.6 - 38.8)
		B	38.4 (8.6 - 39.4)
		C	37.9 (10.6 - 39.4)
		D	16.8 (5.9 - 35.3)
PF 1+2	0.4 - 1.1 nmol/l	A	6.7 (2.4 - 11.6)
		B	6.7 (2.0 - 9.1)
		C	6.5 (2.6 - 11.4)
		D	3.6 (1.6 - 11.4)

There were several significant correlations when haemostatic markers were compared with ET levels. When all patients were examined, there was a significant negative correlation between PF 1+2 immediately before aortic clamp release and big ET-1 at 5 minutes after aortic clamp release ($r= -0.554$, $p= 0.044$). In survivors of rupture, there was a significant negative correlation between PF 1+2 immediately before ($r= -0.695$, $p= 0.038$) and 5 minutes after aortic clamp release ($r= -0.678$, $p= 0.045$) and big ET-1 at 5 minutes after aortic clamp release. The number of patients who did not survive was too small to make any valid statistical conclusions.

11.5 Discussion

The present study is the first to examine the relationship between peri-operative ET levels, organ failure and mortality in patients undergoing ruptured AAA repair. The principal finding is that, contrary to our original hypothesis, patients who died had significantly lower peri-operative ET levels than survivors.

Previous studies of the endothelin response to lower torso ischaemia and reperfusion are few and contradictory. Antonucci *et al* (224) examined the effect of intra-operative nifedipine infusion on endothelin-dependent renal vasoconstriction in five patients undergoing non-ruptured infrarenal AAA repair, and demonstrated a transient but significant increase in plasma ET-1 and -2 levels at the end of the period of aortic cross-clamping. The authors concluded that nifedipine prevented the renal vasoconstrictor response to ET as there was no significant difference in creatinine clearance and glomerular filtration rate post-operatively compared to pre-operatively. Fukuda *et al* (225) measured arterial and iliac vein ET-1 levels in seven patients undergoing elective aortic aneurysm repair. There was no significant change in arterial ET-1 levels, but a significant increase in iliac vein ET-1 levels occurred immediately after aortic clamp release and perfusion of the first limb. Venous ET-1 levels showed a significant correlation with venous O_2 content, pH, pO_2, O_2 saturation and base excess suggesting that ET-1 production occurred secondary to lower limb ischaemia. Lintott *et al* (226) were the first to attempt to examine the relationship between ET-1 levels and outcome in 21 patients who required supracoeliac and eight who required infrarenal aortic clamping for repair of non-ruptured aortic aneurysm. Unlike the studies by Antonucci *et al* and Fukuda *et al*, plasma ET-1 was undetectable during the period of aortic clamping and 30

minutes after aortic declamping. After two hours of reperfusion, ET-1 levels were significantly higher in patients who subsequently developed ARF after supracoeliac clamping, and at 8 hours, ET-1 levels were significantly higher in the supracoeliac clamp group compared with the infrarenal clamp group. In a canine model of infrarenal aortic clamping, Edwards *et al* (227) failed to demonstrate a significant increase in plasma ET-1 levels during ischaemia, but there was a significant increase during reperfusion and subsequent exsanguination.

In the present study, survivors had increased plasma ET levels during the periods of lower torso ischaemia and reperfusion. Furthermore, over half of the survivors had increased ET levels pre-operatively. There was also a significant increase in ET levels after 6 hours of reperfusion in survivors and non-survivors. The increased physiological insult of ruptured AAA repair may explain why, unlike previous studies of non-ruptured AAA repair, elevated ET levels were detected before, during and after operation.

It is interesting to speculate from these data that the ET-dependent vasoconstrictor response to haemorrhagic shock, ischaemia and reperfusion has a homeostatic and protective role in ruptured AAA. Patients who manifest a good vasoconstrictor response (which is partly due to ET) may have a higher probability of survival than those patients who mount an inferior response. This hypothesis would be in keeping with what the majority of vascular surgeons know intuitively: that is, intense vasoconstriction (as well as 'controlled' hypotension, aortic tamponade and the generation of a prothrombotic state) is one of the principal mechanisms which allows patients with ruptured AAA to survive to reach hospital and then undergo successful aneurysm repair.

The reasons for low ET levels in non-survivors as well as those patients who developed ARF are not immediately obvious. Ruptured AAA repair is associated with many factors

known to stimulate ET synthesis and secretion: intra-operative haemorrhage and haemodilution, hypoxia and metabolic acidosis, increased sympathetic discharge and catecholamine release, increased cytokine and endotoxin release, thrombin generation, and impaired renal excretion. In the present study, there was no apparent difference in the duration of symptoms of rupture, severity of pre-operative shock, duration of lower torso ischaemia or peri-operative haematocrit between survivors and non-survivors.

Haynes and colleagues (282) reported a significant, and similarly unexpected, association between high plasma ET-1 levels and survival in cardiac arrest patients. They proposed several explanations for their findings: poor peripheral blood flow and local tissue acidosis may adversely affect production and activity of ET-1, or lead to a local increase in ET-1 which does not enter the circulation; increased NO production may inhibit ET production; and reduced pulsatile shear stress in may lead to selective endothelial cell dysfunction. The low ET levels in non-survivors of ruptured AAA and cardiac arrest may therefore be an early manifestation of irreversible whole body hypoperfusion.

Data from this and previous chapters demonstrate that survivors and non-survivors of ruptured AAA repair have evidence of increased thrombin generation, and increased levels of the endothelial products, t-PA, PAI and vWF. ET is one of the major contributors to vasoconstriction, the first event in haemostasis, and its release may stimulate or be stimulated by thrombin generation. In the present study, however, a negative correlation existed between intra-operative PF 1+2 and ET levels.

These data, together with the fact that increased NO production has been demonstrated in animal models of infrarenal aortic cross-clamping (232,283), lend support to the hypothesis that selective endothelial cell dysfunction may lead to downregulation of the ET response in some patients with ruptured AAA and this may predispose to the

development of fatal organ dysfunction. The procoagulant state in ruptured AAA repair may interact with ET production and contribute to the low ET levels in non-survivors. Impaired ET release may also be a marker of increased NO synthesis which exerts its injurious effect through the production of oxygen-free radicals. The NO response to ruptured AAA repair requires investigation.

Although the majority of the current literature concludes that ET release has a pathological role in critical illness, the findings of the present study do not support the hypothesis that an increased ET response predisposes to poor outcome in patients undergoing ruptured AAA repair. By contrast, elevated peri-operative ET levels were associated with survival. Low ET levels may be an early marker of severe and irreversible whole body hypoperfusion in this group of patients. Alternatively, increased circulating ET levels may occur as part of a homeostatic and protective response to haemorrhage, ischaemia and reperfusion in patients with ruptured AAA. The measurement of plasma ET levels before and during operation may help to identify patients with ruptured AAA who are at an increased risk of developing fatal post-operative MOF.

11.6 Summary

- Patients who died from organ dysfunction after ruptured AAA repair had significantly lower peri-operative ET levels than survivors.
- Survivors had increased plasma ET levels during the periods of lower torso ischaemia and reperfusion, and over half had increased levels pre-operatively.
- Increased circulating ET levels present in survivors may occur as part of a homeostatic and protective response to haemorrhage, ischaemia and reperfusion.
- Low ET levels present in non-survivors may represent an early manifestation of severe and irreversible whole body hypoperfusion.
- Peri-operatively, elevated ET levels were associated with reduced PF 1+2.
- The measurement of plasma ET levels before and during operation may help to identify patients who are at an increased risk of developing fatal post-operative organ dysfunction.

Chapter 12

Soluble TNF receptors and haemostasis in ruptured and non-ruptured AAA repair

12.1 Introduction

The pro-inflammatory cytokine, TNF, has been implicated in the systemic inflammatory response syndrome and MOF (35,228). It also has an important role in haemostasis and can induce a procoagulant and hypofibrinolytic state by increasing TF expression, vWF release (53,54) and PAI release while reducing t-PA release (72-75,77,82,83,150).

Binding of TNF to target cells leads to the cleavage and release of TNF receptors into the circulation as soluble TNF receptors (sTNF-Rs). Circulating TNF is frequently difficult to demonstrate and it has been suggested that the levels of sTNF-Rs may better reflect the degree of TNF-induced tissue injury (230,231). Circulating TNF has been demonstrated in elective and emergency aortic surgery (75,161,162,236-241), and elevated levels of sTNF-Rs have been detected in patients undergoing repair of non-ruptured AAA (241,242), and post-operatively in ruptured AAA (241). To date, however, no study has examined sTNF-R levels during the periods of shock, ischaemia and early reperfusion which occur in ruptured AAA repair. In an animal model of AAA repair, administration of exogenous sTNF-R before aortic cross-clamping has been shown to ameliorate the adverse effects of TNF (232). In humans, however, elevated levels of endogenous sTNF-Rs are actually associated with MOF and increased mortality (233-235). While exogenous sTNF-R therapy may have beneficial effects in elective aortic surgery where endogenous levels are low, it may be ineffective in patients undergoing ruptured AAA repair if endogenous levels are high.

12.2 Aims

To examine serial changes in sTNF-Rs occurring during repair of ruptured and non-ruptured AAA, and to examine the relationship between sTNF-R levels and markers of coagulation, fibrinolysis and endothelial activation.

12.3 Methods

Patients

Sixteen patients (14 men and 2 women of median age 75, range 65-86, years) operated for ruptured and 10 patients (8 men and 2 women of median age 69, range 58-80, years) operated for asymptomatic non-ruptured infrarenal AAA were prospectively studied. In patients operated for ruptured AAA, the median (range) delay between the onset of symptoms of rupture and hospital admission was 5 (3-14) hours. All patients had at least one documented episode of hypotension (systolic blood pressure less than 100mmHg) prior to surgery. In patients undergoing operation for asymptomatic non-ruptured AAA, the median (range) antero-posterior diameter of the aneurysm measured by abdominal ultrasonography was 6.5 (5.5-8.0) cm. Co-morbidity data are shown in Table 12.1.

Operative methods

Thirteen patients had retroperitoneal rupture and three had retroperitoneal and intraperitoneal rupture. The operations were performed as previously described. A dacron tube graft was inserted in 17 patients (12 rupture, 5 non-rupture), an aorto-bi-iliac graft in five (1 rupture, 4 non-rupture) and aorto-bifemoral graft in four patients (3 rupture, 1 non-rupture).

Sample collection and assay methods

For estimation of serum levels of sTNF-Rs p55 and p75, blood was sampled immediately prior to the induction of anaesthesia (sample A); immediately before release of the aortic clamp (sample B); and five minutes (sample C), 6 hours (sample D) and 24 hours

(sample E) after aortic clamp release. For estimation of t-PA activity, PAI activity, PF 1+2 and vWF levels, blood was sampled immediately prior to the induction of anaesthesia (sample A); immediately before release of the aortic clamp (sample B); five minutes (sample C) and 24 hours (sample D) after aortic clamp release. All samples were prepared and assays were performed as described above.

Definition of major post-operative complications

These were defined as described above.

Statistical analysis

The Mann-Whitney U test and Kruskall-Wallis one-way analysis of variance were used. Where levels of sTNF-Rs were below the limit of detection of the assay, the minimum detection concentration was assigned to that sample and statistical analysis was performed using this convention.

TABLE 12.1

Co-morbidity in patients operated for ruptured and non-ruptured AAA.

	Ruptured AAA (n=16)	**Non-ruptured AAA** (n=10)
Co-morbidity		
None	5	1
Myocardial infarction	2	-
Angina pectoris	3	2
Coronary artery bypass graft	-	1
Hypertension	5	2
Congestive cardiac failure	1	-
Atrial fibrillation	1	-
Stroke	2	1
Peripheral arterial occlusive disease	1	2
Venous thrombo-embolism	-	1
Chronic obstructive airways disease	2	2
Non-insulin dependent diabetes mellitus	-	1
Cigarette smoking		
Non-smoker	9	1
Ex-smoker	3	5
Current smoker	4	4
Medications		
None	7	3
Aspirin	5	5
Diuretic	5	-
Nitrate	1	1
Angiotensin converting enzyme inhibitor	1	-
Calcium-channel blocker	1	-
Beta-adrenoceptor blocker	-	2
Bronchodilator	2	1

12.4 Results

Clinical data

Clinical and operative data for both groups of patients are summarized in Table 12.2. All patients with ruptured AAA were admitted to the ITU. The median (range) duration of ITU stay was 3 (0.5 - 24.1) days. The median (range) duration of ventilatory support was 0.9 (0.4 - 18.8) days and seven patients were ventilated for more than 4 days. All patients operated for non-ruptured AAA were admitted to the intermediate care unit post-operatively and no patient was admitted to ITU or required ventilatory support.

Thirteen patients operated for rupture and four operated for non-ruptured AAA developed major post-operative complications (Table 12.3). Three patients with rupture and 6 with non-rupture had no post-operative complications. All patients survived to 24 hours after repair. Five (31.2%) patients with ruptured AAA died in hospital. There were no deaths after repair of non-ruptured AAA.

TABLE 12.2

Clinical and operative data in patients with ruptured and non-ruptured AAA.

	Ruptured AAA median (range) (n=16)	**Non-ruptured AAA** Median (range) (n=10)	**p value** *
Pre-operative			
Crystalloid administration (l)	0.5 (0.1 - 4.0)	-	-
Colloid administration (l)	0 (0 - 1.5)	-	-
Intra-operative			
Operation time (mins)	110 (70 - 250)	160 (85 - 285)	NS
Total aortic clamp time (mins)	75 (30 - 180)	75 (30 - 150)	NS
Measured blood loss (l)	3.1 (1.0 - 11.0)	2.4 (0.4 - 6.0)	NS
Crystalloid administration (l)	2.3 (0.5 - 6.0)	1.9 (1.0 - 4.0)	NS
Colloid administration (l)	1.5 (0 - 2.3)	1.5 (1.0 - 4.1)	NS
RCC administration (units) [2]	8 (5 - 22)	4 (0 - 10)	0.004
FFP administration (units) [3]	2 (0 - 12)	0 (0 - 2)	0.0003
Platelet administration (bags) [4]	1 (0 - 1)	0 (0 - 1)	0.0007

KEY: * = Mann-Whitney U test, [2] RCC = 300 ml, [3] FFP = 300ml, [4] one bag of platelet transfusion = 4 pooled units (250 ml)

TABLE 12.3

Post-operative complications and procedures in ruptured and non-ruptured AAA.

	Ruptured AAA (n= 13/16)	**Non-ruptured AAA** (n= 4/10)
Cardiovascular		
AF	5	1
CCF	5	2
MI	1	0
CVA	2	0
CLI	3	1
DVT	2	1
Respiratory		
Pneumonia	11	2
Respiratory failure	5	0
ARDS	1	0
Acute Renal Failure	6 [1]	1
Coagulopathy	2	0
Sepsis syndrome	3	0
Colon ischaemia	1	0
Total parenteral nutrition	5	0
Inotropic support		
Adrenaline	5	0
Renal dose dopamine	11	0
Re-operation	3 [2]	1 [3]
TOTAL	13 / 16 (81%)	4 / 10 (40%)

KEY: [1] = 3 of 6 patients who developed ARF received haemofiltration
[2] = patient 1 - laparotomy for haemorrhage, femoral thrombectomy, Hartmann's procedure for colon ischaemia, drainage of infected pelvic haematoma; patient 2 - negative laparotomy for suspected colon ischaemia; patient 3 - bilateral BKA for CLI; [3] = popliteal embolectomy and fasciotomies

Assays of sTNF-Rs and haemostatic markers

The median (range) values of sTNF-Rs p55 and p75 are shown in Table 12.4. The values for t-PA activity, PAI activity, PF 1+2 and vWF are shown in Table 12.5.

Both types of sTNF-R were detectable at one or more sampling points in all patients with ruptured AAA and 9 of 10 patients with non-ruptured AAA. Five minutes and 24 hours after aortic clamp release, levels of sTNF-R p55 were significantly higher in patients with ruptured compared with non-ruptured AAA. There was no significant change in the levels of sTNF-R p55 as a function of time in either group (Figure 12.1). At all sampling points, levels of sTNF-R p75 were significantly higher in patients with ruptured compared with non-ruptured AAA, and there was a significant increase in the levels of sTNF-R p75 during reperfusion in both groups of patients (Figure 12.2). Six hours after aortic clamp release, levels of sTNF-R p75 were significantly higher in non-survivors of ruptured AAA compared with survivors and patients operated for non-ruptured AAA (Figure 12.3). There was no significant difference in levels of sTNF-R p75 between non-survivors of rupture, survivors of rupture and patients operated for non-ruptured AAA at any other sampling point. There was also no correlation between aortic clamp time and sTNF-R levels in ruptured or non-ruptured AAA.

In patients operated for rupture, sTNF-R p55 levels immediately before ($p=0.015$) and 5 minutes after aortic clamp release ($p=0.034$) were significantly lower in six patients who subsequently developed ARF compared with 10 patients who did not. There was no significant difference in sTNF-R p75 levels among patients who did and did not have complications.

TABLE 12.4

sTNF-Rs p55 and p75 levels.

	Sample point	Ruptured AAA median (range) (n=16)	Non-ruptured AAA Median (range) (n=10)	p value *
sTNF-R p55	A	1.3 (0.2 - 4.9)	0.8 (0.2 - 1.4)	NS
	B	1.1 (0.2 - 2.8)	0.6 (0.2 - 1.1)	NS
	C	1.1 (0.2 - 3.5)	0.5 (0.2 - 1.2)	0.018
	D	1.5 (0.3 - 5.3)	0.7 (0.2 - 4.0)	NS
	E	1.6 (0.2 - 4.4)	0.8 (0.2 -2.1)	0.042
	Significant differences between samples **	None	None	
sTNF-R p75	A	4.0 (2.0 -14.7)	2.1 (2.0 - 9.0)	0.028
	B	3.3 (2.0 - 9.1)	2.0 (2.0 - 2.2)	0.0005
	C	3.2 (2.0 - 9.7)	2.0 (2.0 - 2.2)	0.0013
	D	10.0 (2.1 - 18.5)	2.5 (2.0 - 5.9)	0.0009
	E	11.1 (2.4 - 23.6)	2.6 (2.0 - 7.3)	0.0022
	Significant differences between samples **	B - D, B - E, C - D, C - E	B - D, B - E	< 0.05

KEY: * = Mann-Whitney U test, ** = Kruskall-Wallis test

TABLE 12.5

PF 1+2, t-PA activity, PAI activity and vWF.

Assay (normal range)	**Sample point**	**Ruptured AAA** median (range) (n=16)	**Non-ruptured AAA** Median (range) (n=10)	**p value** *
PF 1+2	A	6.7 (2.4 - 11.6)	1.9 (0.7 - 7.1)	< 0.001
(0.4-1.1 nmol/l)	B	6.3 (2.0 - 9.1)	1.3 (0.9 - 4.0)	< 0.001
	C	6.5 (2.6 - 11.4)	1.8 (1.0 - 4.9)	< 0.001
	D	3.6 (1.6 - 11.4)	1.9 (1.3 - 5.6)	NS
t-PA activity	A	0.24 (0.06 - 7.9)	0.53 (0.14 - 3.2)	NS
(0.2-2.0 IU/ml)	B	0.28 (0.08 - 6.4)	1.07 (0.34 - 4.65)	0.001
	C	0.32 (0.09 - 7.3)	1.91 (0.19 - 5.62)	0.018
	D	0.84 (0.15 - 2.3)	0.46 (0.21 - 1.45)	NS
PAI activity	A	35.2 (2.6 - 38.8)	6.7 (3.2 - 21.7)	0.001
(less than 15 AU/ml)	B	38.3 (8.6 - 39.4)	10.1 (2.8 - 38.9)	0.003
	C	37.5 (10.6 - 39.4)	12.3 (2.2 - 28.7)	0.001
	D	13.5 (5.0 - 35.3)	16.5 (5.7 - 22.4)	NS
vWF	A	1.9 (0.48 - 3.21)	1.4 (0.44 - 3.6)	NS
(0.42 - 1.22 IU/ml)	B	0.81 (0.14 - 2.12)	0.96 (0.3 - 3.06)	NS
	C	0.88 (0.21 - 1.7)	0.86 (0.29 - 2.75)	NS
	D	1.68 (0.8 - 3.12)	1.89 (1.5 - 3.99)	NS

KEY: * = Mann-Whitney U test

FIGURE 12.1

Individual data points for sTNF-R p55 immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C), 6 hours (sample D) and 24 hours (sample E) after aortic clamp release.

Patients with ruptured AAA are represented by the red squares and patients with non-ruptured AAA by the yellow circles.
The normal range for sTNF-R p55 (< 0.2 ng/ml) is shown by the horizontal line. NS = not significant

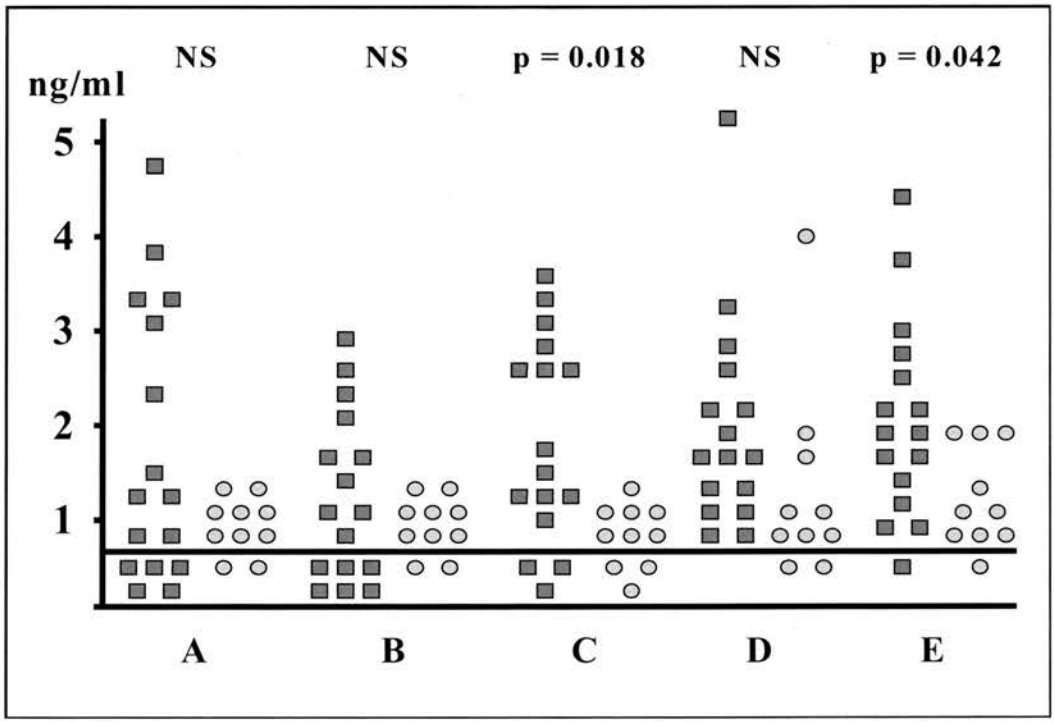


FIGURE 12.2

Individual data points for sTNF-R p75 immediately before induction of anaesthesia (sample A), immediately before release of the aortic clamp, (sample B), and five minutes (sample C), 6 hours (sample D) and 24 hours (sample E) after aortic clamp release.

Patients with ruptured AAA are represented by the red squares and patients with non-ruptured AAA by the yellow circles.
The normal range for sTNF-R p75 (< 2 ng/ml) is shown by the horizontal line.

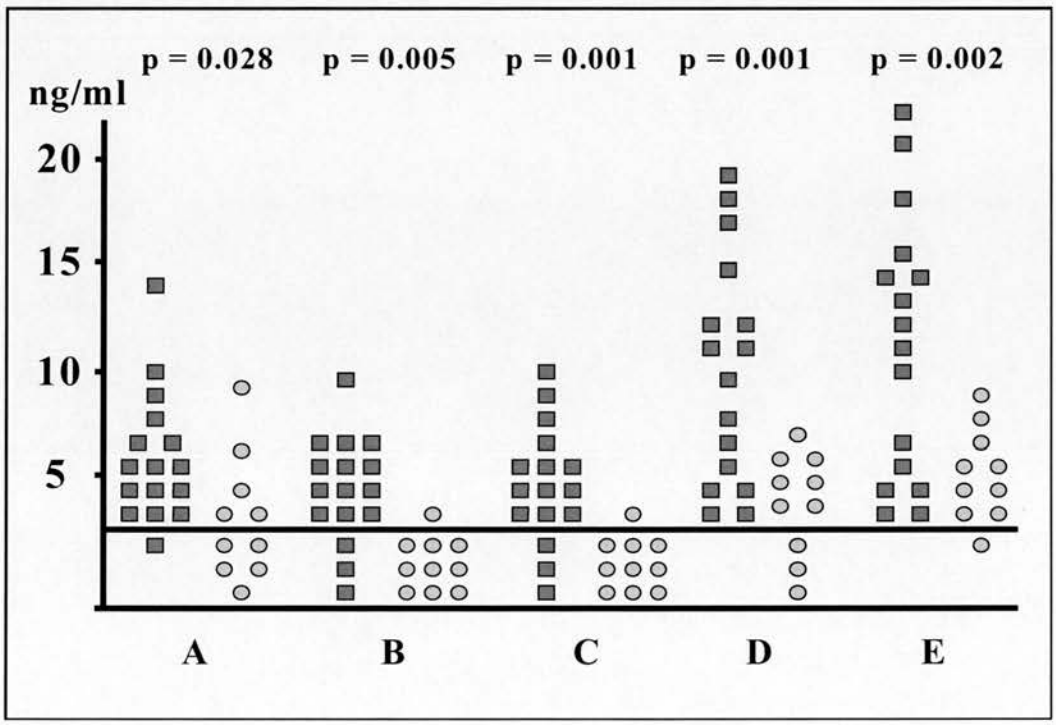
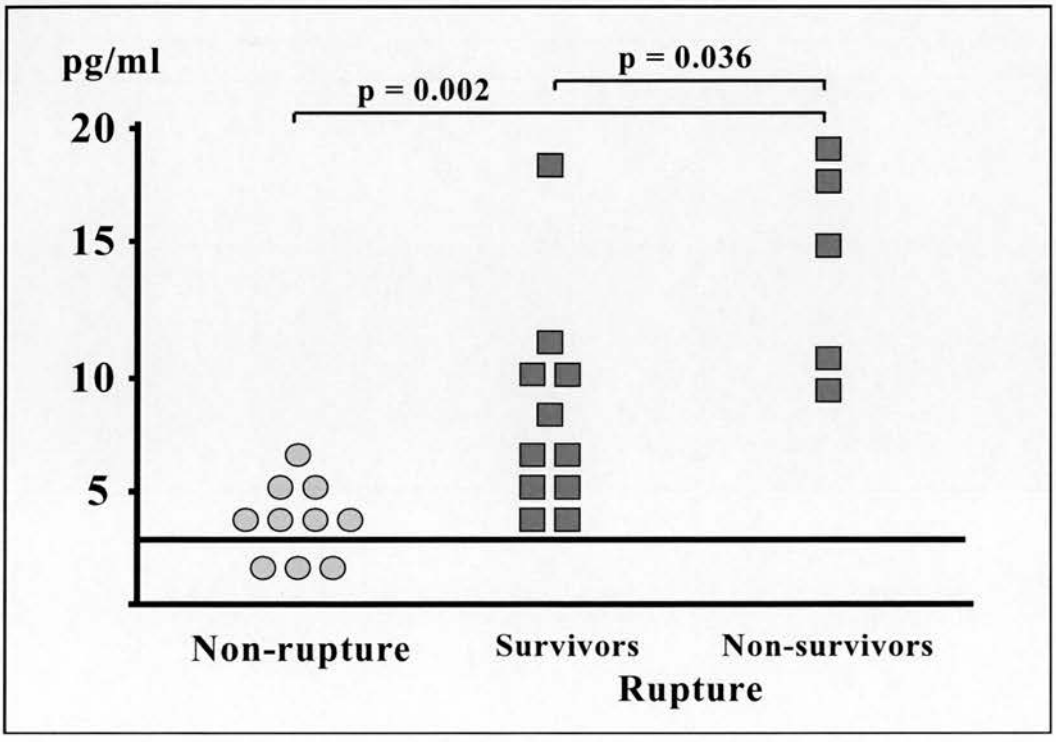


FIGURE 12.3

Individual data points for sTNF-R p75 at six hours after aortic clamp release in non-survivors of ruptured AAA compared with survivors and patients operated for non-ruptured AAA.

Patients with ruptured AAA are represented by the red squares and patients with non-ruptured AAA by the yellow circles.
The normal range for sTNF-R p75 (< 2 ng/ml) is shown by the horizontal line.



There were several significant correlations when haemostatic markers were compared with sTNF-R levels. When all patients were examined, immediately before aortic clamp release there was a significant negative correlation between sTNF-R p55 and t-PA activity (r= -0.535, p= 0.033) and a significant positive correlation between sTNF-R p55 and PAI activity (r= +0.552, p= 0.027). At 24 hours, there was a significant positive correlation between sTNF-R p75 and t-PA activity (r= +0.639, p= 0.008). Five minutes after aortic clamp release, there was a significant positive correlation between sTNF-R p75 and PF 1+2 (r= +0.505, p= 0.046). There was a significant positive correlation between sTNF-R p55 and vWF pre-operatively (r= +0.589, p= 0.016) and at 24 hours (r= +0.699, p= 0.003). There was also a significant positive correlation between sTNF-R p75 and vWF at 5 minutes after aortic clamp release (r= +0.575, p= 0.02). In survivors of rupture, there was a significant positive correlation between sTNF-R p55 and vWF at 24 hours (r= +0.691, p= 0.019). The number of patients who did not survive was too small to make any valid statistical analyses.

12.5 Discussion

The present study has demonstrated, for the first time, that haemorrhagic shock, lower torso ischaemia and early reperfusion occurring in the course of ruptured AAA repair is associated with elevated levels of sTNF-Rs p55 and p75, and that elevated levels of sTNF-R p75 during the period of early reperfusion are associated with increased post-operative mortality. Furthermore, sTNF-Rs appeared rapidly after the onset of haemorrhagic shock and lower torso ischaemia, a finding which may be more indicative of a direct relationship between sTNF-R levels and increased mortality (284) than in a previous study where sTNF-Rs p55 and p75 levels were measured daily for 5 days after operation (241). As circulating TNF is frequently difficult to demonstrate, studies reporting the time course of TNF release in response to lower extremity ischaemia and reperfusion are few and contradictory. Animal studies have either demonstrated no significant increase in TNF levels during ischaemia or reperfusion (163), a transient increase within minutes of reperfusion due to 'washout' from the lower extremities (164), or increased levels during ischaemia but not reperfusion (232). Human studies which have included a statistical analysis of the time course of TNF release have failed to demonstrate a significant increase during early reperfusion in elective aortic reconstruction (237-239). To date, two studies have examined sTNF-R release before and after aortic aneurysm repair: Froon and colleagues (241) measured sTNF-R p55 and p75 levels in 21 patients with ruptured and nine with non-ruptured AAA, and demonstrated significantly higher levels of both sTNF-Rs in shocked patients and non-survivors; Soong and colleagues (242) measured sTNF-R p55 levels in 11 patients with non-ruptured AAA, and demonstrated that levels were lower in four non-survivors but

that the post-operative increase was significantly greater at 48 hours when compared with survivors. The present study is the first, therefore, to examine sTNF-R release before, during and early after ruptured and non-ruptured AAA repair. Although there was no significant change in sTNF-R p55 levels as a function of time, there was a significant increase in sTNF-R p75 levels in both groups of patients at 6 and 24 hours after aortic clamp release.

In contrast to the findings of Froon *et al* (241), there was no significant positive association between serum creatinine and sTNF-R levels in the present study. Moreover, sTNF-R p55 levels before and after aortic clamp release were significantly lower in patients with rupture who later developed renal failure compared with those who did not. There was, however, no significant difference in sTNF-R levels among patients with rupture who did and did not have cardiovascular, respiratory or septic complications.

TNF has been shown to induce a procoagulant and hypofibrinolytic state by increasing TF expression, vWF release and PAI release while reducing t-PA release. Clotting tests from femoral and central venous blood have been shown to be similar suggesting that lower limb ischaemia does not play a direct role in the pathogenesis of haemostatic derangement (144). Hypoxia combined with reperfusion induces procoagulant activity in vascular endothelium (166) and this may occur indirectly through the synthesis and release of cytokines (52-54,72-75,77,82,83,150). There is evidence to suggest that TNF and IL-1 may be responsible for activation of the fibrinolytic system in both lower torso ischaemia and endotoxaemia (71,160). In the present study, only sTNF-R p75 was associated with increased thrombin generation during ruptured AAA repair, as demonstrated by elevated PF 1+2 levels. Both types of sTNF-R, however, were associated with increased vWF release in ruptured AAA repair. There was evidence of an

association between sTNF-R p55 and p75 and a hypofibrinolytic state before and during operation as demonstrated by a negative correlation with t-PA activity and a positive correlation with PAI activity. At 24 hours, however, sTNF-R p75 was associated with increased fibrinolysis, as demonstrated by a positive correlation with t-PA activity.

The association between elevated sTNF-R levels and increased mortality in patients operated for ruptured AAA is similar to that observed by other investigators in acute inflammatory conditions or severe injury (233-235). Elevated levels of sTNF-Rs may indicate that the endogenous pool of sTNF-Rs is replete (233), and this may partly explain the disappointing results achieved with exogenous sTNF-R p55 and p75 in patients with sepsis syndrome and septic shock (285). It is not possible to determine from the present study whether therapeutic intervention with exogenous sTNF-Rs would ameliorate the adverse effects of TNF in lower extremity ischaemia and ruptured AAA repair (162-164,241). However, a recent animal study of infrarenal aortic cross-clamping demonstrated a significant reduction in circulating TNF levels, nitric oxide production and subsequent lung injury when exogenous sTNF-R p55 was administered before aortic clamp placement (232). In the present study, endogenous sTNF-R levels were significantly lower in patients undergoing repair of non-ruptured AAA compared with those operated for ruptured AAA. One can speculate that exogenous sTNF-R therapy may ameliorate the adverse effects of TNF in elective aortic surgery where endogenous levels of sTNF-Rs are low, but may have limited efficacy in patients presenting with haemorrhagic shock before ruptured AAA repair (233), where the concentrations of sTNF-Rs observed in the present study would have been sufficient to effectively antagonise the effects of circulating TNF *in vitro* (286).

In conclusion, these data demonstrate that haemorrhagic shock, lower torso ischaemia and early reperfusion occurring in the course of ruptured AAA repair is associated with elevated levels of the sTNF-Rs, and that elevated levels of sTNF-R p75 during the period of early reperfusion are associated with increased mortality. Furthermore, in patients who survive to 24 hours, elevated levels of sTNF-Rs were associated with increased thrombin generation, increased vWF release and a hypofibrinolytic state before and during ruptured AAA repair. Finally, the fact that the endogenous pool of sTNF-Rs may be replete and elevated endogenous levels are associated with increased mortality suggests that sTNF-R therapy may have limited efficacy in patients with ruptured AAA.

12.6 Summary

- Ruptured AAA repair is associated with elevated levels of sTNF-Rs, and elevated levels of sTNF-R p75 during the period of early reperfusion are associated with increased post-operative mortality.
- There was no significant change in sTNF-R p55 levels as a function of time, but there was a significant increase in sTNF-R p75 levels in ruptured and non-ruptured AAA at 6 and 24 hours after aortic clamp release.
- sTNF-R p55 levels immediately before and after aortic clamp release were significantly lower in patients with rupture who later developed ARF.
- Before and during ruptured AAA repair, elevated sTNF-R levels were associated with increased PF 1+2, vWF and PAI activity and reduced t-PA activity in patients who survive to 24 hours.
- As the endogenous pool of sTNF-Rs may be replete and elevated endogenous levels are associated with increased mortality, exogenous sTNF-R administration may have limited efficacy in patients undergoing ruptured AAA repair.

Chapter 13

Summary

Haemostasis before and after aortic surgery has been the subject of many studies, but there are few detailed investigations of the peri-operative changes in the coagulation and fibrinolytic pathways in patients undergoing elective AAA repair, and to our knowledge no published reports in patients undergoing repair of ruptured AAA.

Thrombotic and haemorrhagic complications such as acute cardiac events, acute renal failure, respiratory failure, coagulopathy and haemorrhage, lower limb ischaemia, stroke and pulmonary embolism were confirmed as the major complications contributing to post-operative mortality in the present study. Despite advances in anaesthesia, surgical techniques, critical care, centralisation of vascular surgical services and a more aggressive surgical approach in recent years there was no improvement in the operative mortality rate or the overall community-based mortality for patients with ruptured AAA in past two decades.

Ruptured AAA repair was shown to be associated with intense peri-operative thrombin generation, increased secondary 'physiological' fibrinolysis and inhibition of systemic fibrinolysis in patients who survived to at least 24 hours after surgery. Prolonged duration of symptoms of rupture were associated with increased thrombin generation which suggests that those patients who mount an increased thrombin response may be more likely to survive long enough to reach hospital. The finding that haemostatic markers were not significantly different between normotensive and hypotensive patients with rupture suggests that haemorrhage, rather than hypotension, may be the principal mechanism which initiates this procoagulant state. Increased operative blood loss was associated with reduced fibrinogen and platelet count, and prolonged clotting times while prolonged aortic clamp time was associated with a fall platelet count early after declamping. In patients with ruptured AAA complicated by peri-operative coagulopathic

haemorrhage, there was also evidence of increased thrombin generation but, unlike those who survived to 24 hours post-operatively, increased systemic fibrinolysis was detectable on admission and/or before aortic declamping in a proportion of patients and this was associated with an extremely poor prognosis. From these findings, patients with ruptured AAA may be classified into two categories according to the pattern of haemostatic derangement. Those who have 'compensated' shock may exhibit thrombin generation and inhibition of systemic fibrinolysis and are likely to survive to at least 24 hours post-operatively. By contrast, those with 'decompensated' shock may exhibit thrombin generation and increased systemic fibrinolysis and are very likely to die intra-operatively or in the immediate post-operative period from coagulopathy.

The present study confirmed increased thrombin generation in the majority of patients undergoing elective repair of non-ruptured AAA. A proportion of patients were also shown to have evidence of increased systemic fibrinolysis during the period of lower torso ischaemia and early reperfusion. Increased operative blood loss was not only associated with reduced fibrinogen and platelet count, prolonged clotting times but also inhibition of systemic fibrinolysis. The relationship between prolonged aortic clamp time and a fall in fibrinogen and platelet count and prolonged of clotting times was confirmed and, for the first time, prolonged clamp time was shown to be associated with increased inhibition of systemic fibrinolysis.

For the first time, haemostasis was examined in a small cohort of patients presenting as an emergency with acutely symptomatic non-ruptured AAA. These patients exhibited reduced thrombin generation, reduced secondary fibrinolysis and increased systemic fibrinolysis compared with patients with rupture, and increased systemic fibrinolysis compared with patients with asymptomatic AAA. There were significant differences in

the markers of coagulation and fibrinolysis between acutely symptomatic patients and normotensive patients with rupture, suggesting that haemorrhage rather than hypotension is responsible for the haemostatic changes.

A hypothesis for the findings in patients with acutely symptomatic and ruptured AAA can be formulated. Impending AAA rupture may be analogous to an aortic dissection which explains the finding of increased systemic fibrinolysis. When actual AAA rupture occurs, haemorrhage triggers the procoagulant and hypofibrinolytic state which is both a homeostatic and pathological response; it acts to minimise local blood loss and allows the patient to survive the initial rupture event and operative repair, but also triggers micro- and macrovascular thrombosis and contributes to the development of major post-operative complications. By contrast, in patients with severe 'decompensated' shock, there is a net increase in systemic fibrinolytic activity which appears to be a pathological response as it is associated with early mortality from coagulopathy and haemorrhage.

The majority of patients with ruptured *and* non-ruptured AAA had elevated peri-operative vWF and sTM levels consistent with endothelial cell activation. These findings contrast with those from a previous study which demonstrated changes in endothelial cell morphology consistent with endothelial cell activation in ruptured, but not non-ruptured, AAA. It is possible that examining changes in endothelial cell morphology is too insensitive to assess endothelial cell activation in this group of patients. There was a significant positive association between peri-operative vWF levels and platelet count in patients undergoing AAA repair and the intra-operative fall in vWF and platelet count were exacerbated by increased operative blood loss and prolonged aortic clamp time. One can speculate that endothelial cell activation and injury leads to vWF release which binds platelets and initiates the formation of occlusive thrombi at sites of endothelial

injury. The resultant consumption of vWF and platelets secondary to macro- and microvascular thrombus formation leads to a fall in their circulating levels. This may explain the relationship between peri-operative thrombocytopenia and poor outcome previously demonstrated in AAA repair.

The hypothesis that haemostatic derangement would contribute to morbidity and mortality from haemorrhagic and thrombotic events in ruptured AAA surgery was confirmed. Life-threatening intra-operative coagulopathy was associated with increased systemic fibrinolysis. By contrast, major post-operative cardiac events as determined by cTn I levels were associated with peri-operative inhibition of systemic fibrinolysis which is consistent with previous studies demonstrating a relationship between myocardial injury and a procoagulant state. The fact that some patients with elevated PAI activity had no myocardial injury and pre-existing cardiac disease, intra-operative hypotension, operative blood loss and aortic clamp time had no demonstrable effect on cTn I levels all indicate that the procoagulant state is only one of many contributory factors in the pathophysiology of peri-operative myocardial injury.

Patients who died from organ dysfunction after ruptured AAA repair had significantly lower peri-operative ET levels compared with survivors. Survivors had increased plasma ET levels during the periods of lower torso ischaemia and reperfusion, and over half had increased levels pre-operatively. The elevated peri-operative ET levels present in survivors were associated with reduced thrombin generation. These findings do not support the hypothesis that an increased ET response has a pathological role in critical illness and predisposes to poor outcome in patients undergoing ruptured AAA repair. The ET-dependent vasoconstrictor response in survivors may occur as part of a homeostatic and protective response to haemorrhage, ischaemia and reperfusion and the

low ET levels in non-survivors may be an early manifestation of severe and irreversible whole body hypoperfusion.

Ischaemia and reperfusion induce procoagulant activity in endothelial cells and this may occur indirectly through the synthesis and release of cytokines such as TNF. As circulating TNF is frequently difficult to demonstrate, the levels of sTNF-Rs were examined. Ruptured AAA repair was associated with elevated levels of sTNF-Rs p55 and p75. Elevated levels of sTNF-R p75 during early reperfusion were associated with increased mortality in ruptured AAA and similar findings have been observed in other acute inflammatory conditions and severe injury.

In ruptured AAA, there was a positive association between increased sTNF-R levels and a procoagulant state as demonstrated by increased thrombin generation and vWF release and inhibition of systemic fibrinolysis. One can speculate from these data that sTNF-Rs have an important stimulatory role in the pathophysiology of haemostatic derangement observed in ruptured AAA.

The findings of this thesis suggest that further study is required to elucidate the triggering mechanisms for the deranged haemostasis evident in patients with AAA. TF expression is the stimulus for thrombin generation and has also been reported to be associated with reduced fibrinolysis. In patients with asymptomatic AAA, no relationship has been demonstrated between pre-operative TF levels and other markers of coagulation and fibrinolysis but further investigation is required. The finding of reduced endothelin levels in non-survivors of ruptured AAA was unexpected but may be secondary to increased NO synthesis. Currently, there are no reports of the NO response to ruptured AAA repair.

The findings of this thesis also suggest that further investigation is required into the effect of therapeutic intervention on the haemostatic abnormalities which occur in ruptured AAA repair. Current data do not support the routine use of antifibrinolytic therapy in ruptured AAA repair, but suggest that peri-operative administration of these agents as well as of FFP, cryoprecipitate and platelets may prove beneficial if targeted at the selected group of patients with a hyperfibrinolytic state. The measurement of peri-operative fibrinolytic function may help to distinguish patients with hypofibrinolysis from those with hyperfibrinolysis and further studies are required to assess whether TEG is of clinical value in this situation.

Cardiac events are the commonest cause of poor outcome in AAA surgery. A large prospective study of prevention, diagnosis and management of myocardial injury in aortic surgery would be valuable. Screening for myocardial injury using cTn I may be of value if a) it predicts post-operative cardiac events and allows these to be treated prophylactically, and b) allows for peri-operative therapeutic intervention either indirectly by correcting the procoagulant and hypofibrinolytic state or directly by emergency percutaneous transluminal coronary angioplasty. Modification of the hypofibrinolytic state is difficult as currently there are few therapeutic options. In elective AAA repair, systemic heparinisation has been shown to be associated with a significant reduction in the incidence of fatal and non-fatal MI. As ruptured AAA is associated with increased thrombin generation, it is possible that judicious use of systemically administered heparin following aortic clamping may partly reverse the procoagulant state and improve outcome.

TABLE 13.1

Clinical correlations in patients operated for ruptured AAA.

		r value	p value
Duration of symptoms	PF 1+2 before operation	+ 0.717	0.02
Operative blood loss	Platelet count at 5 mins post-declamping	-0.569	0.027
Operative blood loss	Fibrinogen pre-declamping	- 0.694	0.026
Operative blood loss	Fibrinogen at 5 mins post-declamping	- 0.75	0.012
Operative blood loss	Platelet count at 5 mins post-declamping	- 0.726	0.018
Operative blood loss	PT pre-declamping	+ 0.823	0.003
Operative blood loss	aPTT pre-declamping	+ 0.787	0.007
Operative blood loss	aPTT at 5 mins post-declamping	+ 0.64	0.046
Operative blood loss	vWF at 5 mins post-declamping	- 0.526	0.03
Aortic clamp time	Platelet count at 5 mins post-declamping	- 0.574	0.02
vWF pre-op.	sTM pre-op.	+ 0.492	0.032
vWF pre-op.	Platelet count pre-op.	+ 0.477	0.033
vWF pre-declamping	Platelet count pre-declamping	+ 0.467	0.044
vWF at 5 mins post-declamping	Platelet count at 5 mins post-declamping	+ 0.495	0.043
PAI activity pre-declamping	cTn I at 6 hrs post-declamping	+ 0.829	0.042
PAI activity pre-declamping	cTn I at 24 hrs post-declamping	+ 0.762	0.028
PAI activity pre-declamping	cTn I at 48 hrs post-declamping	+ 0.829	0.042
PAI activity at 5 mins post-declamping	cTn I at 6 hrs post-declamping	+ 0.943	0.005
PAI activity at 5 mins post-declamping	cTn I at 24 hrs after declamping	+ 0.881	0.004
PF 1+2 pre-declamping	Big ET-1 at 5 mins post-declamping	- 0.554	0.044
PF 1+2 pre-declamping	Big ET-1 at 5 mins post-declamping	- 0.695	0.038
PF 1+2 at 5 mins post-declamping	Big ET-1 at 5 mins post-declamping	- 0.678	0.045
sTNF-R p55 pre-op.	vWF pre-op.	+ 0.589	0.016
sTNF-R p55 pre-declamping	t-PA activity pre-declamping	- 0.535	0.033
sTNF-R p55 pre-declamping	PAI activity pre-declamping	+ 0.552	0.027
sTNF-R p55 at 24 hrs post-declamping	vWF at 24 hrs post-declamping	+ 0.699	0.003
sTNF-R p75 at 5 mins post-declamping	vWF at 5 mins post-declamping	+ 0.575	0.02
sTNF-R p75 at 5 mins post-declamping	PF 1+2 at 5 mins post-declamping	+ 0.505	0.046
sTNF-R p75 at 24 hrs post-declamping	t-PA activity at 24 hrs post-declamping	+ 0.639	0.008

TABLE 13.2

Clinical correlations in patients operated for non-ruptured AAA.

		r value	p value
Operative blood loss	Fibrinogen pre-declamping	- 0.678	0.045
Operative blood loss	Fibrinogen at 5 mins post-declamping	- 0.711	0.032
Operative blood loss	Platelet count pre-declamping	- 0.728	0.026
Operative blood loss	PT at 5 mins post-declamping	+ 0.728	0.026
Operative blood loss	t-PA activity at 5 mins post-declamping	- 0.753	0.019
Aortic clamp time	Fibrinogen pre-declamping	- 0.812	0.008
Aortic clamp time	Fibrinogen at 5 mins post-declamping	- 0.711	0.032
Aortic clamp time	Platelet count pre-declamping	- 0.678	0.045
Aortic clamp time	PT pre-declamping	+ 0.72	0.029
Aortic clamp time	PT at 5 mins post-declamping	+ 0.828	0.006
Aortic clamp time	t-PA activity pre-declamping	- 0.837	0.005
Aortic clamp time	t-PA activity at 5 mins post-declamping	- 0.72	0.029
Aortic clamp time	PAI activity at 5 mins post-declamping	+ 0.686	0.041
Aortic clamp time	vWF at 5 mins post-declamping	- 0.632	0.05
vWF pre-op.	Haematocrit pre-op.	+ 0.721	0.019
vWF pre-op.	Platelet count pre-op.	+ 0.794	0.006
vWF pre-declamping	Platelet count pre-declamping	+ 0.648	0.043
vWF at 5 mins post-declamping	Platelet count at 5 mins post-declamping	+ 0.634	0.048

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Publications, presentations and abstracts

Publications

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Association of Surgeons of Great Britain and Ireland, Birmingham, 2001.

Community and hospital outcome from ruptured abdominal aortic aneurysm within the catchment area of a regional vascular surgical service

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Objective: The objective of this study was to examine patterns of referral, management, and outcome of patients with ruptured abdominal aortic aneurysm (RAAA) within the catchment area of this regional vascular unit (RVU).
Methods: Referral, management, and outcome data regarding 972 consecutive patients admitted to the hospital or certified deceased in the community because of RAAA between January 1, 1989, and December 31, 1995, were retrieved from prospectively gathered computerized national and local databases.
Results: Of 381 (39.2%) patients admitted to this unit, 316 (82.9%) underwent surgery, and of those, 188 (59.5%) survived. There was no significant difference in overall mortality between patients who were admitted directly to this unit (152 of 310, 49%) and those who were transferred from elsewhere (41 of 71, 58%). Surgical patients traveled significantly farther to the RVU than nonsurgical patients ($P < .001$), but there was no significant difference in traveling distance between surgical patients who survived and those who did not. Of 372 (38%) patients who were admitted to other units and not transferred, 24 (6.4%) underwent surgery and 14 (3.8%) survived. Of 972 patients, the overall community mortality from RAAA was 770 (79%).
Conclusion: Transferring patients from outlying units did not appear to prejudice operative outcome in this RVU. However, less than half of all RAAA patients were transferred, and only a small minority of those not transferred underwent surgery. Although the overall community mortality from RAAA was similar to that reported in earlier studies from other regions and countries where centralization has not occurred, centralization of vascular surgical services may be associated with an inappropriately low operation and survival rate for those patients who are not transferred to the regional center. The effect of centralization on the community outcome of emergent vascular surgical conditions requires further investigation. (J Vasc Surg 1999;30:922-8.)

It is widely believed that surgical and anesthetic subspecialization leads to better patient outcomes. Vascular surgery is no exception, with the reduction in operative mortality associated with ruptured abdominal aortic aneurysm (RAAA) and the improving results of carotid endarterectomy being cited as evidence of the benefits of special expertise.[1,2] As a result of these clinical data as well as other clinical, political, and economic factors, there is an increasing tendency in the United Kingdom to centralize vascular surgical services within regional vascular units serving populations of over 500,000.[3] Since 1983, the Edinburgh Regional Vascular Surgery Unit (ERVSU) has been the sole provider of 24-hour, 365-day vascular surgical services for a population of 1.2 million living in an area of approximately 4,500 square miles in southeast Scotland (Fig 1). However, to concentrate vascular expertise within a small number of individuals at limited geographical sites inevitably leads to a depletion, or even

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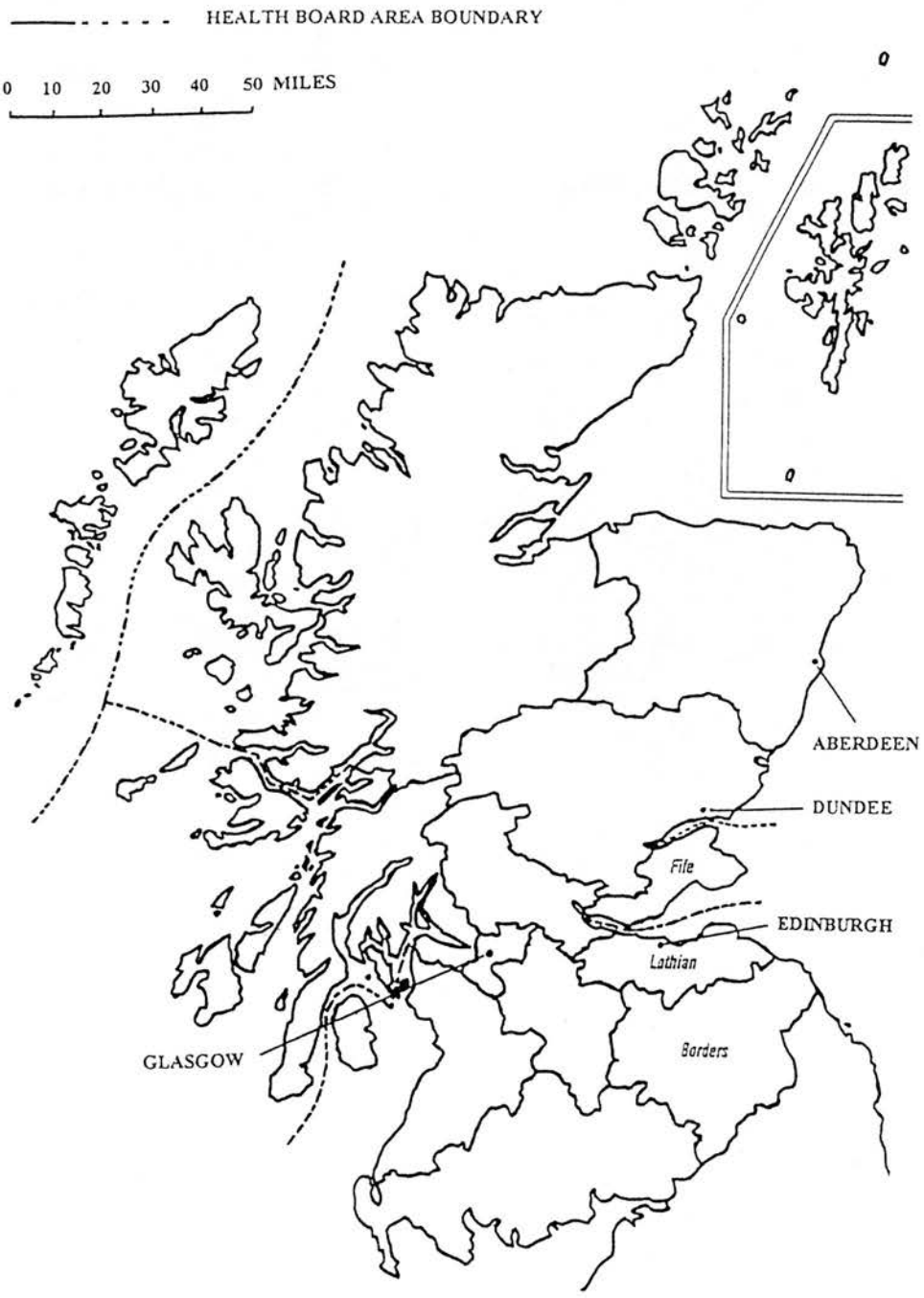

Fig 1. Catchment area of the Edinburgh Regional Vascular Surgery Unit: Lothian, Borders, and Fife Health Board regions.

an absence, of expertise elsewhere. For example, of nine hospitals conducting "general" surgery in this catchment area, only this and one peripheral unit are staffed by vascular surgeons. In these circumstances, ensuring equality of access to specialist care may become increasingly difficult.[4] Although specialization may improve individual patient outcomes for specific procedures performed within specialist units, it is equally important to demonstrate that centralization does not prejudice the overall community-based outcome for the underlying condition. In contrast to North America, where a minority of AAA operations are performed for rupture, this patient group in the ERVSU accounts for no fewer than

45% of all the AAA repairs performed.[5] Although reasons for this are complex, it is clear that there is a low public awareness of the condition and a low index of suspicion among physicians such that only a minority of patients initially seen with RAAA have previously been diagnosed as having an aortic aneurysm.[6] Therefore, RAAA is a major problem in the United Kingdom, and decisions on how vascular services are to be distributed must be based, at least in part, on consideration of how these unstable, high-risk patients are most appropriately managed. The aim of this study, therefore, was to examine for the first time the patterns of referral, management, and outcome of patients identified as having RAAA within the catchment area served by this regional vascular unit.

METHODS

This prospective study was conducted between January 1, 1989, and December 31, 1995 (the most recent year for which complete population data are available). All residents of the catchment area of the ERVSU—who were admitted to any hospital in the catchment area with a diagnosis of RAAA (International Classification of Diseases ninth revision codes 441.3, 441.5, and 441.1, if coded in addition to 441.3 or 441.5), or who were certified deceased as a result of RAAA, either in the hospital or in the community—were identified through the Information and Statistics Division of the National Health Service in Scotland using the Scottish Morbidity Records 1 (hospital discharge records) and General Registrar Office (Scotland) mortality records. Scottish Morbidity Records 1 are linked to each other, and to the General Registrar Office (Scotland) mortality records by the Information and Statistics Division using probability matching,[7] and they provide a patient database that includes hospital admission and mortality data. The Lothian Surgical Audit database was used to identify all residents from the catchment area who were admitted to the ERVSU with RAAA.[8] Residents of the catchment area who were admitted to hospitals outside the catchment area (n = 32) and residents of other catchment areas who were admitted to hospitals within the area (n = 20) were excluded from analysis. Patterns of referral and management, as well as outcome data and post-codes (equivalent to ZIP codes), were retrieved for each patient.

It was not possible, in most instances, to ascertain the patient's precise location at the time of rupture, so it was assumed that rupture had occurred near the home address rather than at a distant site. Travel distance for patients admitted directly to the ERVSU was, therefore, defined as the distance by land from the center of the individual's post-code region of residence to this unit. For those admitted indirectly, travel distance was defined as the distance from the center of the individual's post-code region of residence to the referring hospital and then to this unit. All patients in the study were transferred by land ambulance. In Scotland, air ambulance is not routinely available for transfer of patients with RAAA.

There is no written protocol regarding the selection of patients to be admitted to the ERVSU because we believe that no written guideline can satisfactorily cover all eventualities. Rather, we encourage medical and surgical colleagues to discuss the patient's condition by telephone so that each patient is considered individually. When the condition of a patient with rupture is discussed with the referring doctor, he or she is advised against any prehospital fluid resuscitation; this may lead to an increase in blood pressure and contribute to the development of coagulopathy and fatal aortic hemorrhage before the patient reaches the operating room and the aorta is clamped. The final decision to admit, transfer, or operate on a patient is made by the on-call consultant vascular surgeon, based on this unit's considerable experience in managing patients with RAAA.[9] The Mann-Whitney (MW) test, χ^2 test, and Fisher's exact test were used for statistical analysis.

A probability value of less than .05 was regarded as statistically significant.

RESULTS

The patterns of referral, management, and outcome for 972 patients who were identified as having RAAA during the 7-year study period are shown in Fig 2. Two hundred nineteen (22.5%) patients were certified dead in the community without being admitted to the hospital, 551 (56.7%) were certified dead in the hospital, and 202 (20.8%) survived. Therefore, the community mortality for RAAA was 79% (770 of 972 patients). The diagnosis was confirmed at operation in 340 (35%) patients and at postmortem examination in 268 (28%). Of the latter, 175 died in the community and 93 died in the hospital without transfer to the ERVSU. In the remaining 364 patients, RAAA was diagnosed and recorded on the death certificate on the basis of clinical examination or investigation.

Three hundred seventy-two (38%) patients were admitted to other units within or outside Edinburgh and were not transferred to the ERVSU. Of these, 24 (6.4%) patients underwent operation and 14

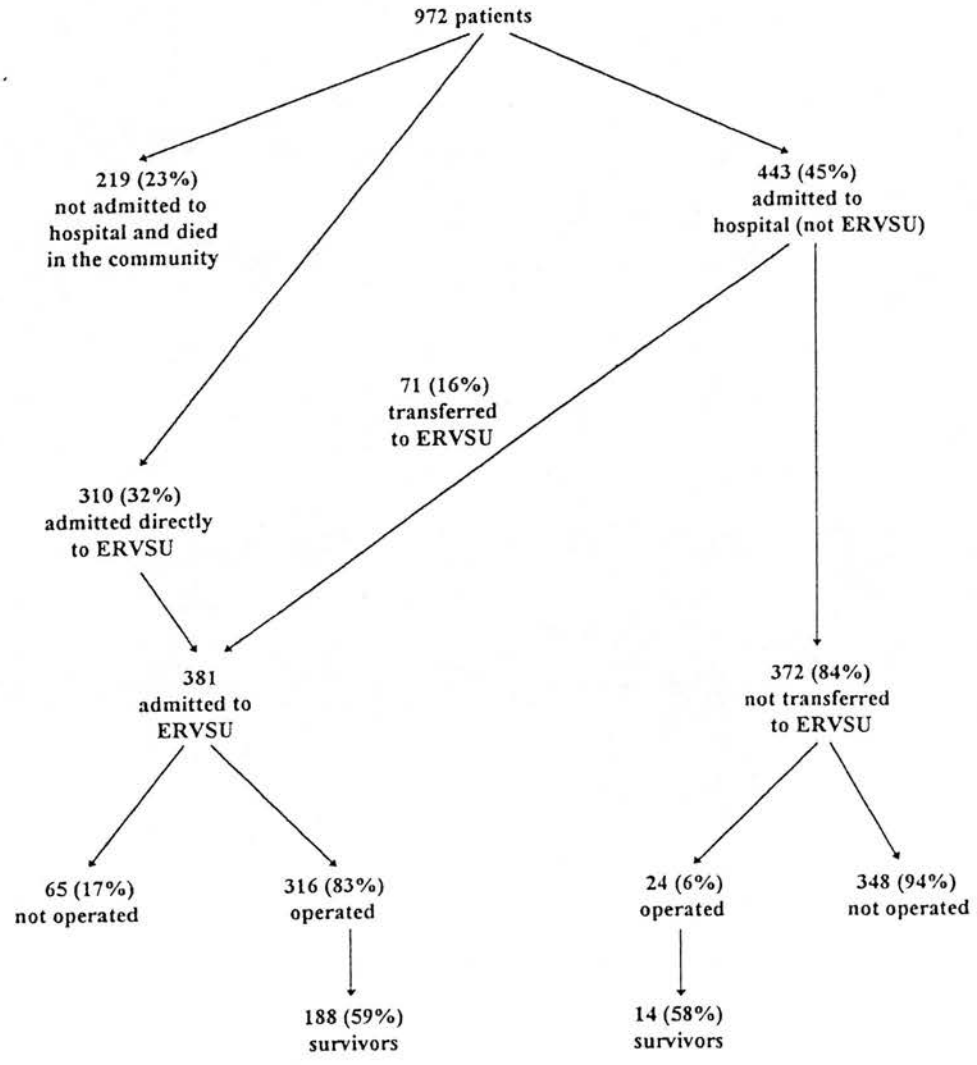

Fig 2. Management of 972 patients diagnosed as ruptured abdominal aortic aneurysm in the catchment area of the Edinburgh Regional Vascular Surgery Unit (ERVSU).

(3.8%) survived. For this group of patients, no data are available to explain the decisions to transfer, perform surgery, or treat conservatively. Three hundred eighty-one (39%) patients (304 men, 77 women; median age, 73 years; range, 46 to 93 years) were admitted to the ERVSU. Of these, 65 (17%) patients did not undergo surgery because they were considered unfit for surgery on the basis of severe co-morbidity (ischemic heart disease, stroke, dementia, renal failure, and carcinoma) or extreme age, their clinical condition had deteriorated such that they were considered unfit for repair (unrecordable blood pressure or loss of consciousness), a decision to operate was made but death occurred before surgery could commence, or the offer of operation was declined.[5] Of 316 (83%) patients who underwent surgery, a graft was inserted in 277 (88%). The overall mortality for all patients admitted to the ERVSU was 193 of 381 (51%), and the operative mortality was 128 of 316 (41%). There was no significant difference in the overall mortality among patients transferred from units outside Edinburgh (25 of 43, 58%), those transferred from units within Edinburgh (16 of 28, 57%), and those admitted directly to the ERVSU (152 of 310, 49%) ($P = .41$, χ^2 test). Overall, 316 surgical patients traveled significantly farther than 65 nonsurgical patients ($P < .001$, MW). There was no significant difference in traveling distance between 188 (59%) surgical patients who survived and the 128 who did not (Table I). Of

Table I. Transfer distance and outcome in 381 patients with ruptured abdominal aortic aneurysm admitted (directly and indirectly) to the Edinburgh Regional Vascular Surgery Unit

Distance traveled (miles)	*No of patients*	*Not operated*	*Operated, graft inserted*	*Operated, no graft inserted*	*Operative mortality (%)*	*Overall mortality (%)*
0 - 5	152	41	95	16	44/111 (40)	85/152 (56)
5 - 10	74	9	58	7	26/65 (40)	35/74 (47)
10 - 15	48	9	34	5	19/39 (49)	28/48 (58)
15 - 20	44	3	39	2	14/41 (34)	17/44 (39)
20 - 25	32	1	27	4	14/31 (45)	15/32 (47)
25+	31	2	24	5	11/29 (38)	13/31 (42)
	381	65	277	39	128/316 (41)	193/381 (51)

310 patients who were admitted directly to the ERVSU, 262 who underwent surgery traveled significantly farther than 48 who did not have surgery ($P < .001$, MW), and there was no significant difference in traveling distance between 160 surgical patients who survived and 102 (40%) who did not.

DISCUSSION

This is the first study to describe the management of patients with RAAA within a single region of the United Kingdom, and to describe the relationship of traveling distance, surgical intervention rates, and outcome. The first principal finding was that there was no significant difference in traveling distance between the operated patients who survived and those who did not; however, patients who did not undergo surgery traveled significantly shorter distances to the hospital than those who did have surgery. One explanation for this may be the preselection of "good-risk" patients for transfer over longer distances. In addition, a proportion of patients sustaining rupture in the immediate vicinity of the ERVSU may have been moribund on arrival and thus did not undergo surgery.

Several studies have attempted to determine whether traveling distance and transfer time has an effect on operative mortality in ruptured aortic aneurysm. Butler and colleagues[9] showed no significant difference in operative mortality between patients admitted from the local catchment area (28 of 48, 58%) and those transferred from other centers (13 of 24, 54%). In 183 patients, Fielding et al[10] reported no significant difference in operative mortality between those transferred less than 5 miles (43 of 85, 50.5%) and those transferred farther than 5 miles (39 of 97, 40.2%). Similarly, Barros D'Sa[11] demonstrated no significant correlation between traveling distance and outcome in 187 surgical patients. Although Yashar et al[12] reported a mortality rate of 27% for patients undergoing surgery within 4 hours of onset of symptoms compared with 80% for those undergoing surgery beyond 4 hours, van Heeckeren[13] was unable to demonstrate a significant correlation between duration of symptoms and mortality in 57 surgical patients, and Amundsen et al[14] failed to demonstrate any correlation between transport time and overall mortality for 114 patients (including 30 who did not undergo surgery). Meyer and colleagues[15] compared 48 patients admitted to a community hospital and 49 admitted to a municipal hospital. They demonstrated that while significantly more patients in stable condition underwent surgery more than 2 hours after diagnosis in the community hospital, significantly more patients who were shocked underwent immediate operation in the municipal hospital, and consequently mortality was significantly higher. However, Ouriel and colleagues[16] demonstrated no significant difference in the delay from the onset of symptoms to hospital arrival for patients admitted to a university or community facility, and they found no significant relationship between operative mortality and the delay from hospital arrival to the start of the operation. In a study of 122 patients, Farooq et al[17] also demonstrated no relationship between operative mortality and duration of symptoms and delay between hospital arrival and the start of the operation. Although more hypotensive patients were operated on within 2 hours of onset of symptoms, this was not associated with a significant increase in mortality.

At first sight, these and present data suggest that centralization does not prejudice the community outcome for RAAA. However, in this 7-year study, 93% of survivors of RAAA were operated on in the ERVSU, fewer than 40% were transferred to this regional vascular unit, and only 6% of those treated outside this unit underwent operation. The operative mortality outside the ERVSU was a very acceptable 10 of 24 (42%). However, almost all of these

Table II. Reported studies estimating the community outcome from ruptured abdominal aortic aneurysm

Author	*Total number*	*Died outside hospital (%)*	*Died in hospital*		*Survivors (%)*
			not operated	*operated*	
Armour,[18] 1977	25	11 (44)	9	1	4 (16)
Ingoldby,[19] 1986	260	158 (61)	1	49	52 (20)
Johansson,[20] 1986	88	24 (27)	51	8	5 (6)
Mealy,[21] 1988	265	169 (64)	18	48	30 (11)
Thomas,[22] 1988	183	64 (35)	44	41	34 (19)
Semmens,[23] 1998	873	379 (43)	211	102	181 (21)
Present study	972	219 (23)	413	138	202 (21)

operations were performed in one peripheral hospital by two general surgeons with a major vascular interest. None of the other seven hospitals were staffed by surgeons with vascular expertise, which presumably explains the low operation rate outside the ERVSU and the other peripheral hospital.

The present study and, indeed, all community studies of RAAA have limitations. The diagnosis of RAAA was confirmed by operation or postmortem examination in only 63% of patients. It is not known what proportion of patients who were not operated on were diagnosed as having RAAA in life. It is likely that there were patients who died suddenly from RAAA in whom the diagnosis was not made, and perhaps there were a few who did not die from rupture but in whom this was the certified cause of death.

The important question raised by these data is whether a broader provision of vascular surgical expertise would have increased the proportion of patients offered and surviving surgery and whether this, in turn, would have had a positive impact on the community survival from the condition. Although the community outcome from RAAA in this series is similar to that reported in earlier studies from regions where centralization has not occurred (Table II), centralization of vascular surgical services may be associated with an inappropriately low operation and survival rate for the majority of patients who are not transferred to the regional center.

The reviewers have specifically requested that the authors discuss whether "rationing" of health care resources in the United Kingdom may explain what they describe as the "excessive mortality" observed in this study. This is a complex issue. However, the United Kingdom spends significantly less money on health education and care than North America and many European countries, and this may have a negative impact on the mortality from AAA in several ways. First, there is low public and physician awareness of the condition such that only a small proportion of patients with AAA are diagnosed and treated before the onset of life-threatening complications. Second, the absence of "round-the-clock" vascular surgical expertise in the majority of "local" hospitals means that, for most patients, the only prospect of survival lies in transfer to a regional center. Third, suboptimal transport of critically ill patients and a lack of intensive therapy beds may be relevant to the outcome for patients with AAA and many other patient groups. Present data indicate that the effect of these factors and the centralization of vascular surgical services on the community outcome of this and other emergent vascular surgical conditions requires further investigation.

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Coagulation and fibrinolysis in patients undergoing operation for ruptured and nonruptured infrarenal abdominal aortic aneurysms

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Purpose: Hemorrhage and thrombosis predisposing to myocardial infarction, multiple organ failure, and thromboembolism account for the majority of the morbidity and mortality associated with repair of ruptured and nonruptured abdominal aortic aneurysms (AAAs). The aim of this study was to examine coagulation and fibrinolysis in patients operated on for ruptured and nonruptured infrarenal AAAs.

Methods: Ten patients operated on for ruptured and 9 patients operated on for nonruptured AAAs were studied. Tissue plasminogen activator (t-PA) antigen, thrombin-antithrombin (TAT), and D-dimer were measured before induction of anesthesia. Plasminogen activator inhibitor (PAI) activity, t-PA activity, and prothrombin fragment (PF) 1+2 were measured before induction of anesthesia, immediately before aortic clamp release, and 5 minutes and 24 hours after aortic clamp release.

Results: Preoperatively, ruptured AAA was associated with significantly elevated t-PA antigen (median 15.7 ng/mL, range 9.0 to 22.1 ng/mL versus nonrupture: median 6.6 ng/mL, range 4.7 to 16.4 ng/mL; $P < .01$, Mann-Whitney test), increased PAI activity (median 36.5 arbitrary units/mL, range 20.6 to 38.8 arbitrary units/mL versus nonrupture: median 8.2 arbitrary units/mL, range 3.2 to 21.7 arbitrary units/mL; $P < .001$), reduced t-PA activity (median 0.12 IU/mL, range 0.06 to 0.4 IU/mL versus nonrupture: median 0.49 IU/mL, range 0.14 to 3.2 IU/mL; $P < .01$), elevated TAT (median 135.5 μg/L, range 61.2 to 209.4 μg/L versus nonrupture: median 21.6 μg/L, range 6.6 to 180.4 μg/L; $P < .02$) and elevated PF 1+2 (median 9.0 nmol/L, range 5.4 to 11.6 nmol/L versus nonrupture: median 2.2 nmol/L, range 0.7 to 7.1 nmol/L, $P < .001$). There was no significant difference in preoperative D-dimer levels (median 3460 ng/mL, range 1236 to 7860 ng/mL versus nonrupture: median 1642 ng/mL, range 728 to 5334 ng/mL; $P = .07$). The differences in PAI activity, t-PA activity, and PF 1+2 persisted throughout the course of surgery, but there was no significant difference between the groups at 24 hours.

Conclusion: These novel data demonstrate that ruptured AAA repair is associated with inhibition of systemic fibrinolysis and intense thrombin generation. Similar changes are seen in nonruptured AAA but are of a lesser magnitude. This procoagulant state may contribute to the microvascular and macrovascular thrombosis that leads to myocardial infarction, multiple organ failure, and thromboembolism. (J Vasc Surg 1999;30:641-50.)

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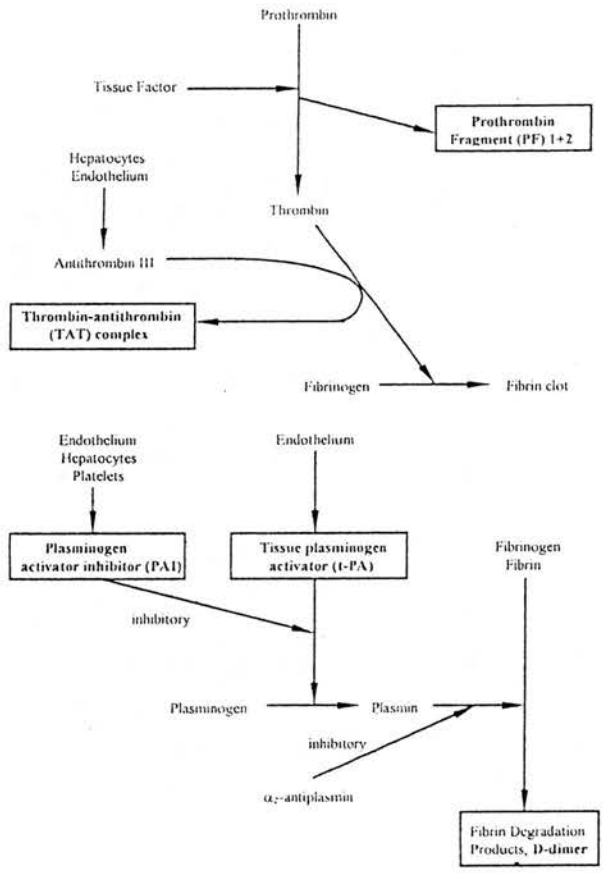

Fig 1. Overview of extrinsic coagulation and fibrinolytic systems. Markers studied are shown in *boxes*. Extrinsic coagulation system: tissue injury, endothelial activation and injury, and monocyte activation lead to tissue factor expression, which triggers the extrinsic coagulation cascade. This leads to conversion of prothrombin to thrombin with release of PF 1+2. Excess thrombin is inactivated by antithrombin III (main thrombin inhibitor) with formation of TAT. Fibrinolytic system: endothelial activation and injury lead to release of t-PA, which converts plasminogen to the active enzyme plasmin, which, in turn, leads to breakdown of fibrinogen, fibrin and fibrin clot to fibrin degradation products such as D-dimer. PAI is the naturally occurring inhibitor of t-PA and is released from endothelium, hepatocytes and platelets.

Repair of ruptured and nonruptured abdominal aortic aneurysms (AAAs) is associated with an operative mortality rate of approximately 35% to 50%[1,2] and 5% to 15%,[3] respectively. The great majority of these deaths are due to myocardial infarction, multiple organ failure, and thromboembolism, all of which may be related to microvascular and macrovascular thrombosis developing as a result of a procoagulant state.[2,4,5] Perhaps surprisingly, therefore, previous authors have suggested that supraceliac aortic cross-clamping and thoracoabdominal aortic aneurysm repair are associated with increased fibrinolysis, which may be due to reduced hepatic blood flow.[6,7] Furthermore, animal studies suggest that infrarenal aortic clamping and isolated lower body ischemia are also associated with elevated levels of fibrinolytic markers.[8] With respect to elective infrarenal aortic reconstruction, studies are few and contradictory.[9-12] Although these data have been used to support the use of antifibrinolytic agents in patients undergoing operation for ruptured AAA,[13] careful review of the literature reveals that the precise nature of the serial changes in hemostatic derangement in such patients has not previously been studied. The aims of this study were to examine serial markers of thrombin generation and fibrinolysis during the course of emergent surgery for ruptured infrarenal AAA and to compare these with those undergoing elective repair of nonruptured infrarenal AAA.

METHODS

Patients. Ten patients (8 men and 2 women of median age 76 years, range 71 to 86 years) operated on for ruptured and 9 patients (8 men and 1 woman of median age 69 years, range 58 to 80 years) operated on for asymptomatic nonruptured infrarenal AAA were prospectively studied. Lothian Region Ethical Committee approval was obtained as was fully informed written consent from all patients.

In patients operated on for ruptured AAA, the median (range) delay between the onset of symptoms of rupture and hospital admission was 5 (3 to 14) hours. All patients had at least one documented episode of hypotension (systolic blood pressure less than 100 mm Hg) before surgery. In patients undergoing operation for nonruptured AAA, the median (range) anteroposterior diameter of the aneurysm measured by abdominal ultrasound scan was 6.5 (5.5 to 8.0) cm. No patient in either group had a history of liver disease or was taking oral anticoagulant medication before admission. Liver function tests were only available preoperatively in elective cases and were all normal. Three patients with ruptured AAA and four with nonruptured AAA were taking regular aspirin before admission.

Operative methods. Ruptured AAA was defined by the presence of fresh retroperitoneal blood at operation. No patient had intraperitoneal rupture. Patients with rupture had general anesthesia, and patients with nonruptured AAA had combined general and epidural anesthesia. All patients underwent AAA repair

Table I. Assays of coagulation and fibrinolysis

Assay	*Tube*	*Volume (mL)*	*Assay*	*Manufacturer*	*Normal range*
Hematocrit					0.37-0.54
Platelet count	EDTA (1.6 mg/mL)	2.7		SYSMEX NE 8000	150-350 × 10^9/L
CRP	Clot activator	9.0	ELISA	DAKO, Denmark	< 10 mg/L
Fibrinogen					1.5-4.0 g/L
PT				ACL 300	10.5-14.5 s
aPTT					28-40 s
TAT				Enzygnost TAT micro; Behring Diagnostics	1.0-4.1 μg/L
PF 1+2	Sodium citrate (0.106 mol/L)	3.0	ELISA	Enzygnost PF 1+2 micro; Behring Diagnostics	0.4-1.1 nmol/L
D-dimer				Asserachrom D-Di; Diagnostica Stago, France	630-850 ng/mL
t-PA antigen				Coaliza t-PA; Chromogenix, Sweden	1-12 ng/mL
PAI activity			Chromogenic assay (amidolytic method)	Coaset PAI; Chromogenix, Sweden	< 15 AU/mL
t-PA activity	Strong acid citrate (Stabilyte; Biopool, Sweden)	4.5		Coatest t-PA; Chromogenix, Sweden	0.2-2.0 IU/mL

EDTA, Ethylenediaminetetraacetic acid; *ELISA*, enzyme-linked immunosorbent assay; *AU*, arbitrary units.

through a transverse supraumbilical incision with infrarenal aortic clamping, which is the standard approach in this institution. No patients required supraceliac aortic clamping. No patients with ruptured AAA were systemically heparinized. Patients with nonruptured AAA were given an intravenous bolus of 5000 units heparin immediately before aortic clamp placement. No patient received protamine sulfate or mannitol infusion because this is the standard policy in this institution. A Dacron tube graft was inserted in 13 patients (nine with rupture, four with nonrupture), aortobi-iliac graft in five (one with rupture, four with nonrupture), and aortobifemoral graft in one patient with nonruptured AAA.

Markers of thrombin generation and fibrinolysis. The extrinsic coagulation and fibrinolytic systems are summarized in Fig 1. Plasma levels of prothrombin fragment (PF) 1+2 (normal range, 0.4 to 1.1 nmol/L) and thrombin-antithrombin complex (TAT) (normal range, 1.0 to 4.1 μg/L) were assayed as markers of thrombin generation, and tissue plasminogen activator (t-PA) activity (normal range, 0.2 to 2.0 IU/mL), t-PA antigen (normal range, 1 to 12 ng/mL), plasminogen activator inhibitor (PAI) activity (normal range, less than 15 arbitrary units [AU]/mL), and fibrin degradation product D-dimer (normal range, 630 to 850 ng/mL) as markers of fibrinolysis. Hematocrit (normal range, 0.37 to 0.54), platelet count (normal range, 150 to 350 × 10^9/L), fibrinogen (normal range, 1.5 to 4.0 g/L), prothrombin time (PT) (normal range, 10.5 to 14.5 seconds) and activated partial thromboplastin time (aPTT) (normal range, 28 to 40 seconds), and C-reactive protein (CRP) (normal range, less than 10 mg/L) were also measured (Table I).

Sample collection. The sampling points were chosen to reflect the maximum effect of each of the three pathophysiologic phases of ruptured AAA repair (Fig 2). Blood was sampled from an indwelling radial arterial line immediately before the induction of anesthesia (sample A), immediately before release of the aortic clamp (sample B), and 5 minutes (sample C) and 24 hours (sample D) after aortic clamp release. t-PA antigen, D-dimer and TAT were measured at sample point A. Hematocrit, platelet count, fibrinogen, PT, aPTT, CRP, t-PA activity, PAI activity, and PF 1+2 were measured at all four sample points. Samples were placed immediately on ice and centrifuged within 30 minutes of collection at 3000 revolutions per minute for 30 minutes at 4°C (equivalent to 1400*g*). Plasma and serum were separated and stored at −70°C for later batch analysis.

Statistical methods. The Mann-Whitney test was used to compare groups of patients. Because the data were not normally distributed, the Spearman rank test was used to correlate the degree of preoperative hypotension and the operative blood loss with the levels of hemostatic markers in patients operated on for ruptured AAA. A probability value of less than .05 was regarded as statistically significant.

RESULTS

Clinical data. Clinical and operative data for both groups of patients are summarized in Table II. During operation, no patients with nonruptured AAA received inotropic support. Three patients with

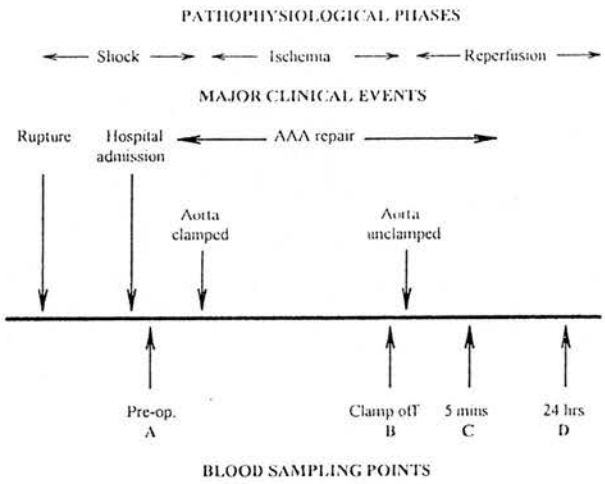

Fig 2. Schematic view of pathophysiology of ruptured AAA repair. Pathophysiology of ruptured AAA repair can be divided into three phases. First, there is a period of whole body hypoperfusion due to hypovolemic shock. Second, there is a period of profound lower body ischemia after aortic clamp placement. Finally, if repair is successful, there is a period of reperfusion.

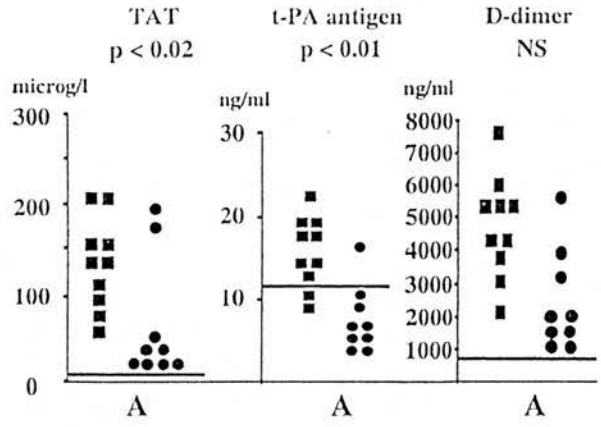

Fig 3. Individual data points for levels of TAT, t-PA antigen, and fibrin degradation product D-dimer immediately before induction of anesthesia (sample A) in 10 patients with ruptured AAA (*black squares*) and 9 patients with nonruptured AAA (*black circles*). Normal ranges for TAT (1.0 to 4.1 μg/L), t-PA antigen (1 to 12 ng/mL), and D-dimer (630 to 850 ng/mL) are shown by *horizontal lines*. *NS*, Not significant.

rupture received adrenaline infusion and five received dopamine infusion. All patients with rupture were admitted to the intensive therapy unit postoperatively for ventilatory support. The median (range) duration of intensive therapy unit stay was 72 (13 to 244) hours. The median (range) duration of ventilatory support was 19 (9 to 142) hours. All patients operated on for nonruptured AAA were admitted to the surgical high dependency unit postoperatively, and no patient was admitted to the intensive therapy unit or required ventilatory support. Seven patients operated on for rupture and four operated on for nonruptured AAA developed major postoperative complications (Table III). All patients survived to 24 hours after repair. Two patients with rupture died in hospital of acute respiratory distress syndrome and acute renal failure on postoperative day 10 and of pneumonia and critical lower limb ischemia on postoperative day 21. There were no deaths after repair of nonruptured AAA.

Hematocrit, platelet count, fibrinogen, PT, aPTT, and CRP. The median (range) values for hematocrit, the standard tests of hemostasis (platelet count, PT, aPTT, and fibrinogen) and CRP are shown in Table IV.

In patients operated on for ruptured AAA, there was no significant relationship between the degree of preoperative hypotension and any of the standard tests of hemostasis. There was, however, a significant negative correlation between operative blood loss and fibrinogen level immediately before ($r = -0.694$, $P = .026$) and 5 minutes after aortic clamp release ($r = -0.75$, $P = .012$), and also platelet count 5 minutes after aortic clamp release ($r = -0.726$, $P = .018$); and a significant positive correlation between operative blood loss and PT immediately before aortic clamp release ($r = +0.823$, $P = .003$), and aPTT immediately before ($r = +0.787$, $P = .007$) and 5 minutes after aortic clamp release ($r = +0.64$, $P = .046$).

Markers of thrombin generation. Before operation, TAT levels were elevated above the normal range in all patients. Levels were significantly higher in patients with ruptured AAA (median 135.5 μg/L, range 61.2 to 209.4 μg/L) than in those with nonruptured AAA (median 21.6 μg/L, range 6.6 to 180.4 μg/L; $P < .02$, Mann-Whitney test) (Fig 3). Before and during operation, PF 1+2 levels were also significantly higher in patients undergoing repair of ruptured AAA when compared with those undergoing repair of nonruptured AAA. At 24 hours there was no significant difference in PF 1+2 levels between the groups (Table V and Fig 4). There was no significant relationship between the degree of preoperative hypotension or operative blood loss and any of the markers of thrombin generation.

Markers of fibrinolysis. Before operation,

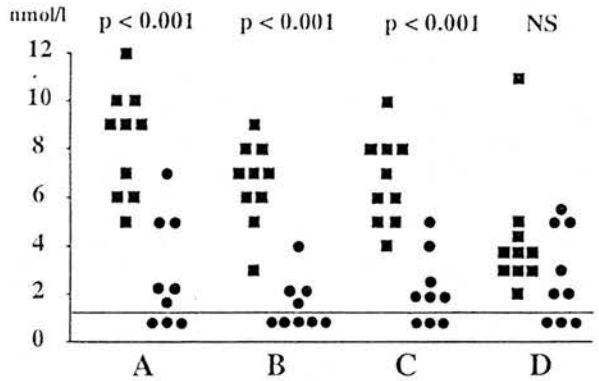

Fig 4. Individual data points for PF 1+2 immediately before induction of anesthesia (sample A), immediately before release of aortic clamp (sample B), and 5 minutes (sample C) and 24 hours (sample D) after aortic clamp release in 10 patients operated on for ruptured (*black squares*) and 9 patients operated on for nonruptured (*black circles*) infrarenal AAA. Normal range for PF 1+2 is 0.4 to 1.1 nmol/L and is shown by the *horizontal line*. *NS*, Not significant.

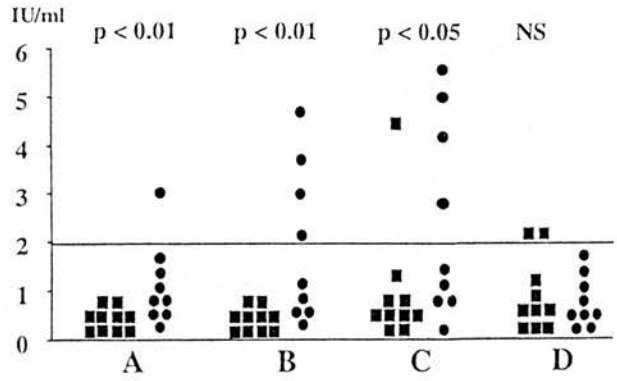

Fig 5. Individual data points for t-PA activity immediately before induction of anesthesia (sample A), immediately before release of aortic clamp (sample B), and 5 minutes (sample C) and 24 hours (sample D) after aortic clamp release in 10 patients operated on for ruptured (*black squares*) and 9 patients operated on for nonruptured (*black circles*) infrarenal AAA. Normal range for t-PA activity is 0.2 to 2.0 IU/mL and is shown by the *horizontal line*. *NS*, Not significant.

Table II. Clinical and operative data in 10 patients operated on for ruptured and 9 patients operated on for nonruptured infrarenal AAAs

	Ruptured AAA, median (range) (n = 10)	*Nonruptured AAA, median (range) (n = 9)*	*P value**
Preoperative			
Crystalloid administration (L)	0.5 (0.1-4.0)	—	
Colloid administration (L)	0 (0-1.5)	—	
Intraoperative			
Operation time (min)	105 (70-205)	160 (85-285)	NS
Aortic clamp time (min)	60 (30-125)	70 (25-150)	NS
Measured blood loss (L)	2.3 (1.0-6.4)	2.8 (1.0-6.0)	NS
Crystalloid administration (L)	2.0 (0.5-3.5)	2.0 (1.0-4.0)	NS
Colloid administration (L)	1.5 (0-2.3)	2.0 (0.5-3.8)	NS
RCC administration (units)	8 (6-11)	4 (0-10)	0.02
FFP administration (units)	2 (0-6)	0 (0-2)	NS
Platelet administration (bags)†	1 (0-1)	0 (0-1)	NS

NS, Not significant; *RCC*, red cell concentrate (300 mL); *FFP*, fresh frozen plasma (300 mL).
*Mann-Whitney test.
†One bag of platelet transfusion = 4 pooled units (250 mL).

t-PA antigen levels were significantly higher in patients with ruptured AAA (median 15.7 ng/mL, range 9.0 to 22.1 ng/mL) compared with those with nonruptured AAA (median 6.6 ng/mL, range 4.7 to 16.4 ng/mL; $P < .005$, Mann-Whitney test). Before operation, there was no significant difference in the D-dimer levels between the ruptured AAA (median 3460 ng/mL, range 1236 to 7860 ng/mL) and nonruptured AAA group (median 1642 ng/mL, range 728 to 5334 ng/mL; $P = .07$, Mann-Whitney test) (Fig 3). Before and during operation, t-PA activity was significantly lower in patients undergoing repair of ruptured AAA when compared with those undergoing repair of nonruptured AAA (Table V and Fig 5). At 24 hours there was no significant difference in t-PA activity between the groups. Before and during operation, PAI activity was significantly higher in patients undergoing repair of ruptured AAA when compared with those undergoing repair of nonruptured AAA. At 24

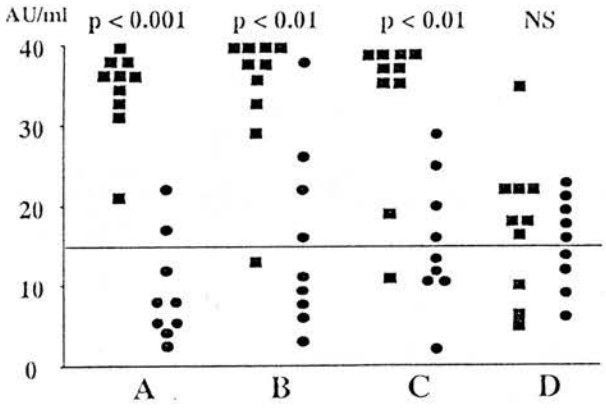

Fig 6. Individual data points for PAI activity immediately before induction of anesthesia (sample A), immediately before release of aortic clamp (sample B), and 5 minutes (sample C) and 24 hours (sample D) after aortic clamp release in 10 patients operated on for ruptured (*black squares*) and 9 patients operated on for nonruptured (*black circles*) infrarenal AAA. Normal range for PAI activity is less than 15 AU/mL and is shown by the *horizontal line*. *NS,* Not significant.

hours there was no significant difference in PAI activity between the groups (Table V and Fig 6). There was no significant relationship between the degree of preoperative hypotension or operative blood loss and any of the markers of fibrinolysis. Coagulation and fibrinolytic data for two patients who died after ruptured AAA repair compared with eight who survived are shown in Table VI.

DISCUSSION

The principal finding of the present study is that emergency repair of ruptured AAA is associated with intense thrombin generation (as demonstrated by elevated TAT and PF 1+2 levels) and inhibition of systemic fibrinolysis (as demonstrated by elevated t-PA antigen, reduced t-PA activity, and elevated PAI activity). This procoagulant state is present before surgery, persists throughout the period of operation, and has largely resolved 24 hours after operation. Furthermore, similar changes are observed in a proportion of patients undergoing elective repair of nonruptured AAA but are of much less magnitude.

At first sight, these novel findings appear to contradict a previous study that reported supraceliac aortic cross-clamping to be associated with increased fibrinolysis.[6] One reason for this apparent discrepancy may be due to the measurement of t-PA antigen as opposed to the measurement of t-PA activity. This failure to measure activity, as well as antigen, in the present study might have led to the erroneous conclusion that ruptured AAA was, indeed, associated with enhanced fibrinolysis. However, it is increasingly apparent that t-PA antigen levels primarily reflect the level of inactive circulating t-PA/PAI complexes. This contention is demonstrated by the present study, where elevated t-PA antigen is, in fact, associated with markedly elevated PAI activity and markedly depressed t-PA activity, consistent with a procoagulant state.[14,15]

Given that repair of ruptured aortic aneurysm and a proportion of elective aneurysm operations are associated with a procoagulant state,[9,10] two important questions need to be addressed.

First, are the presence and the intensity of the procoagulant state associated with poor outcome as a result of microvascular and macrovascular thrombotic events? Although the changes in coagulation and fibrinolysis observed in the present study have been associated with myocardial injury,[14,16-19] multiple organ failure,[20,21] and stroke[22] in other patient groups, it is not possible to answer this question directly from the present data for two reasons. Only data for patients who survived for 24 hours are included in the present analysis. Data for those who

Table III. Postoperative complications and procedures in 7 patients operated on for ruptured and 4 patients operated on for nonruptured infrarenal AAAs

	Ruptured AAA (n = 7)	*Nonruptured AAA (n = 4)*
Cardiovascular		
Atrial fibrillation	3	1
Congestive cardiac failure	4	2
Myocardial infarction	1	0
Stroke	1	0
Lower limb critical ischemia	2	1
Deep venous thrombosis	0	1
Respiratory		
Chest infection	6	2
Respiratory failure	3	0
Acute respiratory distress syndrome	1	0
Acute renal failure	2	1
Disseminated intravascular coagulopathy	1	0
Sepsis syndrome	1	0
Colon ischemia	1	0
Total parenteral nutrition	3	0
Inotropic support		
Adrenaline	3	0
Renal dose dopamine	6	0
Reoperation	1*	1†

*Laparotomy for intra-abdominal hemorrhage, femoral thrombectomy, Hartmann's procedure for colon ischemia, drainage of infected pelvic hematoma.
†Popliteal thrombectomy and fasciotomies.

Table IV. Hematocrit, platelet count, fibrinogen, PT, aPTT, and CRP in patients operated on for ruptured and nonruptured AAAs

Assay (normal range)	*Sample point*	*Ruptured AAA, median (range) (n = 10)*	*Nonruptured AAA, median (range) (n = 9)*	*P value**
Hematocrit (0.37-0.54)	A	0.31 (0.13-0.34)	0.42 (0.33-0.47)	.0004
	B	0.27 (0.18-0.44)	0.30 (0.25-0.37)	NS
	C	0.28 (0.22-0.42)	0.30 (0.23-0.35)	NS
	D	0.34 (0.26-0.42)	0.34 (0.25-0.39)	NS
Platelet count (150-350 × 10⁹/L)	A	230 (119-303)	182 (75-744)	NS
	B	120 (81-189)	132 (103-541)	NS
	C	108 (59-146)	135 (91-577)	NS
	D	97 (50-133)	127 (85-604)	NS
Fibrinogen (1.5-4.0 g/L)	A	2.27 (0.86-3.75)	2.80 (1.59-6.02)	NS
	B	1.12 (0.88-2.51)	1.68 (0.72-5.39)	NS
	C	0.97 (0.46-1.82)	1.45 (0.36-5.44)	NS
	D	3.29 (1.76-4.63)	3.70 (2.50-8.98)	NS
PT (10.5-14.5 s)	A	14 (11-35)	12 (11-14)	.009
	B	20 (15-26)	17 (15-26)	NS
	C	20 (17-31)	20 (14-23)	NS
	D	16 (13-18)	14 (12-21)	NS
aPTT (28-40 s)	A	32 (28-126)	31 (25-49)	NS
	B	50 (34-210)	176 (56-240)	.006
	C	55 (42-210)	210 (79-240)	.008
	D	39 (32-76)	36 (29-39)	.02
CRP (< 10 mg/L)	A	6.7 (2.6-178.3)	4.3 (0.3-18.6)	NS
	B	3.0 (0.9-116)	5.0 (1.1-13.4)	NS
	C	3.8 (1.9-11.4)	2.0 (1.3-8.2)	NS
	D	105 (41.8-141.8)	92.4 (41.5-180.8)	NS

Sample A, Immediately before induction of anesthesia; *sample B*, immediately before release of aortic clamp; *sample C*, 5 minutes after aortic clamp release; *sample D*, 24 hours after aortic clamp release; *NS*, not significant.
*Mann-Whitney test.

Table V. PF 1+2, t-PA activity, and PAI activity in patients operated on for ruptured and nonruptured AAAs

Assay (normal range)	*Sample point*	*Ruptured AAA, median (range) (n = 10)*	*Nonruptured AAA, median (range) (n = 9)*	*P value**
PF 1+2 (0.4-1.1 nmol/L)	A	9.0 (5.4-11.6)	2.2 (0.7-7.1)	.0008
	B	6.7 (3.3-8.9)	1.0 (0.9-4.0)	.0003
	C	6.5 (4.2-9.6)	2.0 (1.0-4.9)	.0007
	D	3.5 (1.9-11.4)	1.9 (1.3-5.6)	NS
t-PA activity (0.2-2.0 IU/mL)	A	0.12 (0.06-0.43)	0.49 (0.14-3.2)	.009
	B	0.27 (0.08-0.8)	0.91 (0.34-4.65)	.0014
	C	0.32 (0.09-4.53)	1.06 (0.19-5.62)	.034
	D	0.41 (0.15-2.1)	0.46 (0.21-1.45)	NS
PAI activity (< 15 AU/mL)	A	36.5 (20.6-38.8)	8.2 (3.2-21.7)	.0003
	B	38.6 (13.0-39.4)	10.8 (2.8-38.9)	.0042
	C	37.2 (10.6-39.4)	12.6 (2.2-28.7)	.0055
	D	18.1 (5.0-35.3)	14.7 (5.7-22.3)	NS

Sample A, Immediately before induction of anesthesia; *sample B*, immediately before release of aortic clamp; *sample C*, 5 minutes after aortic clamp release; *sample D*, 24 hours after aortic clamp release; *NS*, not significant.
*Mann-Whitney test.

died intraoperatively or within the first 24 hours after operation are the subject of an ongoing study. Furthermore, the number of patients and adverse clinical outcomes is small (Table III). However, both of the patients with rupture who died had PF 1+2 levels 24 hours after operation that were considerably higher than survivors, and one who died 10 days after surgery had very high t-PA activity and very low PAI activity (indicating increased systemic fibrinolysis) during and 24 hours after the operation. A larger prospective study of the relationship between hemostatic derangement and outcome in patients undergoing repair of ruptured and nonruptured AAAs is currently underway in our institution.

Table VI. Coagulation and fibrinolytic data for 2 patients who died after ruptured AAA repair compared with 8 patients who survived

Assay (normal range)	*Sample point*	*Nonsurvivor (ARDS, ARF)*	*Nonsurvivor (pneumonia, CLI)*	*Survivors, median (range) (n = 8)*
Platelet count (150-350 × 10⁹/L)	A	119	178	249 (156-303)
	B	81	116	125 (96-189)
	C	66	84	117 (59-146)
	D	86	72	102 (50-133)
Fibrinogen (1.5-4.0 g/L)	A	2.18	1.87	2.52 (0.86-3.75)
	B	1.2	0.94	1.20 (0.88-2.51)
	C	0.77	0.85	1.20 (0.46-1.82)
	D	1.87	2.57	3.35 (1.76-4.63)
PT (10.5-14.5 s)	A	16	14	14 (11-35)
	B	23	18	20 (15-26)
	C	27	24	20 (17-31)
	D	18	15	16 (13-18)
aPTT (28-40 s)	A	40	35	30 (28-126)
	B	120	52	53 (34-210)
	C	108	210	55 (42-123)
	D	51	49	39 (32-76)
PF 1+2 (0.4-1.1 nmol/L)	A	10.1	9.3	7.7 (5.4-11.6)
	B	7.0	8.9	6.2 (3.3-8.4)
	C	4.8	9.6	6.5 (4.2-8.0)
	D	11.4	4.8	3.4 (1.9-4.4)
t-PA activity (0.2-2.0 IU/mL)	A	0.13	0.11	0.17 (0.06-0.43)
	B	0.8	0.12	0.27 (0.08-0.41)
	C	4.53	0.43	0.28 (0.09-1.27)
	D	2.1	0.25	0.41 (0.15-2.08)
PAI activity (< 15 AU/mL)	A	31.0	36.4	36.7 (20.6-38.8)
	B	13.0	38.9	38.6 (29.4-39.4)
	C	10.6	38.2	37.2 (19.4-39.4)
	D	5.9	22.2	18.1 (5.0-35.3)

Sample A, Immediately before induction of anesthesia; *sample B*, immediately before release of aortic clamp; *sample C*, 5 minutes after aortic clamp release; *sample D*, 24 hours after aortic clamp release; *ARDS*, acute respiratory distress syndrome; *ARF*, acute renal failure; *CLI*, critical lower limb ischemia.

The second question is whether therapeutic intervention may ameliorate the adverse effects of this procoagulant state and thus improve outcome. Apart from the fact that patients in this study with ruptured AAA sustained a period of preoperative hypovolemic shock, the most obvious difference between the groups relates to the use of systemic heparin during aneurysm repair. The effects of heparin on the coagulation parameters examined in the present study include slight (5% to 10%) reduction in platelet count in a small proportion of patients, prolongation of the aPTT, marginal prolongation of the PT in a small proportion of patients, elevation of the fibrinogen level and a reduction in D-dimer levels due to a reduction in fibrin deposition, reduced PF 1+2 due to inhibition of thrombin generation by antithrombin III, and increased binding of thrombin to antithrombin III but no increase in TAT levels. Other than t-PA, no other fibrinolytic component appears to be affected by heparin; whereas the majority of clinical and volunteer studies have shown that repeated administration of unfractionated heparin over a number of days increases t-PA antigen, short-term studies have shown increases in t-PA activity similar in magnitude to what might be expected due to diurnal variation and that may be artifactual.[23] Surgeons are naturally reluctant to systemically heparinize a patient with a ruptured aortic aneurysm, but these data suggest that after control of bleeding by aortic clamping, judicious use of heparin may reverse the procoagulant state and may, therefore, improve outcome from thrombotic complications.[24] It is important to note, however, that patients with ruptured AAA exhibited very elevated levels of TAT, which indicate that there is already a considerable, albeit ineffective, attempt by nature to inhibit thrombin activation. This may limit the efficacy of heparin in this situation. Other possible therapeutic interventions include angiotensin-converting enzyme inhibitors (which may decrease PAI and increase t-PA levels) and specific inhibitors of PAI activity.[25] There have been suggestions that patients with ruptured AAA might benefit from antifibrinolytic therapy, specifically with aprotonin. However, this has not been shown to be of therapeutic value in

reducing blood loss or blood transfusion requirement in elective infrarenal aortic surgery,[13] and the findings of the present study strongly suggest that such therapy is contraindicated in patients undergoing operation for ruptured aortic aneurysm.

At the present time, the triggering mechanisms leading to this procoagulant state are unknown, but, as it is present before operation in patients with rupture, it is presumably related to whole body hypoperfusion as a result of hemorrhagic shock. Although there was no significant relationship between the degree of preoperative hypotension and any of the coagulation and fibrinolytic parameters studied, there was a significant negative correlation between operative blood loss and fibrinogen level and platelet count during the operation, and a significant positive correlation between operative blood loss and PT and aPTT during the operation. The finding that certain patients undergoing elective repair of nonruptured AAA also have elevated levels of TAT and PF 1+2 (indicating pathologic levels of thrombin generation) is difficult to explain but may be related to the presence of thrombus within the aneurysm sac.[26] Indeed, both we and a number of previous authors have suggested that patients with AAA have a low-grade coagulopathy that may make them particularly sensitive to the effects of hypotension and ischemia.[5,27,28] Previous work from this group has demonstrated morphologic evidence of endothelial cell activation before operation in patients with ruptured AAA, suggesting that this may be an early event in this group of patients.[29] Tissue factor expression is associated with thrombin generation and reduced fibrinolysis,[30,31] and its role in the pathophysiology of the hemostatic derangement in patients with ruptured AAA is currently being investigated.

In conclusion, these novel data demonstrate that ruptured AAA repair is associated with inhibition of systemic fibrinolysis and intense thrombin generation. This procoagulant state may contribute to microvascular and macrovascular thrombosis that, in turn, lead to the common causes of perioperative morbidity and mortality, namely myocardial infarction, multiple organ failure, and thromboembolism.

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INVITED COMMENTARY

Regarding "Coagulation and fibrinolysis in patients undergoing operation for ruptured and nonruptured infrarenal abdominal aortic aneurysms"

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The rupture of an abdominal aortic aneurysm is associated with death in the vast majority of patients in whom it occurs. One of the most eye-opening demonstrations of the lethality of the problem is the population-based report from Malmö in which 88% of the patients with ruptured aortic aneurysms died, most of whom did not survive to reach the hospital.[1] Lowering the rate of mortality would require the development of a screening program to identify individuals with aortic aneurysms so that elective repair could be performed before rupture. The reality of such an approach is uncertain, and the cost effectiveness is questionable. As such, the institution of global methods to identify and repair the great number of asymptomatic aneurysms is a matter of public policy and poorly controllable by the vascular practitioner.

By contrast, vascular surgeons have the potential to alter patient survival rates for those patients who arrive at the hospital alive. Anecdotally, it appears that intraoperative mortality rate has, indeed, decreased over time, but the chance of surviving to discharge remains only one in two for these patients.[2] Vascular surgeons and anesthesiologists have become remarkably effective at getting patients with ruptured aneurysms through the operation itself, but the perioperative mortality rate remains depressingly high. Thrombotic complications predominate during the early postoperative period, including myocardial infarction, lower extremity and intestinal ischemia, stroke, and venous thromboembolism. The frequency of these events must be limited if a decrease in the in-hospital mortality rate is to be achieved.

The work of Adam and colleagues sheds light on possible mechanisms to explain the increased incidence of thrombotic events in patients who undergo repair of ruptured aortic aneurysms. The crux of their work is based on the assumption that thrombotic events occur as a result of an imbalance between thrombogenesis (thrombin generation vs natural anticoagulant pathways) and endogenous thrombolysis (plasminogen activation vs plasminogen activator inhibition and antiplasmin activity). Elucidation of these mechanisms provides critical information that can be used to design treatments directed at the prevention of intravascular thrombosis, thereby decreasing the rate of associated perioperative thrombotic complications. In this regard, it is likely that similar mechanisms underlie the pathophysiology of thrombotic events after many peripheral vascular procedures, as well as major operative procedures in general.

There are two major findings of this study, both of which relate to the development of a hypercoagulable state before and during the repair of ruptured aneurysms. First, the investigators detected markers of intense thrombin generation in these patients, with elevation of thrombin-antithrombin complexes and prothrombin fragments 1 and 2. Second, reduced endogenous tissue plasminogen activator (t-PA) activity was seen, explainable by marked elevations in plasminogen activator inhibitor (PAI). The PAI activity was high enough to account for the diminished t-PA activity in spite of an increase in the levels of circulating t-PA antigen. This apparent paradox is easily understood when one considers that total t-PA antigen measures both unbound (active) t-PA as well as

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the inactive t-PA bound to inhibitors such as PAI-1. In fact, the importance of measuring t-PA activity rather than antigen underlies apparent contradictions between this study and a previous investigation of our own, where elevations in t-PA antigen were thought to account for an increased incidence of bleeding after supraceliac aortic cross clamping.

It is important to note that the present study compared the levels of markers of coagulation and fibrinolysis in patients with ruptured versus elective aneurysm repair. Interesting findings are revealed when the levels in the elective group are compared with the normal range. For instance, even the group of patients who underwent elective aneurysm repair manifested mild elevations in prothrombin fragments 1 and 2, indicative of ongoing thrombin generation. t-PA and PAI were, however, within the range of normal before, during, and after operation. These findings suggest that a procoagulant state is present at baseline in patients with aneurysms, primarily related to increased thrombin generation. This contention, however, may be spurious, related to an increase in prothrombin fragments in blood drawn from the arterial lines of patients with aneurysms rather than the potentially less traumatic venous sticks used for determining the normal range. Nevertheless, it corroborates previous work from a variety of investigators, documenting significant coagulation derangements in patients with abdominal aortic aneurysms.[3,4]

There are some potential limitations of the authors' work. There exist unavoidable demographic, anatomic, and pharmacologic differences in patients with ruptured versus non-ruptured aneurysms. For instance, the patients with ruptured aneurysms may be older, with an increased frequency of coexistent medical illnesses. As well, these patients have larger aneurysms with a greater amount of potentially thrombogenic surface area exposed to blood flow. Lastly, the pharmacologic profile implemented in patients with ruptured and non-ruptured aneurysms is quite different, most importantly with respect to the use of heparin, but also with regard to vasoactive agents, such as epinephrine and dopamine—agents that can have significant effects on coagulation, platelet function, and endothelial physiology.

Despite these potential drawbacks, the present work represents a carefully designed and well-executed study of coagulation and fibrinolytic derangements in patients with ruptured aortic aneurysms. Although the observations must be corroborated by subsequent, larger studies, a major value of the present study resides in the fact that it will generate cognizance of these hemostatic derangements. Clinical results can only be improved through the acquisition of sound research data gained from investigations such as this. Novel techniques and strategies are, of necessity, formulated and implemented on the basis of fundamental research. It is incumbent on us to assure a continuing supply of well-trained vascular surgeons with education sufficient to critique research studies and, in many cases, design and conduct investigations themselves. Training of academically inclined vascular surgeons will ensure adequate growth in the fund of knowledge relating to vascular disease and, ultimately, bring about great improvements in patient care and clinical outcome.

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Please see related article by Adam et al on pages 641-50.

Hemostatic markers before operation in patients with acutely symptomatic nonruptured and ruptured infrarenal abdominal aortic aneurysm

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Background: In patients with acutely symptomatic but nonruptured abdominal aortic aneurysm (AAA), emergent repair is associated with an increased mortality rate as compared with semi-elective repair. Previous results have shown that ruptured but not asymptomatic AAA repair is associated with intense thrombin generation and inhibition of systemic fibrinolysis. The purpose of this study was to determine whether circulating markers of coagulation and fibrinolysis may be used to distinguish acutely symptomatic nonruptured and ruptured AAA.

Methods: A prospective study was performed of 44 patients who underwent emergency AAA repair for suspected rupture. Platelet count, fibrinogen level, prothrombin time, activated partial thromboplastin time, tissue plasminogen activator (t-PA) activity, plasminogen activator inhibitor (PAI) activity, prothrombin fragment (PF) 1+2 level, and D dimer level were measured before surgery.

Results: When compared with ruptured AAAs (n = 37), acutely symptomatic nonruptured AAAs (n = 7) were associated with increased fibrinogen level (P = .033), reduced activated partial thromboplastin time (P = .043), increased t-PA activity (P = .023), reduced PAI activity (P = .005), reduced PF 1+2 level (P = .001), and reduced D dimer level (P = .005; all P values determined with Mann-Whitney test). The differences in t-PA activity (P = .01), PAI activity (P = .004), and PF 1+2 level (P = .01) persisted in patients whose conditions were normotensive. In all patients, a PF 1+2 level of greater than or equal to 2.5 nmol/L was associated with a sensitivity, specificity, and positive and negative predictive value for rupture of 89%, 86%, 97%, and 60%, respectively. In patients whose conditions were normotensive, PAI activity of greater than or equal to 16 AU/mL was associated with a sensitivity, specificity, and positive and negative predictive value of 83%, 100%, 100%, and 88%, respectively.

Conclusion: These data show that acutely symptomatic nonruptured AAA is associated with increased systemic fibrinolysis (caused by reduced fibrinolytic inhibition) and reduced thrombin generation as compared with rupture. Preoperative hemostatic markers, particularly PF 1+2 level and PAI activity, may distinguish acutely symptomatic nonruptured from ruptured AAA. (J Vasc Surg 2002;35:661-5)

In the United Kingdom, more than 50% of all abdominal aortic aneurysm (AAA) repairs are performed on an emergency basis because the surgeon believes, or is unable to exclude the possibility, that rupture has occurred. However, in approximately 20% of these patients, the AAA is found to be intact, and sudden expansion or impending rupture are presumed to be responsible for the patient's symptoms.[1] The operative mortality rate for this group of patients is twice that of symptomatic patients in whom rupture is not suspected and who do not undergo operation on an emergency basis.[1-4]

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Competition of interest: nil.

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At present, acutely symptomatic nonruptured AAAs are differentiated from ruptured AAAs primarily on the basis of history and examination, but even the most experienced of vascular surgeons cannot always confidently exclude rupture with clinical assessment alone.[5] Many clinicians have advocated the use of emergency computed tomographic (CT) scanning in this situation, but previous results from this group have shown that, in cases of true clinical uncertainty, CT scan has an unacceptably low sensitivity and specificity.[6] Previous results from this group have also shown that ruptured AAA is associated with intense thrombin generation and inhibition of systemic fibrinolysis.[7] To date, there are no reports of hemostatic function in patients with acutely symptomatic nonruptured AAA. We hypothesised that, because acutely symptomatic nonruptured AAA is not associated with extramural hemorrhage, this group of patients would not manifest the same pattern of hemostatic derangement as patients with rupture. As such, hemostatic variables might aid clinical decision-making regarding the timing of surgery in patients with acutely symptomatic nonruptured AAA. The aims of this study were two-fold. The first aim was the examination of whether patients who undergo repair of acutely symptomatic nonruptured AAA exhibited the same hemostatic derangement as those who underwent opera-

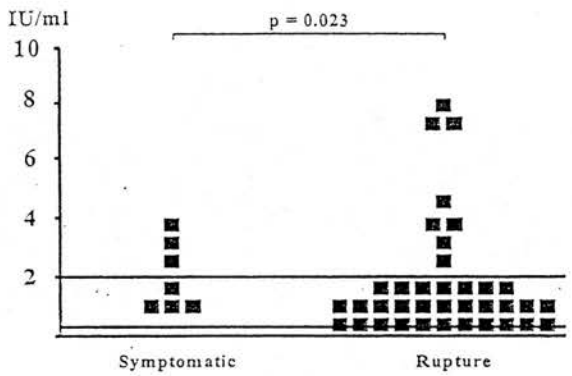

Fig 1. Preoperative tissue plasminogen activator activity in acutely symptomatic and ruptured abdominal aortic aneurysm. Upper and lower limits of healthy range are shown with *horizontal lines.*

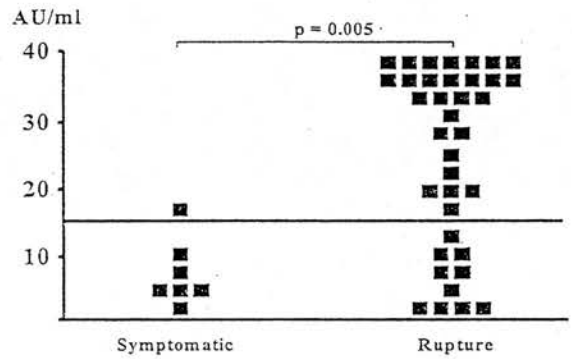

Fig 2. Preoperative plasminogen activator inhibitor activity in acutely symptomatic and ruptured abdominal aortic aneurysm. Upper limit of healthy range is shown with *horizontal line.*

tion for rupture. The second aim was the determination of the feasibility of hemostatic markers as diagnostic adjuncts.

METHODS

Patients. Forty-four patients who underwent emergency operation for suspected rupture of infrarenal AAA were prospectively studied. At operation, 37 patients (33 men and four women; median age, 74 years; range, 63 to 87 years) were found to have ruptured AAAs and seven men (median age, 68 years; range, 65 to 74 years) had nonruptured AAAs. Thirty-one patients with rupture had at least one documented episode of hypotension (systolic blood pressure, <100 mm Hg) before surgery. Seven patients with acutely symptomatic nonruptured AAAs and six patients with rupture were not hypotensive before surgery. The clinical data for both groups of patients are shown in Table I. Rupture was defined by the presence of fresh retroperitoneal or intraperitoneal blood in the presence of an aortic aneurysm with no other identifiable cause for the findings. In patients with acutely symptomatic nonruptured AAAs, sudden expansion or impending rupture were presumed to be responsible for the clinical findings because there were no other identifiable causes.

Table I. Clinical data for patients with acutely symptomatic nonruptured and ruptured abdominal aortic aneurysm

	Ruptured AAA (n = 37)	*Nonruptured AAA (n = 7)*
Median age (y)	74 (range, 63 – 87)	68 (range, 65 – 74)
Gender	33 M, 4 F	7 M
Hypotension (systolic BP, <100 mm Hg)	31	–
Comorbidity		
None	6	3
Myocardial infarction	13	–
Angina pectoris	10	1
Coronary artery bypass graft	–	1
Hypertension	13	2
Congestive cardiac failure	2	–
Atrial fibrillation	2	–
Stroke	4	–
Peripheral arterial occlusive disease	2	–
Venous thromboembolism	–	1
Chronic obstructive airways disease	6	–
Non–insulin-dependent diabetes mellitus	1	–
Cigarette smoking		
Nonsmoker	17	3
Ex-smoker	2	1
Current smoker	12	3
Medications		
None	6	4
Aspirin	15	1
Diuretic	12	2
Nitrate	10	1
Angiotensin-converting enzyme inhibitor	6	1
Calcium-channel blocker	4	1
Beta-adrenoceptor blocker	1	1
Bronchodilator	6	–

AAA, Abdominal aortic aneurysm; *M,* male; *F,* female; *BP,* blood pressure.

Sample collection. Blood was sampled from an indwelling arterial line immediately before the induction of anesthesia. No patients underwent a blood transfusion before blood sampling. The samples were placed immediately on ice and centrifuged within 30 minutes of collection at 3000 revolutions per minute for 30 minutes at 4°C (equivalent to 1400*g*). Plasma was separated and stored at –70°C for later batch analysis.

Markers of thrombin generation and fibrinolysis. Platelet count, fibrinogen level, prothrombin time (PT), and activated partial thromboplastin time (aPTT) were measured in the routine hematology laboratory. Plasma prothrombin fragment (PF) 1+2 (healthy range, 0.4 to 1.1 nmol/L) was assayed as a marker of thrombin generation. Plasma tissue plasminogen activator (t-PA) activity (healthy range, 0.2 to 2.0 IU/mL) and plasminogen activator inhibitor (PAI) activity (healthy range, <15 AU/mL) were assayed as markers of primary systemic fibrinolysis. D dimer (healthy range, 630 to 850 ng/mL) was assayed as a marker of secondary fibrinolysis. The healthy range for each hemostatic marker was determined by the manufacturer of the assay. Details of the commer-

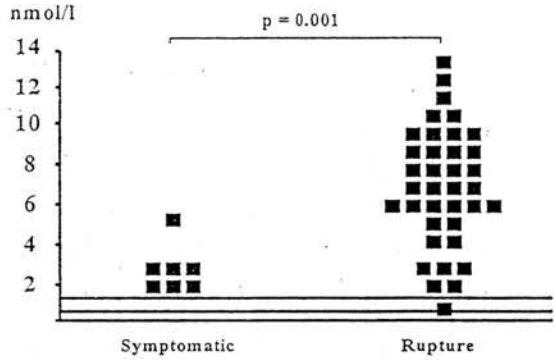

Fig 3. Preoperative prothrombin fragment 1+2 level in acutely symptomatic and ruptured abdominal aortic aneurysm. Upper and lower limits of healthy range are shown with *horizontal lines.*

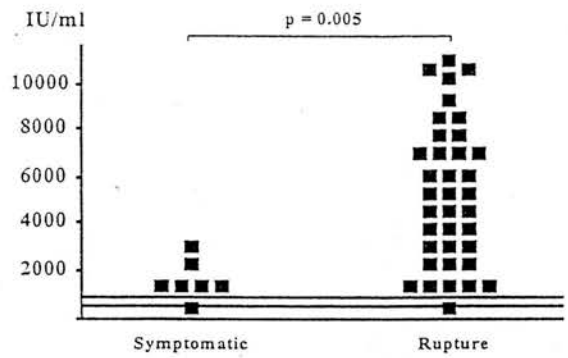

Fig 4. Preoperative D dimer level in acutely symptomatic and ruptured abdominal aortic aneurysm. Upper and lower limits of healthy range are shown with *horizontal lines.*

Table II. Fibrinogen, platelet count, prothrombin time, and activated partial thromboplastin time in patients who underwent operation for acutely symptomatic nonruptured and ruptured abdominal aortic aneurysm (median; range)

Assay	*Healthy range*	*Ruptured AAA (n = 37)*	*Nonruptured AAA (n = 7)*	P *value**
Fibrinogen level	1.5 – 4.0 g/L	2.59 g/L (0.46 – 6.31 g/L)	4.85 g/L (1.61 – 7.9 g/L)	.033
Platelet count	150 – 350 × 10^9/L	178 × 10^9/L (40 – 360 × 10^9/L)	183 × 10^9/L (75 – 292 × 10^9/L)	NS
PT	10.5 – 14.5 seconds	14.3 seconds (9 – 126 seconds)	11.8 seconds (10 – 100 seconds)	NS
aPTT	28 – 40 seconds	33 seconds (26 – 210 seconds)	29 seconds (26 – 36 seconds)	.043

**P* value determined with Mann-Whitney test.
AAA, Abdominal aortic aneurysm; *PT,* prothrombin time; *aPTT,* activated partial thromboplastin time.

cially available assays of coagulation and fibrinolysis have been described in a previous report.[7]

Statistical methods. The Mann-Whitney test was used for the comparison of groups of patients. A probability value of less than .05 was regarded as statistically significant. The sensitivity, specificity, and positive and negative predictive values of each assay (alone and in combination) for the diagnosis of ruptured AAA as compared with operative findings were determined.

RESULTS

Clinical data. There were no deaths after repair of acutely symptomatic nonruptured AAA. The inhospital mortality rate for repair of ruptured AAA was 16 of 37 patients (43%): seven patients died during surgery or within 24 hours of repair, and nine patients died in the late postoperative period. There were no deaths among the six patients with ruptured AAAs whose conditions were normotensive before surgery.

Markers of fibrinolysis and thrombin generation. The median and range values for preoperative platelet count, fibrinogen level, PT, and aPTT are shown in Table II. The median and range values for preoperative t-PA activity, PAI activity, PF 1+2, and D dimer levels are shown in Table III. When compared with ruptured AAA, acutely symptomatic nonruptured AAA was associated with significantly higher fibrinogen level (P = .033), reduced aPTT (P = .043), increased t-PA activity (P = .023; Fig 1), reduced PAI activity (P = .005; Fig 2), reduced PF 1+2 level (P = .001; Fig 3), and reduced D dimer level (P = .005; Fig 4).

When the seven normotensive patients with acutely symptomatic nonruptured AAA were compared with the six normotensive patients with rupture, there was no significant difference in platelet count, fibrinogen level, PT, aPTT, or D dimer level. However, the differences in t-PA activity (nonruptured: median, 1.7 IU/mL; range, 0.75 to 3.2 IU/mL; versus ruptured: median, 0.22 IU/mL; range, 0.11 to 1.0 IU/mL; P = .01), PAI activity (nonruptured: median, 6.3 AU/mL; range, 3.2 to 15.4 AU/mL; versus ruptured: median, 30.3 AU/mL; range, 12.1 to 37.6 AU/mL; P = .004), and PF 1+2 level (nonruptured: median, 2.1 nmol/L; range, 1.1 to 5.2 nmol/L; versus ruptured: median, 5.3 nmol/L; range, 2.5 to 6.8 nmol/L; P = .01) were maintained.

In ruptured AAA, there was no significant difference in any of the markers between patients with hypotensive and normotensive conditions or between patients with

Table III. Tissue plasminogen activator activity, plasminogen activator inhibitor activity, prothrombin fragment 1+2 level, and D dimer level in patients who underwent operation for acutely symptomatic nonruptured and ruptured abdominal aortic aneurysm (median; range)

Assay	*Healthy range*	*Ruptured AAA (n = 37)*	*Nonruptured AAA (n = 7)*	P *value**
t-PA activity	0.2 – 2.0 IU/mL	0.28 IU/mL (0.06 – 9.6 IU/mL)	1.7 IU/mL (0.75 – 3.2 IU/mL)	.023
PAI activity	<15 AU/mL	31 AU/mL (0.1 – 39.4 AU/mL)	6.3 AU/mL (3.2 – 15.4 AU/mL)	.005
PF 1+2 level	0.4 – 1.1 nmol/L	6.4 nmol/L (1.1 – 13.3 nmol/L)	2.1 nmol/L (1.1 – 5.2 nmol/L)	.001
D dimer level	630 – 850 ng/mL	4108 ng/mL (155 – 25,947 ng/mL)	1633 ng/mL (753 – 3014 ng/mL)	.005

**P* value determined with Mann-Whitney test.
AAA, Abdominal aortic aneurysm; *t-PA,* tissue plasminogen activator; *PAI,* plasminogen activator inhibitor; *PF,* prothrombin fragment.

retroperitoneal or intraperitoneal hemorrhage. Nonsurvivors had significantly lower platelet counts (nonsurvivors: median, 126×10^9/L; range, 40 to 321×10^9/L; versus survivors: median, 224×10^9/L; range, 97 to 360×10^9/L; $P = .005$), lower fibrinogen levels (nonsurvivors: median, 2.03 g/L; range, 0.46 to 6.31 g/L; versus survivors: median, 2.8 g/L; range, 0.86 to 5.9 g/L; $P = .007$), and prolonged aPTT (nonsurvivors: median, 39 seconds; range, 28 to 210 seconds; versus survivors: median, 31 seconds; range, 26 to 142 seconds; $P = .01$) as compared with survivors. There was no significant difference in t-PA activity, PAI activity, or PF 1+2 level between survivors and nonsurvivors, but D dimer level was significantly higher in nonsurvivors ($P = .046$).

When all patients were examined, PF 1+2 level was the most accurate assay for distinguishing nonruptured from ruptured AAA. With the upper limit of the healthy laboratory range for PF 1+2 level as a diagnostic cut-off (>1.1 nmol/L), the test had a high sensitivity (36 of 37; 97%) and positive predictive value (36 of 41; 88%) but low specificity (1 of 7; 14%) and negative predictive value (1 of 2; 50%) for the diagnosis of ruptured AAA. When the cut-off for PF 1+2 was increased to greater than or equal to 2.5 nmol/L, the test had a high sensitivity (33 of 37; 89%), specificity (6 of 7; 86%) and positive predictive value (33 of 34; 97%), but low negative predictive value (6 of 10; 60%). When only patients with normotensive conditions were examined, PAI activity was the most accurate assay for distinguishing acutely symptomatic nonruptured from ruptured AAA. PAI activity higher than the healthy range (>15 AU/mL) had a high sensitivity (5 of 6; 83%) and specificity (6 of 7; 86%) and a high positive (5 of 6; 83%) and negative predictive value (6 of 7; 86%) for the diagnosis of rupture. When the cut-off for PAI activity was increased to greater than or equal to 16 AU/mL, the sensitivity and specificity was 5 of 6 (83%) and 7 of 7 (100%) and the positive and negative predictive values were 5 of 5 (100%) and 7 of 8 (88%), respectively.

DISCUSSION

Hemostatic derangement is central to the thrombotic and hemorrhagic complications that are responsible for the considerable majority of the major morbidity and mortality associated with aortic aneurysm surgery.[7-9] Before surgery, asymptomatic AAA is associated with increased thrombin generation[10-13] and essentially healthy systemic fibrinolytic activity in most cases,[14-16] although a proportion of cases show increased systemic fibrinolysis.[7] By contrast, hypotensive patients with ruptured AAA have evidence of increased thrombin generation and inhibition of systemic fibrinolysis.[7] The findings of this study corroborate the finding of a previous report,[7] provide further insight into the pathophysiology of hemostasis associated with ruptured AAA, and reveal novel data regarding hemostasis in patients with acutely symptomatic nonruptured AAA.

The principal finding of this study is that acutely symptomatic nonruptured AAA is associated with increased primary systemic fibrinolysis, reduced secondary fibrinolysis, and reduced thrombin generation as compared with ruptured AAA. The lower t-PA activity in ruptured AAA may be caused by increased inhibition of systemic fibrinolysis (as shown with elevated PAI activity), a finding that was not present in the patients with acutely symptomatic nonruptured AAA. D dimer is a marker of secondary fibrinolysis, which occurs in response to thrombus formation and acts to restore microvascular patency. D dimer and PF 1+2 levels were elevated in both groups of patients but to a greater extent in the ruptured AAA group.

These differences in coagulation and fibrinolysis were also observed when the normotensive patients with acutely symptomatic nonruptured AAA were compared with normotensive patients with rupture. No such differences were present when normotensive and hypotensive patients with ruptured AAA were compared. These findings suggest that hemorrhage rather than hypotension may be one of the principal mechanisms that triggers the generalized procoagulant state in patients with ruptured AAA.

The findings of this study may be explained if one considers that sudden expansion or impending rupture of an aortic aneurysm is analogous to an aortic dissection. Fibrinolytic gene expression and focal fibrinolytic activity have been shown within the aortic wall of patients with asymptomatic AAA,[17,18] and aortic adventitia is associated with elevated fibrinolytic activator activity.[19-21] Sudden expansion leads to bloodflow in the marginal thrombus adjacent to the diseased aortic wall, and as the surface area of aortic adventitia exposed to blood flow increases, there is increased local and systemic fibrinolysis.[19] Aneurysm rupture and subsequent hemorrhage trigger a procoagulant and hypofibrinolytic state that acts to minimize local blood loss but has the detrimental effect of stimulating microvascular and macrovascular thrombosis.

Many clinicians have advocated the use of emergency CT scanning to differentiate acutely symptomatic nonruptured AAA from rupture. Although these early studies reported acceptable diagnostic accuracy, the conclusions were limited by the fact that a significant proportion of patients did not undergo operation and thus CT scan findings could not be correlated with operative findings.[6] Previous results from this group have shown that, in cases of true clinical uncertainty, CT scanning has an unacceptably low sensitivity and specificity of 79% and 77%, respectively, in patients with hemodynamically stable conditions.[6] There are no data to show that advances in CT technology have been associated with improved diagnostic accuracy in this clinical situation.

In this study, there was no significant difference in platelet count, fibrinogen level, PT, aPTT, or D dimer level between normotensive patients with nonruptured and ruptured AAA. There were, however, significant differences in t-PA activity, PAI activity, and PF 1+2 level. Furthermore, preoperative PAI activity greater than or equal to 16 AU/mL was more accurate than our experience with emergency CT scanning in these patients with hemodynamically stable conditions. Although the measurement of PAI activity may distinguish acutely symptomatic nonruptured AAA from ruptured AAA, currently, the time taken to prepare the plasma and perform the assay (approximately 2 hours) precludes its use as a diagnostic adjunct. The small numbers of patients studied with nonruptured AAA do not allow the authors to reach strong conclusions or make firm clinical recommendations. However, should a rapid assay for PAI activity become available, then it may be of value in distinguishing rupture from nonrupture in normotensive patients who are seen with acutely symptomatic aneurysms.

In conclusion, these novel data show that patients with acutely symptomatic nonruptured AAAs do not manifest the same pattern of hemostatic derangement evident in patients with rupture. Preoperative PAI activity higher than the healthy range was more accurate than our experience with CT scanning in patients who were hemodynamically stable with suspected ruptured AAAs.

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Plasma endothelin levels and outcome in patients undergoing repair of ruptured infrarenal abdominal aortic aneurysm

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Background: Endothelin-1 (ET-1) is the most potent known vasoconstrictor. Elevated plasma levels have been demonstrated in patients with myocardial infarction, cardiogenic and septic shock, and respiratory, heart, and kidney failure, as well as in those undergoing elective abdominal aortic aneurysm (AAA) repair. However, endothelin levels have not previously been examined in patients undergoing repair of ruptured AAA. We hypothesized that hemorrhagic shock, lower torso ischemia, and reperfusion associated with ruptured AAA repair lead to increased synthesis and secretion of ET-1, which, in turn, predispose to organ failure, one of the principal causes of death in this condition.

Methods: Fourteen patients were studied. Plasma levels of big ET-1 and ET-1 were measured immediately before operation and immediately before, 5 minutes, and 6 hours after aortic clamp release.

Results: All patients survived for at least 24 hours after operation. Big ET-1 levels were above the normal range at one or more sample points in all patients, and the ET-1 levels were above the normal range in all survivors and four of five nonsurvivors. Five patients who died of organ failure had significantly lower big ET-1 levels at all sample points and significantly lower ET-1 levels after 5 minutes of reperfusion when compared with survivors. Preoperative ET-1 levels were significantly lower in eight patients who subsequently developed kidney failure than in six patients who did not.

Conclusion: Contrary to our original hypothesis, these novel data demonstrate that patients with ruptured AAA in whom fatal postoperative organ failure develops have significantly lower perioperative endothelin levels than survivors.
(J Vasc Surg 2001;33:1242-6.)

Endothelin-1 (ET-1) is the most potent known vasoconstrictor. It is principally secreted abluminally from vascular endothelial cells but may enter the circulation if concentrations are high at the endothelial cells–vascular smooth muscle interface.[1] ET-1 leads to vasoconstriction in resistance vessels, especially the coronary, cerebral, and renal circulation, by acting on ET_A receptors in vascular smooth muscle cells, and ET_B receptors on vascular smooth muscle and endothelial cells. Big ET-1 is the immediate precursor of ET-1, and its conversion to biologically active ET-1 by endothelin-converting enzymes occurs mainly in the vessel wall.[2,3] Big ET-1 is detectable in the plasma for considerably longer than ET-1,[4] and increased plasma levels of big ET-1 are considered to represent increased ET-1 generation. Elevated plasma endothelin levels may form part of a homeostatic response to maintain systemic blood pressure[5] and have been demonstrated in critically ill patients with myocardial infarction,[6] cardiogenic shock,[7] septic shock,[8] adult respiratory distress syndrome,[9] heart failure,[10,11] and acute kidney failure.[12] To date, several animal and human studies of septic shock have suggested that endothelin release may be pathologic[13-18] or homeostatic.[18,19] Increased plasma endothelin levels have been demonstrated in patients undergoing nonruptured abdominal aortic aneurysm (AAA) repair with infrarenal[20,21] and supraceliac aortic cross-clamping,[22] as well as in one animal model of infrarenal aortic clamping and subsequent exsanguination.[23] To date, however, there are no reports of the endothelin response in patients undergoing repair of ruptured AAA. The aim of this study was to examine, for the first time, perioperative changes in plasma levels of big ET-1 and ET-1 in patients undergoing repair of ruptured AAA. We hypothesized that hemorrhagic shock, ischemia, and reperfusion would lead to increased synthesis and secretion of endothelin, which would predispose to the development of organ failure, one of the principal causes of death in this group of patients.

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METHODS

Patients. Fourteen consecutive patients (13 men and 1 woman of median age 74 years; range, 65-86) who underwent repair of ruptured infrarenal AAA and survived to at least 24 hours after surgery were prospectively studied. Lothian Research Ethics Committee approval was

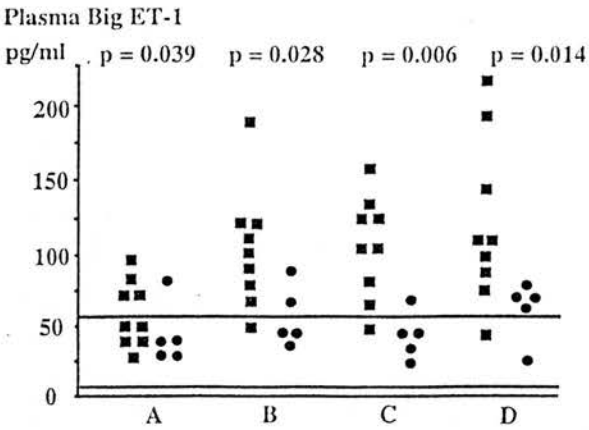

Fig 1. Individual data points for big ET-1 immediately before induction of anesthesia (sample A), immediately before release of aortic clamp, (sample B), and 5 minutes (sample C), and 6 hours (sample D) after aortic clamp release in 14 patients who underwent operation for ruptured AAA. Survivors (n = 9) are represented by *black squares* and nonsurvivors (n = 5) by *black circles*. Normal laboratory range for big ET-1 (10-60 pg/mL) is shown by *parallel horizontal lines*. Mann-Whitney *U* test was used to test whether medians of samples in survivors and nonsurvivors were significantly different from each other at each sample point. A *P* value less than .05 was regarded as statistically significant.

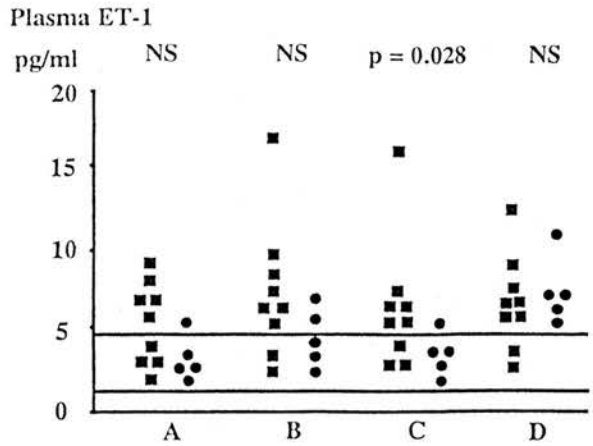

Fig 2. Individual data points for ET-1 immediately before induction of anesthesia (sample A), immediately before release of aortic clamp, (sample B), and 5 minutes (sample C), and 6 hours (sample D) after aortic clamp release in 14 patients who underwent operation for ruptured AAA. Survivors (n = 9) are represented by *black squares* and nonsurvivors (n = 5) by *black circles*. Normal laboratory range for ET-1 (1.5-4.5 pg/mL) is shown by *parallel horizontal lines*. Mann-Whitney *U* test was used to test whether medians of samples in survivors and nonsurvivors were significantly different from each other at each sample point. A *P* value less than .05 was regarded as statistically significant. *NS*, Not significant.

obtained. The median (range) delay between the onset of symptoms of rupture and hospital admission was 5 (3-14) hours. All patients had at least one documented episode of hypotension (systolic blood pressure < 100 mm Hg) before surgery.

Operative methods. Ruptured AAA was defined by the presence of fresh retroperitoneal blood at operation. No patient had intraperitoneal rupture. All patients underwent AAA repair under general anesthesia through a transverse supraumbilical incision with infrarenal aortic cross-clamping. No patient required suprarenal aortic clamping. An aortoaortic graft was inserted in 10 patients, an aortobifemoral graft in three, and an aortobiiliac graft in one patient.

Sample collection and assay methods. Blood (8.2 mL) was obtained from an indwelling radial arterial line and placed into ethylenediamine tetra-acetic acid (2.7 mL) for estimation of hematocrit, and lithium heparin (5.5 mL) for estimation of big ET-1 and ET-1. Blood was sampled immediately before the induction of anesthesia (sample A), immediately before aortic clamp release (sample B), and 5 minutes (sample C) and 6 hours (sample D) after aortic clamp release. Samples were placed immediately on ice and spun in a centrifuge within 30 minutes of collection at 1400*g* for 30 minutes at 4°C. Plasma was separated and stored at −70°C for later batch analysis.

Hematocrit was estimated in the routine hematology laboratory with the fully automated Sysmex NE 8000 analyzer (Sysmex, Milton Keynes, UK). Plasma immunoreactive big ET-1 and ET-1 concentrations were measured by use of an acetic acid extraction technique[24] and a modified commercial radioimmunoassay with rabbit antihuman big ET-1 or ET-1 (Peninsula Laboratories Europe, St Helens, UK). Sample extract was incubated with either big ET-1 or ET-1 antibody for 24 hours at 4°C. After incubation, 125-labeled big ET-1 (Peninsula Laboratories Europe) or ET-1 (NEN Life Science Products, Boston, Mass) was added, and incubation was continued for an additional 20 minutes at 4°C. Complexes were precipitated with Amerlex donkey antirabbit antibody (Amersham Life Sciences Limited, UK) and counted for radioactivity. The lower limits of detection for the big ET-1 and ET-1 assays are 1 pg/mL and 0.25 pg/mL, respectively. The normal laboratory range for big ET-1 is 10 to 60 pg/mL, and for ET-1 1.5 to 4.5 pg/mL.

Definitions of postoperative organ failure. Cardiac failure was defined as arrhythmia requiring pharmacologic treatment to maintain cardiovascular stability and/or sustained periods of hypotension (mean arterial pressure ≤ 60 mm Hg) requiring fluid resuscitation and inotropic support. Respiratory failure was defined as hypoxia requiring mechanical ventilatory support for more than 4 days. Kidney failure was defined as elevated serum creatinine level greater than or equal to 250 μmol/L and/or the requirement for renal replacement therapy. Disseminated intravascular coagulation was defined as clinical evidence of hemorrhage accompanied by laboratory evidence of thrombocytopenia, prolonged clotting times, hypofibrino-

Table I. Clinical and operative data

	Survivors (n = 9)		*Nonsurvivors (n = 5)*		
	Median	*Range*	*Median*	*Range*	*P value**
Preoperative					
Duration of symptoms (h)	4	(3-14)	6	(5-12)	NS
Serum creatinine level (μmol/L)	138	(82-176)	115	(77-183)	NS
Intravenous fluid administration before clamping (L)	0.5	(0.2-5.5)	0.7	(0.1-1.0)	NS
Intraoperative					
Total operation time (min)	140	(75-240)	105	(75-200)	NS
Aortic clamp time (min)	90	(40-185)	75	(55-135)	NS
Measured blood loss (L)	4.0	(1.0-6.4)	3.3	(1.5-11.0)	NS
Crystalloid and colloid administration (L)	3.4	(1.5-8.0)	3.8	(3.5-5.0)	NS
pRBC administration (units)	8	(5-11)	8	(6-22)	NS
FFP administration (units)	2	(2-6)	2	(2-12)	NS
Platelet administration (bags)†	1	(1)	1	(0-1)	NS

pRBC, Packed red blood cells (300 mL); *FFP*, fresh frozen plasma (300 mL).
*Mann-Whitney *U* test.
†One bag of platelet transfusion = 4 pooled units (250 mL).

genemia, and elevated levels of fibrin/fibrinogen degradation products.

Statistical methods. The Mann-Whitney *U* test was used to test whether the medians of samples in survivors and nonsurvivors were significantly different from each other and to examine whether there was a difference in levels between patients who had postoperative organ failure compared with patients who did not. The Kruskal-Wallis one-way analysis of variance was used to examine whether assay levels changed significantly over the four sampling points in survivors and nonsurvivors. A *P* value of less than .05 was regarded as statistically significant.

RESULTS

Clinical data. All patients were admitted to the intensive therapy unit after operation. All patients survived for at least 24 hours after operation, but five (36%) died in the postoperative period. Clinical and operative data for survivors and nonsurvivors are summarized in Table I, and postoperative complications are shown in Table II.

Plasma levels of big ET-1 and ET-1. There was no significant difference in hematocrit between survivors and nonsurvivors at any of the sample points. The values of big ET-1 and ET-1 in survivors and nonsurvivors are shown in Figs 1 and 2, respectively. The big ET-1 level was above the normal laboratory range at one or more sampling points in all patients, and the ET-1 level was above the normal range in all survivors and four of five nonsurvivors. When compared with nonsurvivors, survivors had significantly higher levels of big ET-1 at all four sampling points and significantly higher levels of ET-1 after 5 minutes' reperfusion than nonsurvivors. When compared with preoperative levels, there was a significant increase in big ET-1 levels after 6 hours of reperfusion in survivors. In nonsurvivors, there was a significant increase in ET-1 levels between 5 minutes and 6 hours after reperfusion. Preoperative ET-1 levels were significantly lower in eight patients who subsequently had kidney failure (median, 3.72; range, 2.76-6.0 pg/mL) than in six patients who did not (median, 5.89; range, 3.86-7.23 pg/mL; $P = .02$). There was no significant difference in big ET-1 or ET-1 levels between patients who did and did not have cardiac failure, respiratory failure, or disseminated intravascular coagulation.

DISCUSSION

This study is the first to examine the relationship between perioperative endothelin levels, organ failure, and death in patients undergoing ruptured AAA repair. The principal finding is that, contrary to our original hypothesis, patients who died had significantly lower perioperative endothelin levels than survivors.

Previous studies of the endothelin response to lower torso ischemia and reperfusion are few and contradictory. Antonucci et al[20] examined the effect of intraoperative nifedipine infusion on endothelin-dependent renal vasoconstriction in five patients undergoing nonruptured infrarenal AAA repair and demonstrated a transient but significant increase in plasma ET-1 and -2 levels at the end of the period of aortic cross-clamping. The authors concluded that nifedipine prevented the renal vasoconstrictor response to endothelin because there was no significant difference in creatinine clearance and glomerular filtration rate after operation compared with before operation. Fukuda et al[21] measured arterial and iliac vein ET-1 levels in seven patients undergoing elective aortic aneurysm repair. There was no significant change in arterial ET-1 levels, but a significant increase in iliac vein ET-1 levels occurred immediately after aortic clamp release and perfusion of the first limb. Venous ET-1 levels showed a significant correlation with venous O_2 content, pH, partial pressure of oxygen O_2, O_2 saturation, and base excess suggesting that ET-1 production occurred because of lower limb ischemia. Lintott et al[22] were the first to attempt to

examine the relationship between ET-1 levels and outcome in 21 patients who required supraceliac and eight who required infrarenal aortic clamping for repair of nonruptured aortic aneurysm. Unlike the studies by Antonucci et al[20] and Fukuda et al,[21] plasma ET-1 was undetectable during the period of aortic clamping and 30 minutes after aortic declamping. After 2 hours of reperfusion, ET-1 levels were significantly higher in patients in whom acute kidney failure developed after supraceliac clamping, and at 8 hours, ET-1 levels were significantly higher in the supraceliac clamp group compared with the infrarenal clamp group. In a canine model of infrarenal aortic clamping, Edwards et al[23] failed to demonstrate a significant increase in plasma ET-1 levels during hind limb ischemia, but there was a significant increase during reperfusion and subsequent exsanguination.

In this study, survivors had increased plasma endothelin levels during the periods of lower torso ischemia and reperfusion. Furthermore, more than half of the survivors had increased endothelin levels before operation. There was also a significant increase in endothelin levels after 6 hours of reperfusion in survivors and nonsurvivors. The increased physiological insult of emergency ruptured AAA repair may explain why, unlike previous studies of nonruptured AAA repair, elevated endothelin levels were detected before, during, and after operation.

It is interesting to speculate from these data that the ET-dependent vasoconstrictor response to hemorrhagic shock, ischemia, and reperfusion has a homeostatic and protective role in ruptured AAA, in that patients who manifest a good vasoconstrictor response (which is partly due to endothelin) have a higher probability of survival than those patients whose response is inferior. This hypothesis would be in keeping with what most vascular surgeons know intuitively: that is, intense vasoconstriction (as well as "controlled" hypotension, aortic tamponade, and the generation of a prothrombotic state) is one of the principal mechanisms that allows patients with ruptured AAA to reach the hospital in better clinical condition and then undergo successful aneurysm repair.

The reasons for low endothelin levels in nonsurvivors, as well as those patients in whom acute kidney failure developed, are not immediately obvious because ruptured AAA repair is associated with many factors known to stimulate endothelin synthesis and secretion: intraoperative hemorrhage and hemodilution, hypoxia, and metabolic acidosis, increased sympathetic discharge and catecholamine release, increased cytokine and endotoxin release, thrombin generation, and impaired renal excretion. In this study, there was no apparent difference in the duration of symptoms of rupture, severity of preoperative shock, duration of lower torso ischemia or perioperative hematocrit between survivors and nonsurvivors. Most patients received renal-dose dopamine in the perioperative period, but, unlike nifedipine, this has not been shown to reduce plasma endothelin levels or maintain glomerular filtration rate in patients undergoing major aortic surgery.[25]

Haynes et al[26] reported a significant, and similarly unexpected, association between high plasma ET-1 levels and survival in patients with cardiac arrest. They proposed several explanations for their findings: poor peripheral blood flow and local tissue acidosis may adversely affect production and activity of ET-1, or lead to a local increase in ET-1 that does not enter the circulation; increased nitric oxide production may inhibit endothelin production; and reduced pulsatile shear stress may lead to selective endothelial cell dysfunction. The low endothelin levels demonstrated in nonsurvivors of ruptured AAA and cardiac arrest[26] may therefore be an early manifestation of irreversible whole body hypoperfusion. Studies from this department have shown that survivors and nonsurvivors of ruptured AAA repair have elevated plasma levels of the endothelial products, tissue plasminogen activator and plasminogen activator inhibitor.[27] These and present data lend support to the hypothesis that selective endothelial cell dysfunction may lead to downregulation of the endothelin response in some patients with ruptured AAA, and this may predispose to the development of fatal organ dysfunction.

Table II. Postoperative complications

	Survivors (n = 9)	*Nonsurvivors (n = 5)*
Cardiovascular		
Cardiac failure	4	3
Myocardial infarction	1	—
Stroke	2	1
Critical lower limb ischemia	1	2
Respiratory		
Respiratory failure	3	4
Acute respiratory distress syndrome	—	1
Pneumonia	6	5
Acute kidney failure	3	5
Disseminated intravascular coagulation	1	2
Sepsis syndrome	1	2
Colon ischemia	1	—
Inotropic support		
Adrenaline	2	3
Dopamine	7	4

Although most of the current literature concludes that endothelin release has a pathologic role in critical illness,[13-18] the findings of this study do not support the hypothesis that an increased endothelin response predisposes to poor outcome in patients undergoing ruptured AAA repair. By contrast, elevated perioperative endothelin levels were associated with survival. Increased circulating endothelin levels may occur as part of a homeostatic and protective response to hemorrhage, ischemia, and reperfusion in patients with ruptured AAA, or alternatively, low endothelin levels may be an early marker of severe and irreversible whole body hypoperfusion in this group of patients.

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Elevated levels of soluble tumor necrosis factor receptors are associated with increased mortality rates in patients who undergo operation for ruptured abdominal aortic aneurysm

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Purpose: Elevated levels of soluble tumor necrosis factor receptors (sTNF-Rs) are associated with multiple organ failure and increased mortality rates in critically ill patients. Paradoxically, experimental data suggest exogenous sTNF-Rs may improve outcome in patients who undergo elective abdominal aortic aneurysm (AAA) repair. This study examines, for the first time, changes in sTNF-R levels during repair of ruptured and nonruptured AAA.

Methods: Sixteen patients who underwent surgical procedures for ruptured AAA and 10 patients who underwent surgical procedures for nonruptured AAA were studied. Levels of sTNF-Rs p55 and p75 were measured before the operation and immediately before and 5 minutes, 6 hours, and 24 hours after aortic clamp release.

Results: When compared with nonruptured AAA, levels of sTNF-R p55 were significantly higher in ruptured AAA 5 minutes ($P < .02$) and 24 hours after aortic clamp release ($P < .05$). Levels of sTNF-R p75 were significantly higher in ruptured AAA before ($P < .05$), during ($P < .001$), and after the surgical procedure ($P < .01$). Six hours after aortic clamp release, sTNF-R p75 levels were significantly higher in nonsurvivors of ruptured AAA when compared with survivors ($P < .05$) and patients who underwent surgical procedures for nonruptured AAA ($P < .01$).

Conclusion: Ruptured AAA repair is associated with increased sTNF-R expression. Furthermore, elevated levels of sTNF-R p75 are associated with increased postoperative mortality rates. (J Vasc Surg 2000;31:514-9.)

The proinflammatory cytokine, tumor necrosis factor (TNF), has been implicated in the pathophysiologic features of the systemic inflammatory response syndrome and multiple organ failure.[1,2] TNF acts by binding to target cells at specific TNF receptors (TNF-Rs) of 55 kDa and 75 kDa molecular mass (p55 and p75), respectively.[3,4] The binding of TNF leads to cleavage of these receptors from target cells and their release into the circulation as soluble TNF receptors (sTNF-Rs),[5] and it has been suggested that the levels of sTNF-Rs may reflect the degree of TNF-induced tissue injury.[6,7] The finding that high concentrations of sTNF-Rs act as endogenous TNF antagonists and that low concentrations may slow down TNF clearance, so prolonging its activity,[7] has led to the suggestion that the administration of exogenous sTNF-Rs may ameliorate the effects of TNF.

Circulating TNF has been demonstrated in elective and emergency aortic surgery,[8-16] and elevated levels of sTNF-Rs have been detected in patients who undergo repair of nonruptured abdominal aortic aneurysm (AAA) [16,17] and after the operation in patients with ruptured AAA.[16] To date, however, no study has examined the levels of sTNF-Rs during the periods of hemorrhagic shock, lower torso ischemia,

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and early reperfusion, which occur in ruptured AAA repair. This is clinically relevant because, although experimental data suggest that the administration of exogenous sTNF-R before aortic crossclamping is associated with a reduction in circulating TNF and subsequent lung injury in an animal model,[18] human studies have shown that elevated levels of endogenous sTNF-Rs are actually associated with the development of multiple organ failure and increased mortality rates in sepsis,[19] pancreatitis,[20] and multiple trauma.[21] Although exogenous sTNF-R therapy may ameliorate the adverse effects of TNF in elective aortic surgery in which endogenous levels are low, it may be ineffective if endogenous levels are high in patients who undergo ruptured AAA repair.[19] The aims of this study were to examine serial changes in sTNF-Rs occurring during emergency repair of ruptured infrarenal AAA and to compare the findings with patients who undergo elective repair of nonruptured AAA.

METHODS

Patients. Sixteen patients (14 men and 2 women; median age, 75 years; range, 65-86 years) who underwent operation for ruptured infrarenal AAA and 10 patients (eight men and two women; median age, 69 years; range, 58-80 years) who underwent operation for asymptomatic nonruptured infrarenal AAA were prospectively studied. Lothian Region Ethical Committee approval and fully informed written consent from all patients were obtained. In patients who underwent operation for ruptured AAA, the median delay between the onset of symptoms of rupture and hospital admission was 5 hours (range, 3-14 hours). All patients had at least one documented episode of hypotension (systolic blood pressure less than 100 mm Hg) before the surgical procedure. In patients who underwent an operation for asymptomatic nonruptured AAA, the median anteroposterior diameter of the aneurysm measured by abdominal ultrasonography was 6.5 cm (range, 5.5-8.0 cm).

Operative methods. Ruptured AAA was defined as the presence of fresh retroperitoneal blood at the time of the operation. Three patients had retroperitoneal and intraperitoneal rupture. Patients with rupture were given general anaesthesia, and patients with nonruptured AAA were given combined general and epidural anesthesia. All patients underwent AAA repair through a transverse supraumbilical incision with infrarenal aortic clamping. A Dacron tube graft was inserted in 17 patients (12 patients with rupture, 5 patients with nonrupture), aortobi-iliac graft in five (one patient with rupture, four patients with nonrupture) and aortobifemoral graft in four patients (three patients with rupture, one patient with nonrupture). Aortic clamp time was defined as the time between aortic clamp placement and the completion of the distal anastomosis for patients who underwent tube graft repair and the completion of the first and second distal anastomoses for patients who underwent repair with a bifurcated graft.

Sample collection. The pathophysiology of ruptured AAA repair can be divided into three phases. First, there is a period of whole-body hypoperfusion as the result of hypovolemic shock. Second, there is a period of profound lower body ischemia after aortic clamp placement. Finally, if repair is successful, there is a period of reperfusion. The sampling points were chosen to reflect the maximum effect of each of the three pathophysiologic phases of ruptured AAA repair. Blood was sampled from an indwelling arterial line immediately before the induction of anesthesia (sample A); immediately before release of the aortic clamp (sample B); and 5 minutes (sample C), 6 hours (sample D), and 24 hours (sample E) after aortic clamp release. For patients who underwent repair with a bifurcated graft, sampling points C, D, and E were relative to reperfusion of the first limb. Blood (9 mL) was collected into a clot activator for the estimation of serum levels sTNF-Rs p55 and p75. Samples were placed immediately on ice and centrifuged within 30 minutes of collection at 3000 revolutions per minute for 30 minutes at 4°C (equivalent to 1400*g*). Serum was separated and stored at −70°C for later batch analysis.

Assays of soluble TNF receptors p55 and p75. Serum levels of sTNF-Rs p55 and p75 were detected by enzyme-linked immunosorbent assay, with monoclonal and polyclonal anti-sTNF-R p55 and p75 antibodies (donated by Dr W. A. Buurman, Maastricht, The Netherlands).[22] Purified sTNF-R p55 and p75 were used to construct standard curves. The lower limit of detection of the sTNF-R p55 assay was 0.2 ng/mL and of the sTNF-R p75 assay was 2 ng/mL.

Statistical methods. The Mann-Whitney *U* test was used to test whether the medians of two samples were significantly different from each other and to examine whether there was a difference in assay levels between patients with ruptured AAA who had (1) one or more cardiovascular complications, (2) one or more respiratory complications, (3) sepsis syndrome, or (4) renal failure compared with patients who did not have these complications.

Table I. Operative patient data

	Ruptured AAA median (range) (n =16)	*Nonruptured AAA median (range) (n =10)*	P *value**
Preoperative			
Crystalloid administration (L)	0.5 (0.1-4.0)	—	—
Colloid administration (L)	0 (0-1.5)	—	—
Intraoperative			
Operation time (min)	110 (70-250)	160 (85-285)	NS
Total aortic clamp time (min)	75 (30-180)	75 (30-150)	NS
Tube graft (min)	69 (35-180)	77 (45-120)	—
Bifurcate graft: first limb (min)	95 (32-130)	68 (38-90)	—
Bifurcate graft: second limb (min)	113 (42-140)	71 (52-110)	—
Measured blood loss (L)	3.1 (1.0-11.0)	2.4 (0.4-6.0)	NS
Crystalloid administration (L)	2.3 (0.5-6.0)	1.9 (1.0-4.0)	NS
Colloid administration (L)	1.5 (0-2.3)	1.5 (1.0-4.1)	NS
Packed red blood cell administration (units)†	8 (5-22)	4 (0-10)	.004
Fresh frozen plasma administration (units)†	2 (0-12)	0 (0-2)	.0003
Platelet administration (bags)‡	1 (0-1)	0 (0-1)	.0007

*Mann-Whitney *U* test.
†300 mL.
‡One bag of platelet transfusion = 4 pooled units (250 mL).
NS, Not significant.

The Kruskall-Wallis one-way analysis of variance was used to examine whether assay levels changed significantly over the five sampling points in the ruptured and nonruptured AAA groups separately. In addition, the differences between the average rankings were examined to determine whether they exceeded a precalculated critical value.[23] In patients who underwent operations for ruptured AAA, Spearman's rank correlation was calculated between the assay levels and the following clinicopathologic variables: duration of symptoms before hospital admission, volume of preoperative fluid resuscitation, preoperative serum creatinine level, operative blood loss, volume of intraoperative fluid resuscitation (crystalloid and colloid and packed red blood cells), and aortic crossclamp time. Multiple regression was used to examine the independent effect of these variables and the assay levels at each of the five sampling points. Age and sex had forced entry, and a forward-stepping procedure was used. A probability value of less than .05 was regarded as statistically significant. When levels of sTNF-Rs were below the limit of detection of the assay, the minimum detection concentration was assigned to that sample, and statistical analysis was performed with this convention.[20]

RESULTS

Clinical data. Operative data for both groups of patients are summarized in Table I. All patients with ruptured AAA were admitted to the Intensive Care Unit (ICU) for postoperative care and ventilatory support. The median duration of ICU stay was 3 days (range, 0.5-24.1 days). The median duration of ventilatory support was 0.9 days (range, 0.4-18.8 days), and seven patients received ventilatory support for more than 4 days. All patients who underwent operations for nonruptured AAA were admitted to the intermediate care unit after the operation, and no patient was admitted to the ICU or required ventilatory support. Major postoperative complications developed in 13 patients who underwent operation for rupture and in 4 patients who underwent operation for nonruptured AAA (Table II). Three patients with rupture and six patients with nonrupture had no postoperative complications. All patients survived to 24 hours after repair. Five patients (31.2%) with ruptured AAA died in the hospital. There were no deaths after the repair of nonruptured AAA.

Assays of soluble TNF receptors p55 and p75. The median values for sTNF-Rs p55 and p75 are shown in Table III. Both types of sTNF-R were detectable at one or more sampling points in all patients with ruptured AAA and in 9 of 10 patients with nonruptured AAA.

Five minutes and 24 hours after aortic clamp release, levels of sTNF-R p55 were significantly higher in patients with ruptured compared with nonruptured AAA. There was no significant change in the levels of sTNF-R p55 as a function of time in either group.

At all sampling points, levels of sTNF-R p75 were significantly higher in patients with ruptured compared with nonruptured AAA, and there was a significant increase in the levels of sTNF-R p75 during reperfusion in both groups of patients (Table III). Six hours after aortic clamp release, levels of sTNF-R p75 were significantly higher in nonsurvivors of ruptured AAA (median, 14.6 ng/mL; range, 9.7-18.0 ng/mL) compared with survivors (median, 6.3 ng/mL; range, 2.1-18.5 ng/mL; $P = .036$) and patients who underwent operation for nonruptured AAA (median, 2.5 ng/mL; range, 2.0-5.9 ng/mL; $P = .002$). There was no significant difference in levels of sTNF-R p75 between nonsurvivors of rupture, survivors of rupture, and patients who underwent operation for nonruptured AAA at any other sampling point.

In patients who underwent operation for rupture, sTNF-R p55 levels immediately before ($P = .015$) and 5 minutes after aortic clamp release ($P = .034$) were significantly lower in six patients who subsequently experienced the development of renal failure compared with 10 patients who did not. There was not a significant difference in sTNF-R p75 levels among patients who did and did not have cardiovascular complications, respiratory complications, sepsis syndrome, or renal failure.

In patients who underwent operation for rupture, Spearman's rank test demonstrated a significant negative association between aortic crossclamp time and sTNF-R p55 level immediately before aortic clamp release ($r = -0.53$), volume of preoperative fluid resuscitation and sTNF-R p75 level at 24 hours ($r = -0.52$), and volume of intraoperative packed red blood cells and sTNF-R p75 level after 5 minutes of reperfusion ($r = -0.55$). After adjustment for age and sex, multiple regression analysis demonstrated an independent association between aortic crossclamp time and sTNF-R p55 level immediately before ($P = .028$) and 5 minutes after aortic clamp release ($P = .028$), volume of intraoperative crystalloid and colloid resuscitation and sTNF-R p55 level at 6 hours after aortic clamp release ($P = .013$), and volume of preoperative fluid resuscitation and sTNF-R p75 level at 5 minutes after aortic clamp release ($P = .008$).

DISCUSSION

The present study has demonstrated, for the first time, that hemorrhagic shock, lower torso ischemia, and early reperfusion occurring in the course of ruptured AAA repair are associated with elevated levels of sTNF-Rs p55 and p75 and that elevated levels of sTNF-R p75 during the period of early reperfusion are associated with increased postoperative mortality rates. Furthermore, sTNF-Rs appeared rapidly after the onset of hemorrhagic shock and lower torso ischemia,[24] a finding that is more indicative of a direct relationship between sTNF-R levels and increased mortality rates than in a previous study where sTNF-Rs p55 and p75 levels were measured daily for 5 days after operation.[16]

Studies that report the time course of TNF and sTNF-R release in response to lower extremity ischemia and reperfusion are few and contradictory. Animal studies have either demonstrated no significant increase in TNF levels during ischemia or reperfusion,[27] a transient increase within minutes of reperfusion as the result of washout from the lower extremities,[28] or increased levels during ischemia but not reperfusion.[18] Human studies that have included a statistical analysis of the time course of TNF release have failed to demonstrate a significant increase during early reperfusion in elective aortic reconstruc-

Table II. Postoperative complications

	Ruptured AAA (n = 16)	*Nonruptured AAA (n = 10)*
Cardiovascular		
Atrial fibrillation	5	1
Congestive cardiac failure	5	2
Myocardial infarction	1	0
Stroke	2	0
Lower limb critical ischemia	3	1
Proximal deep venous thrombosis	2	1
Respiratory		
Pneumonia	11	2
Respiratory failure	5	0
Acute respiratory distress syndrome	1	0
Acute renal failure	6*	1
Disseminated intravascular coagulopathy	2	0
Sepsis syndrome	3	0
Colon ischemia	1	0
Total parenteral nutrition	5	0
Inotropic support		
Adrenaline	5	0
Renal dose dopamine	11	0
Reoperation	3†	1‡
Total	13/16 (81%)	4/10 (40%)

*Three of six patients who experienced the development of acute renal failure received hemofiltration.
†Patient 1, laparotomy for hemorrhage, femoral thrombectomy, Hartmann's procedure for colon ischemia, drainage of infected pelvic hematoma; patient 2, negative laparotomy for suspected colon ischemia; patient 3, bilateral below knee amputations for critical limb ischemia.
‡Popliteal thrombectomy and fasciotomies.

Table III. Serum levels of soluble sTNF-Rs p55 and p75

	*Ruptured AAA** *median (range)* *(n = 16)*	*Nonruptured AAA†* *median (range)* *(n = 10)*	P *value**
sTNF-R p55 level (ng/mL)			
Immediately before induction of anesthesia (A)	1.3 (0.2-4.9)	0.8 (0.2-1.4)	NS
Immediately before aortic clamp release (B)	1.1 (0.2-2.8)	0.6 (0.2-1.1)	NS
5 minutes after aortic clamp release (C)	1.1 (0.2-3.5)	0.5 (0.2-1.2)	.018
6 hours after aortic clamp release (D)	1.5 (0.3-5.3)	0.7 (0.2-4.0)	NS
24 hours after aortic clamp release (E)	1.6 (0.2-4.4)	0.8 (0.2-2.1)	.042
Significant differences between samples†	None	None	
sTNF-R p75 level (ng/mL)			
Immediately before induction of anesthesia (A)	4.0 (2.0-14.7)	2.1 (2.0-9.0)	.028
Immediately before aortic clamp release (B)	3.3 (2.0-9.1)	2.0 (2.0-2.2)	.0005
5 minutes after aortic clamp release (C)	3.2 (2.0-9.7)	2.0 (2.0-2.2)	.0013
6 hours after aortic clamp release (D)	10.0 (2.1-18.5)	2.5 (2.0-5.9)	.0009
24 hours after aortic clamp release (E)	11.1 (2.4-23.6)	2.6 (2.0-7.3)	.0022
Significant differences between samples†	B-D, B-E, C-D, C-E	B-D, B-E	<.05

NS, Not significant.
*Mann-Whitney *U* test.
†Kruskall-Wallis test.

tion.[12-14] To date, two studies have examined sTNF-R release before and after aortic aneurysm repair. Froon et al[16] measured sTNF-R p55 and p75 levels in 21 patients with ruptured AAA and 9 patients with nonruptured AAA and demonstrated significantly higher levels of both sTNF-Rs in shocked patients and nonsurvivors; Soong et al[17] measured sTNF-R p55 levels in 11 patients with nonruptured AAA and demonstrated that levels were lower in four nonsurvivors but that the postoperative increase was significantly greater at 48 hours when compared with survivors. The present study is the first therefore to examine sTNF-R release before, during, and early after ruptured and nonruptured AAA repair. Although there was no significant change in sTNF-R p55 levels as a function of time, there was a significant increase in sTNF-R p75 levels in both groups of patients at 6 and 24 hours after aortic clamp release.

In contrast to the findings of Froon et al,[16] there was no significant positive association between serum creatinine and sTNF-R levels in the present study. Moreover, sTNF-R p55 levels immediately before and after aortic clamp release were significantly lower in patients with ruptured AAA who later developed renal failure compared with those patients who did not. There was, however, no significant difference in sTNF-R levels among patients with rupture who did and did not have cardiovascular, respiratory, or septic complications.

The association between elevated sTNF-R levels and increased mortality rates in patients who underwent operation for ruptured AAA is similar to that observed by other investigators in acute inflammatory conditions or severe injury.[19-21] Elevated levels of sTNF-Rs may indicate that the endogenous pool of sTNF-Rs is replete[19] and this may partly explain the disappointing results achieved with exogenous sTNF-R p55 and p75 in patients with sepsis syndrome and septic shock.[25,26] It is not possible to determine from the present study whether therapeutic intervention with exogenous sTNF-Rs would ameliorate the adverse effects of TNF in lower extremity ischemia and ruptured AAA repair.[10,16,27,28] However, a recent animal study of infrarenal aortic crossclamping demonstrated a significant reduction in circulating TNF levels, nitric oxide production, and subsequent lung injury when exogenous sTNF-R p55 was administered before aortic clamp placement.[18] In the present study, endogenous sTNF-R levels were significantly lower in patients who underwent repair of nonruptured AAA compared with those who underwent operation for ruptured AAA. One can speculate that exogenous sTNF-R therapy may ameliorate the adverse effects of TNF in elective aortic surgery where endogenous levels of sTNF-Rs are low but may have limited efficacy in patients with hemorrhagic shock before ruptured AAA repair,[19] in which the concentrations of sTNF-Rs observed in the present study would have been sufficient to effectively antagonize the effects of circulating TNF in vitro.[29]

In conclusion, these data demonstrate that hemorrhagic shock, lower torso ischemia, and early reperfusion that occur in the course of ruptured

AAA repair are associated with elevated levels of the sTNF-Rs and that elevated levels of sTNF-R p75 during the period of early reperfusion are associated with increased mortality rates. The fact that the endogenous pool of sTNF-Rs may be replete and that elevated endogenous levels are associated with increased mortality rates suggests that sTNF-R therapy may have limited efficacy in patients who undergo ruptured AAA repair.

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