Coagulopathy and Haemostasis in Surgery for Abdominal Aortic Aneurysm

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Declaration

The work which is described in this thesis is my own and was carried out while I was employed as a Clinical Research Fellow in the department of Vascular Surgery in the Royal Infirmary of Edinburgh. My employment was funded by the Scottish National Blood Transfusion Service (SNBTS).

My personal contribution to the work in thesis is as follows:- The concept and design of all the studies were entirely my own, except for the fibrin sealant trial which was designed in collaboration with the staff of the clinical trials unit of the SNBTS. I carried out all patient recruitment, clinical observation and follow-up, tissue and blood sampling and I performed assays for glycocalicin. I performed observation, interpretation and analysis of the material examined by electron microscopy. I carried out all statistical analysis and interpretation and drew all illustrations and graphs.

Abstract

The mortality rate for patients undergoing operation for ruptured abdominal aortic aneurysm is about 30-50% and has not significantly decreased over the last 20 years. Rupture may be complicated by a coagulopathy in up to 50% of cases, the presence of which is associated with a mortality rate in excess of 80%. Little is known about the pathogenesis of this coagulopathy and treatment remains empirical. The aim of this thesis is to investigate the pathological process which leads to this coagulopathy and thus provide a rational basis for the development of new therapeutic strategies.

A retrospective case-control study of patients requiring re-operation for control of haemorrhage following elective and emergency aortic aneurysm repair was performed. The incidence of haemorrhage was 1.9% after elective surgery and 2.7% after operation for rupture. The mortality rate was 58% in the study group and 21% in the control group. The patients requiring re-operation appeared to have undergone more complicated primary operations and nearly all patients had a coagulopathy at the end of the first procedure which may have been the cause of the haemorrhage.

Coagulation studies were performed in a series of 24 patients with asymptomatic abdominal aortic aneurysm. Levels of fibrinogen were high but within the normal range and there was no evidence of systemic activation of the soluble coagulation or fibrinolytic system. Platelet counts were low but usually within the normal range and levels of glycocalicin were elevated suggesting either increased platelet turnover or increased activation.

Coagulation studies were performed in a series of six patients undergoing elective aortic aneurysm repair. It was found that thrombin, fibrinolytic and platelet activation increases during the period of initial dissection in a time dependent manner. After the administration of heparin and the application of the aortic cross

clamp, thrombin, fibrinolytic and platelet activation reduced. After reperfusion there was an increase in platelet activation which correlated with the duration of cross-clamping.

Coagulation studies were performed in a series of 22 patients presenting with ruptured aortic aneurysm. At time of admission there was marked activation of platelets and thrombin in all patients which increased greatly by the end of operation. Platelet count at the end of operation was significantly lower in patients who died than in survivors.

Samples of subcutaneous fat and skeletal muscle were taken at the start of operation in six patients with ruptured aneurysm. Transmission electron microscopy was used to examine the endothelial cells in small vessels in these samples. When compared with samples from six control patients undergoing elective aortic surgery it was found that there were significant ultrastructural differences in the endothelial cells from patients with rupture.

A randomised controlled study of fibrin sealant as a topical haemostatic agent at vascular anastomoses was carried out in 57 patients undergoing aortic aneurysm repair, carotid endarterectomy or arterial bypass graft using polytetrafluoroethylene bypass graft. The time taken to achieve haemostasis at the anastomoses was significantly reduced in patients treated with fibrin sealant. The reduction was most marked in patients undergoing carotid endarterectomy.

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Chapter 1

General Introduction

Historical Review

Surgery for abdominal aortic aneurysm

There are accounts of arterial aneurysms as long ago as Egyptian times but the first recorded operations were carried out in Roman times. Antyllus, who lived in the 2nd century A.D., is widely regarded as performing the first operations for aneurysms.[1] He described both true and false aneurysms and clearly perceived the difference between them. The operative technique he described involved proximal and distal ligature with opening of the sac and evacuation of it contents. This technique, with some variations, remained the standard operation until the middle of the Twentieth century when arterial reconstruction became possible.

These early operations were performed on peripheral aneurysms and it was not until the Nineteenth century that operations on the abdominal aorta were attempted. The techniques used were variations on those described by Antyllus. The first such operation was attempted in 1817 by Sir Astley Cooper who had considerable previous experience of arterial ligation, including the first successful ligation of a carotid artery in 1808 and a number of successful external iliac ligations.[2] He had also demonstrated that ligation of the aorta was compatible with survival in several animal experiments. In 1817 a 38 year old porter with a rapidly enlarging iliac artery aneurysm was admitted to Guy's Hospital. This aneurysm had ulcerated through the skin, bleeding externally intermittently over a period of 5 days. Sir Astley Copper, foreseeing the possible need to ligate the aorta, experimented with various means of exposing the aorta in the post-mortem room. When the bleeding became so profuse that the patient's life was in immediate danger he first attempted to ligate the feeding artery by opening the sac and placing his finger in the orifice of the feeding artery.

This proved unsuccessful and in a bold attempt he exposed the aorta through a transperitoneal approach and placed a silk ligature above the bifurcation. This was not a true aortic aneurysm but as the ligature was placed on the aorta it can be considered the first aortic operation. The patient initially recovered but unfortunately died some 40 hours post-operatively.

Despite this lack of success further attempts were made, though on occasion these met with disapproval from the surgical community at large.[3-8] One such attempt [8] was reported in an editorial in the Lancet as "*an appalling operation, and we hope not to hear of it repetition*" [9] Despite this exhortation, surgeons persevered such that by 1891 eleven such procedures had been carried out, all with fatal results.[10] However it should be noted that some achieved limited success with one patient in 1842 surviving for 10 days before succumbing to secondary haemorrhage.[5]

The first successful ligation of the abdominal aorta was reported in 1925 by Rudolph Matas, more than one hundred years after the first attempt by Sir Astley Cooper.[11,12] This patient survived for 1 year, 5 months and 5 days before succumbing to pulmonary tuberculosis. Aortic ligation did not become widely popular and by 1940 there were only 24 recorded cases of which 5 had been successful.[13] A notable proponent of ligation was Brooks who described several successful cases and carried out animal experiments on the effects of aortic ligation.[14,15]

During this time another approach to the treatment of aortic aneurysm was also being pursued; the promotion of thrombosis within the aneurysm by various means. The first such attempt was in 1831 when Velpeau introduced three pairs of knitting needles into an aortic aneurysm.[16] In 1864 Moore passed 26 yards of iron wire into an aneurysm[17] and Corradi modified this technique by passing an electric current through the wire in an attempt to induce thrombosis. This manoeuvre became known as the Moore-Corradi technique and, with some variation, continued to be

popular until the early part of the Twentieth century.[18] Indeed a series of patients treated by wiring was reported recently as 1968.[19] However, it was recognised that although these procedures relieved pain they did not prevent rupture and thus the technique fell out of favour as other forms of management became more successful.

It had been shown both experimentally and clinically that complete occlusion of the abdominal aorta below the renal arteries was compatible with survival but it was thought that it would be preferable to maintain blood flow to the lower limbs.[14] Various techniques were developed such as partial ligation of the aorta [20] or ligation with fascia lata which would later loosen and allow restoration of flow.[21] It was the introduction of arterial reconstruction using arterial homografts that finally allowed the development of modern aortic surgery.[22] The first successful aortic resection and reconstruction was reported in 1952 by Dubost.[23] The use of arterial homografts was soon superseded by the development of prosthetic grafts. The early development of these grafts was largely the work of Voorhees [24] but it was DeBakey who introduced the knitted dacron graft which, with some modifications, is still used today.[25]

The final step towards aortic surgery as we now know it was the development of the "inlay" operative technique, first described by Creech, which avoided the extensive dissection required to excise the aneurysm. This technique greatly reduced intraoperative bleeding and operating time and led to a reduction in the mortality rate.[26]

In parallel with improvement in surgical technique there have been considerable advances in anaesthesia and intensive care with the introduction of adequate anaesthesia, artificial ventilators, muscle relaxants, invasive monitoring, intravenous fluids, heparin and blood transfusion not to mention other aspects of modern care such as oxygen, antibiotics, x-rays, angiography, ultrasound scanning and routine

biochemical and haematological investigation. This has led to a reduction in mortality and broadening of the indications for operation. When considering the sophistication of modern anaesthetic and intensive care required in aortic surgery, it is interesting to note that during the first aortic procedure carried out by Sir Astley Cooper, the anaesthetic consisted of brandy and ether. It is now difficult to envisage undertaking aortic surgery in those circumstances.

Acquired coagulopathy

The earliest recognition of inherited bleeding disorders was in the 2nd century A.D. when Rabbi Judah exempted from circumcision a new-born boy whose brothers had died from bleeding following circumcision.[27] In comparison, the recognition of acquired bleeding disorders as a clinical entity is relatively recent.

Early philosophers and medical practitioner proposed a variety of hypotheses concerning how and why blood clotting occurs. The first step forward in determining the true nature of coagulation was in the mid-seventeenth century when Malpighi (1686) separated fibrin from blood clot. The origin of fibrin remained a subject of debate over the next century. Some believed that it was produced from albumin, others that it was derived from red cell nuclei but the views of Hewson (1846) and Babington (1830) who believed that fibrin was formed from a precursor in plasma eventually became accepted. The plasma precursor was later extracted, purified and given the name fibrinogen.[27]

Buchanan, in 1845, observed that hydrocoele fluid clotted upon the addition of fresh serum and concluded that serum contained some agent which converted fibrinogen into fibrin.[29] Some years later this agent, thrombin, was identified. It was soon realised that this also must exist in an inactive form otherwise it would cause the coagulation of circulating blood. This precursor, prothrombin, was sought and identified.[30]

It had previously been demonstrated that blood clotted rapidly both in vivo and in vitro on the addition of tissue suspensions [31] and Schmidt (1892) drew upon this observation to formulate a hypothesis that tissue contained some factor, which he termed tissue thromboplastin, which converted prothrombin to thrombin.[32]

Platelets were first described by Donné in 1842 [33] but their function was unknown.

The role of platelets in haemostasis was suggested by the observation that some patients with purpura were thrombocytopenic.[34] Osler (1874) observed that platelets tended to clump together [35] and Hayem (1882) suggested that this process might play a role in haemostasis.[36] This was confirmed by the observations of Bizzozero (1882) and Eberth and Schimmelbusch (1886) that platelets adhered to injured vessel walls and that more platelets then adhered to these clumps.

Thus by the beginning of the Twentieth century the basic haemostatic mechanism was understood and classic theory of coagulation was formulated.[37] It was also at this time that the first acquired coagulopathies were described. The Hippocratic school had observed that the blood of those who die suddenly or violently may be incoagulable,[27] an observation also made by Hunter at the end of the Eighteenth century,[28] but there were no descriptions of acquired bleeding disorders in living patients.

It is interesting that disseminated intravascular coagulation was described and investigated in an animal model long before it was recognised clinically. In a classic and much repeated experiment de Blainville (1834) injected an intravenous bolus of brain tissue suspension into dogs.[31] This led to the immediate death of the animals and post mortem examination demonstrated widespread fibrin deposition within blood vessels. In a modification of this experiment Woolridge in 1881 slowly administered tissue extract intravenously and found that, rather than clotting, the blood became incoagulable.[38] It was later demonstrated that the animal's blood was depleted of fibrinogen.[39-41]

It was in field of obstetrics that acquired bleeding disorders as a clinical entity were first described and investigated. In 1901 DeLee reported a case of a patient with abruptio placenta whose blood failed to clot even several days later.[42] He described this condition as temporary haemophilia. Defective clotting in patients with both

abruptio placenta and placenta praevia was noted by Williams (1915) who likened the abnormal bleeding to that found after snake bites and hypothesised that it might be due to the release of some toxin, although it was suggested that the pathological injury was to the blood vessel wall rather than the coagulation system.[43] Subsequent investigations revealed that these patients had markedly reduced levels of fibrin and this was proposed as the cause of the excessive bleeding.[44] In animal experiments it was shown that the infusion of placental extract caused a clotting defect [45] and the causative factor was identified as thromboplastin.[46,47] The hypothesis evolved that the escape of placental thromboplastin into the maternal circulation caused activation of the coagulation cascade producing defibrination.[48]

At the same time as these studies in obstetric patients were being carried out, a fortuitous observation led MacFarlane (1937) to investigate the changes in the coagulation systems of patients following general surgical operations.[49] He noticed that a clotted blood sample taken from a patient following cholecystectomy and left overnight was fluid the following morning. The importance of this was that it was not the case that the blood did not clot but that the blood had clotted and subsequently lysed. This phenomenon had in fact been observed in 1893 by Dastre in dogs which had bled extensively and he gave it the name "fibrinolysis".[50] These and other studies demonstrated that fibrinolytic activity was increased in patients following major surgery.[51] This activity was attributed to the action of plasmin, a proteolytic enzyme described by Christensen and McLeod (1945).[52]

Early reports describing excessive bleeding during and after general surgical operations laid the blame on blood transfusion.[53] The bleeding tendency was attributed to "citrate intoxication" or thrombocytopenia secondary to massive transfusion. However Coon and Hodgson formulated an alternative hypothesis.[54] The presence of excessive fibrinolysis in patients following surgery, trauma and shock was well established by this time [49,51,55,56] and combining this with the

current understanding of coagulopathy in obstetric patients, they hypothesised that the bleeding disorder was due to hypofibrinogenaemia secondary to excessive fibrinolysis caused by shock. Subsequent clinical investigation indicated that in most cases clinical bleeding was due to hypofibrinogenaemia and consumption of clotting factors and that the effects of blood transfusion were less important.[57]

Coagulopathy and aortic aneurysm

Fine *et al* (1967) are generally accredited with the first account of coagulopathy in association with an aortic aneurysm but there are earlier reports.[58-60] Krevans and Jackson (1955) in a study of massive transfusion reported two cases of coagulopathy complicating aortic surgery; a 50 year old man who, following elective abdominal aortic aneurysm repair, developed excessive bleeding from the wound, rectal bleeding and haematuria with thrombocytopenia and prolonged clotting times, and a 64 year old man who bled post-operatively following elective abdominal aortic aneurysm repair and was found to have thrombocytopenia and hypofibrinogenaemia.[61] Both these patients died in the early post-operative period.

The next report was published some 12 years later by Phillips.[62] A patient was described who developed excessive bleeding during abdominal aortic aneurysm repair that was associated with prolonged clotting times, thrombocytopenia and hypofibrinogenaemia. This patient died 5 hours postoperatively from cardiac arrest. There are also many early accounts of coagulopathy in patients with thoracic aortic aneurysms and even femoral artery aneurysms.[58,59,63,64]

It was not until 1974 that more rigorous investigation of patients undergoing aortic aneurysm revealed that coagulopathy was quite commonly found in patients post-operatively.[65] The first report of coagulopathy occurring pre-operatively in a patient with a ruptured abdominal aortic aneurysm was by ten Cate *et al* who described "*markedly low levels of coagulation factors, decreased level of fibrinogen, an increase of fibrin/fibrinogen degradation products and a distinct thrombocytopenia*" at the time of admission in a 71 year old woman with ruptured abdominal aortic aneurysm.[66]

There has been some debate as to whether clinical disseminated intravascular

coagulation is a complication of intact abdominal aortic aneurysms.[60] In 1969 a case was reported of a 76 year old man with a chronic bleeding disorder and an abdominal aortic aneurysm.[67] However it was subsequently reported by Siebert and Natelson that this patient continued to have DIC after operative repair and was subsequently found to have pancreatic carcinoma.[68] A second patient was reported by these authors; a 68 year old man whose coagulopathy did resolve after operation but this case is complicated by the fact that he also had liver cirrhosis and underwent splenectomy at the time of aortic surgery.

Siebert and Natelson recommended that the presence of a coagulopathy complicating abdominal aortic aneurysm should prompt the search for another cause of the coagulopathy such as occult carcinoma. It should also be noted that DIC may be idiopathic and that should the coagulopathy not correct after aneurysm repair, the two conditions may have been coincidental.[76] These authors recommended four criteria for the diagnosis of coagulopathy complicating intact abdominal aortic aneurysm:-

1. The presence of a chronic acquired bleeding disorder.
2. Laboratory evidence of DIC.
3. Correction of coagulopathy after operative repair.
4. Maintenance of normal coagulation for three months after surgery.

Many of the reported cases in the literature do not meet these criteria because the aneurysm was never operated on[58,64,67,69,70] or there was concurrent liver disease[67,68] and many quoted cases were in fact patients with thoraco-abdominal aneurysms.[58,64,70] However there are a few reported cases that do fulfil these criteria.[71-75]

In a prospective study Fisher *et al* looked for clinical and laboratory evidence of DIC in a series of 76 patients prior to aortic surgery.[77] Three patients with clinical

bleeding disorders were described but these were all patients with thoraco abdominal aneurysms. None of the 26 patients with abdominal aortic aneurysms had clinical or laboratory evidence of DIC, although some did have mild thrombocytopenia or elevated fibrin degradation products. It must be considered that, although clinical DIC may be present with an intact abdominal aortic aneurysm, it is a rare complication.

Surgical haemostasis

The earliest technique used to achieve haemostasis was cautery which remained common up to the Eighteenth century. The more sophisticated technique of applying ligatures to bleeding vessels was first described by Sushruta who lived in the period 800 - 600 B.C. in ancient India.[78] The use of ligatures was also described in the writings of several eminent Roman surgeons, Celsus, Rufus, Antyllus and Galen, around the second century A.D.[79] However this knowledge was lost and, although ligatures were described by several authors after this time, cautery, either by application of hot irons or boiling oil, remained the haemostatic technique of choice until the rediscovery of the ligature by Ambroise Paré in 1552.[80] Thereafter there was a period of debate as to whether cautery, compression or ligature was the best means to control haemorrhage. By the end of the Eighteenth century, ligature was generally accepted as the preferable technique.

These early techniques were devised to overcome the massive haemorrhage associated with trauma and early surgical procedures such as amputation. The development of increasingly sophisticated surgical procedures demanded better haemostatic techniques to allow the control of haemorrhage without causing extensive tissue damage. This was especially the case in neurosurgery where a wide variety of techniques were developed. Sir Victor Horsley, one of the founders of modern neurosurgery, described the use of wax to seal bone; a method still used today.[81] Harvey Cushing, another pioneer of neurosurgery, described a wide variety of haemostatic techniques: hot cotton, pieces of muscle (also described by Horsley), well-solidified blood clots and silver wire clips.[82] In this paper Cushing also suggested that fibrin might be a useful agent, a suggestion taken up by Grey as mentioned below.[83]

An important technique promoted by Cushing, in association with Bovie, was

electrocautery and electrocoagulation; a return to the principles of cautery but in a more controlled manner.[84] In early clinical use an electric current was used to heat an element which was then applied to the bleeding area, electrocautery.[86] This is fundamentally no different from the application of a hot iron, as used many centuries earlier. A more sophisticated technique was developed in which current was passed through the patient producing heat in the tissues by electrical power dissipation.[85] This technique reduces damage in surrounding tissues, produces more secure haemostasis and quickly superseded earlier techniques.[87] Since then equipment has been refined and electrocoagulation is universally employed in nearly all branches of surgery.

Thus present haemostatic methods are fundamentally the same as those employed by Sushruta, a combination of ligature and cautery, albeit much more refined. The introduction of topical haemostatic agents, such as fibrin sealant, therefore represents a radical new approach.

Fibrin sealant - Historical aspects

It may be thought that fibrin based adhesives and sealants are likely to be the ideal agents for surgical haemostasis and tissue adhesion since fibrin plays a central role in the physiological processes of haemostasis and wound healing. Thus it might be possible to avoid the problems of foreign body reaction, delayed healing and fibrosis which are associated with other topical haemostatic agents such as those based on collagen or oxidised cellulose. The first experiments using fibrin to control haemorrhage were performed by Bergel in 1909.[88] The use of sheep fibrin as a haemostatic agent in neurosurgical animal experiments was reported by Grey in 1915.[83] Sheep fibrin was used because it was readily available as a by-product of materials used to perform Wassermann tests. The combination of fibrinogen and

thrombin was first described in 1944, but poor adhesive effect was reported, probably due to low concentration of fibrinogen.[89,90]

The advent of technology to extract concentrated and purified clotting factors allowed the development of the fibrin sealant that is in current use.[91] This consists of two principle components, fibrinogen and thrombin, which are mixed, in the presence of factors VIII and XIII, fibrinectin and calcium, to produce insoluble fibrin. Thus the principle components of the final stage in the physiological pathway of coagulation are present. Fibrin sealant of this form was first described in 1972.[91]

Fibrin sealant was initially developed as a tissue adhesive in nerve repairs but its possibilities as a haemostatic agent were soon investigated. The haemostatic properties of fibrin sealant were first clinically employed in cardiothoracic surgery.[92] Since then it has been used in a wide variety of settings but has only achieved common use in cardiothoracic surgery, especially for aortic dissection, and in otorhinolaryngology for procedures such as middle ear reconstructions.[93]

Fibrin sealant appears to be extensively used throughout Europe but it is only used in the UK by a small number of enthusiasts. To date there is no fibrin sealant preparation with a product licence on the market in the UK.

Epidemiology, Natural History and Management of Abdominal Aortic Aneurysm

Epidemiology

Attempts to determine the precise prevalence of aortic aneurysms within a given population founder upon the problem of the definition of an aneurysm. An aneurysm can be defined as "*an abnormal dilatation of a blood vessel*" but the point at which dilatation becomes abnormal is the debatable issue. Aortic diameter within a population is a continuum and any measurement at which the aorta is called aneurysmal is arbitrary.[94] Furthermore an aorta of 2.5 cm in a large male may be considered normal but an aorta of the same diameter in a small female would be considered pathological. Some epidemiological studies have set an arbitrary size at which the aorta is considered abnormal while others have considered the infrarenal aorta to be aneurysmal if it is larger,[95] 5mm larger [96] or more than 50% greater than the suprarenal diameter.[97] The next difficulty is the detection of such aneurysms. The most practical method is to use ultrasound scanning, which has the advantage of being non-invasive but has a margin of error of 0.5 cm.[98]

Most studies have examined high risk populations, as predicted by age,[96] sex,[96] family history [99,100] and a history of vascular disease.[101,102] One of the best estimates of community prevalence comes from the Oxford screening programme; a large community based study in men aged 65-74 years.[96] This study reported a 2.3 per cent prevalence of aneurysm greater than 4 cm. A similar study, the Birmingham Community Aneurysm Screening Project, examined 2669 men aged 65-75 and found an aortic diameter >29mm in 8.4% of patients and aortic diameter >40mm in 3.0% of patients.[103] A study of the incidence of aneurysm in patients aged greater than 50

years undergoing abdominal ultrasound scan for non-vascular disease demonstrated a prevalence of aortic diameter greater than 3cm of 8.8 per cent in men and 2.1 per cent in women.[104] However the population in this study is clearly a biased sample of the general population.

Another approach to epidemiology of aortic aneurysms is to look at the end point - death from ruptured aortic aneurysm - as shown on death certificates or as determined by post-mortem examination. Studies such as these give figures in the range of 2.9-14.1 per 100,000 population per year.[105-115] The wide variation between studies may be methodological in nature. The rate of post-mortem examination varies between countries. People dying suddenly and unexpectedly, as is often the case with ruptured aneurysms, are more likely to undergo post-mortem examination and therefore the observed incidence may be an overestimate in communities which have a low overall rate of post-mortem examination. In some studies a low or unstated community post-mortem rate gives rise to some doubt over the validity of the conclusions. However there may be genuine differences in incidence between different countries. Most of the studies come either from the UK or Sweden and mortality rates are consistently higher in the UK.

The prevalence of aortic aneurysm is strongly related to age, male sex, hypertension cigarette smoking, prior vascular disease and family history.[116] The mortality from aortic aneurysm in men aged more than 80 years is ten times that of men aged 55-64 years and one hundred times that of men aged below 55 years.[117]

The incidence of aortic aneurysm appears to be increasing. This has been shown by an increase in the incidence of diagnosis of aortic aneurysm in the general population in Scotland from 25.8 per 100,000 in 1971 to 63.6 per 100,000 in 1984.[118] Similar increases have been reported in Australia, Sweden and the USA.[112,119,120] The mortality from aortic aneurysm has risen twenty-fold in men and eleven-fold in

women between 1950 and 1984.[121] The incidence of ruptured aortic aneurysm has likewise increased.[108,122] These trends appear to be continuing. A more recent study shows a steady rise in the numbers of elective and emergency aneurysm repairs and mortality from aortic aneurysm in Scotland during the period 1980-91.[123] While some these changes can be attributed to a greater awareness of the diagnosis and greater ease of detection, due to ultrasound scanning, it does appear that there is an underlying increase in incidence.

Natural history

The classic study of the natural history of abdominal aortic aneurysms was published in 1950 by Estes.[124] This study comprised 102 patients and demonstrated that the 5 year survival was 19 per cent, with 63 per cent dying from rupture. Aneurysms increase in diameter with time, although the rate of increase is highly variable between individuals and within the same individual at different times.[125-127] The growth rate is in the order of 0.2 - 0.5 cm diameter per year. Laplace's law states that the tension in the wall varies with the square of the diameter. It can thus be predicted that the risk of rupture should be related to diameter of aneurysm. This prediction has been bourne out in practice; patients with larger aneurysms have a greater risk of rupture and post-mortem examinations reveal that aneurysms that have ruptured tend to be large.[128-130] However even small aneurysms can rupture. Some authors have considered that the shape of the aneurysm, the presence of intraluminal thrombus and atherosclerosis may be related to the risk of rupture.[131] An important factor that is related both to rate of growth of aneurysms and risk of rupture is hypertension.[130,132]

The accurate determination of the risk of rupture with an aneurysm of a given size has remained elusive. One study reported that the risk of rupture was 6% at 5 years

and 8% at 10 years, with zero risk in aneurysms of less than 5cm diameter and 25% risk in aneurysms over 5 cm.[127] A more recent study involving 300 patients described a 1 per cent cumulative incidence per year of rupture for aneurysms less than 4cm and 2.9 per cent for those 4-4.9 cm.[133]

Treatment and outcome of ruptured aortic aneurysm

As stated above the natural history of aortic aneurysms is that they eventually rupture unless death from another cause intervenes or they are repaired surgically. The mortality rate for those who rupture in the community has been estimated at 90-95% as most patients do not survive to reach hospital.[107,108,112,134] Of those who reach hospital alive a considerable number, 20-30%, are deemed unfit for surgery. Of those who undergo emergency aortic aneurysm repair the mortality rate is about 30-50% (Table 1). Some investigators have shown that those who undergo a successful aneurysm repair have a normal quality of life and normal life expectancy.[135,136] In a more recent study it was found that late survival was poorer in patients having undergone successful operation for rupture compared with those who had undergone elective aortic surgery.[137] However the figures were not corrected for confounding variables, such as age, which might lead to reduced survival rates in patients following operation for rupture.

Direct comparison between series is not valid as patient selection varies widely. For example Gloviczki *et al* include patients admitted with ruptured aneurysm but who died without operation and 24% of patients in their series had suffered a cardiac arrest prior to operation. Other series only include patients who undergo operation and it is likely that some centres were more selective than others and did not operate on patients with poor prognosis. These difficulties in comparison led to the proposal of a classification system for ruptured aortic aneurysms but this has not become

widely adopted.[157] Direct comparison is not valid but it is noteworthy that the mortality rate from rupture has not decreased significantly over the last 30 years. Some studies that compare different time periods within the same hospital report that their mortality rate has decreased despite the fact that a higher proportion of patients admitted with rupture undergo operation,[122,152,155] although one group report no improvement in mortality rate over an eleven year period.[156] This suggests that although the performance of individual units may improve with increasing experience, there have been no major advances that have improved the outcome for these patients.

The most common causes of death are post-operative myocardial infarction, uncontrolled intra-operative haemorrhage, continued post-operative haemorrhage and multisystem failure.[144,158] As well as a high mortality rate, surgery for ruptured aortic aneurysm carries considerable post-operative morbidity. Respiratory failure, renal failure, sepsis, stroke, lower limb ischaemia and ischaemic colitis are common in the early post-operative period.

Table 1 *Reported results of operation for ruptured aortic aneurysm*

Author	Year	No. patients	Mortality rate
De Bakey [138]	1964	117	34%
Mannick *et al* [139]	1964	26	32%
Kouchoukos *et al* [140]	1967	36	61%
Hicks *et al* [141]	1975	56	55%
Marsh [142]	1980	29	41%
Diehl *et al* [143]	1983	34	26%
Fielding *et al* [144]	1984	198	43%
Donaldson *et al* [145]	1985	81	43%
Ingoldby *et al* [107]	1986	101	48%
Jenkins *et al* [122]	1986	151	28%
Shackleton *et al* [146]	1987	106	41%
Amundsen *et al* [147]	1989	114	60%
Murphy *et al* [148]	1990	172	49%
Ouriel *et al* [149]	1990	243	55%
Harris *et al* [150]	1991	113	64%
Johansen *et al* [151]	1991	186	70%
Cohen *et al* [152]	1975-85	70	76%
	1986-89		54%
Gloviczka *et al* [153]	1992	214	45%
McCready *et al* [154]	1993	208	49%
D'Angelo *et al* [155]	1966-78	16	69%
	1978-87	93	60%
	1988-90	61	40%
Johnston [137]	1994	147	51%
Katz *et al* [156]	1994	1829	50%

Prognostic indicators

Given the high mortality rate for emergency surgery, a number of studies have been carried out to identify the factors that are associated with poor outcome.[150,159,160] Such studies may help to guide decision making as to which patients should be offered operation and indicate patients in whom current treatment is inadequate and for whom new treatment strategies should be developed.

Pre-operative factors that have consistently been associated with poor prognosis are increasing age, hypotension on admission, low admission haematocrit and chronic obstructive airways disease. The intra-operative factors associated with poor outcome are free intraperitoneal rupture, length of operation, blood loss and blood transfusion. The post-operative factors associated with late death from multi-organ failure are immediate post-operative thrombocytopenia and organ system failure score (GORIS score) determined 48 hours post-op.[161]

A recent large multicentre prospective study of ruptured aneurysms showed that the variables associated with outcome were pre-induction systolic blood pressure, creatinine, total intraoperative urine output, total volume of blood administered, site of aortic cross clamp and cross clamp time.[137] However no combination of factors was associated with 100% mortality and it was thus concluded that the decision as to whether to undertake operation could not be made on the basis of these factors.

Coagulopathy has been shown to be an indicator of poor outcome in a number of papers.[142] Davies *et al* examined this issue in a prospective study. Patients with ruptured aneurysms had blood samples taken at the time of admission.[162] A platelet count, PTR, APPT, fibrinogen assay and d-dimer assay were performed on this sample. Patients were followed until death or discharge. It was found that the platelet count was closely associated with mortality. It is important to note that the

cause of death in these patients was not restricted to bleeding complications but that a significant number of patients died in the post-operative period from myocardial infarction and multi-organ failure. Four patients had DIC at time of admission with thrombocytopenia, prolonged clotting times and elevated D-dimers and all four patients died.

Two further clinical studies have supported these findings. Johnson *et al* reported that the presence of a coagulopathy at the end of operation is associated with increased mortality rate.[137] A coagulopathy was present in 12% of patients and these patients had a mortality rate of 76%, the remaining 88% who did have a coagulopathy had a mortality rate of only 25%. Bradbury *et al* in a study of 65 patients presenting with ruptured aortic aneurysm demonstrated a direct correlation between platelet count at time of admission and mortality and between platelet count at the end of operation and multi-organ failure.[163] There was a 93% mortality rate in those with an admission platelet count of less than 150 x 10^9/l compared with 24% in those with a platelet count greater than 150 x 10^9/l.

It is difficult to interpret these findings in terms of cause and effect; it may be that the derangement in the coagulation system is simply a marker for blood loss, prolonged hypotension etc. or it may be that there is a causal relationship between coagulopathy and death.

Current Understanding of Disseminated Intravascular Coagulation

Introduction

Disseminated intravascular coagulation (DIC) is a clinical condition which is associated with many diverse disease states (Table 2).[164] DIC is characterised clinically by platelet-fibrin thrombi deposition in the microvasculature of various organs and a bleeding tendency, with evidence on laboratory testing of thrombocytopenia, consumption of coagulation factors as demonstrated by prolonged clotting times, low levels of plasma fibrinogen and elevated fibrin degradation products.

There is spectrum of clinical presentation with varying degrees of thrombotic and haemorrhagic features. There are three broad groups: firstly a chronic, predominately thrombotic disorder associated with malignancy or connective tissue disease, secondly an acute thrombotic disorder with multi-organ failure associated mainly with infection and thirdly an acute haemorrhagic disorder associated with obstetric accidents, trauma, head injury and ruptured aortic aneurysm.

The pathogenesis of DIC is a complex and confusing subject. DIC is thought to arise when an otherwise normal haemostatic system is subjected to an abnormal, overwhelming stimulus or when some component of the haemostatic mechanism is defective. The haemostatic mechanism is complex with elaborate systems of activation and control. It has several features, such as powerful positive feedback mechanisms, which are necessary for optimal function but which also make it susceptible to the development of DIC when it is subjected to certain stimuli. Thus a clear understanding of the normal physiological controls on haemostasis is required

in order understand why this mechanism can produce DIC when subjected to abnormal stimuli.

Table 2 *Causes of DIC*

Causes of DIC	
Infection[164]	Meningococcal septicaemia
	Streptococcal septicaemia
	Rocky mountain spotted fever[165]
Neoplasia	Acute promyelocytic leukemia[166]
	Disseminated carcinoma
Trauma	Head injury[167,168]
	Burns
	Heat stroke
	Multiple injuries
Surgery	Ruptured aortic aneurysm
	Liver resection
Obstetric	Abruptio placenta
	Amniotic fluid embolism
	Retained dead foetus
Snake venom [169]	*Vipera Russell*
	Echis Carinatus
	plus many others
Liver disease	Liver failure
	Liver transplantation

Normal haemostasis

The purpose of the haemostatic mechanism is to seal defects in blood vessels walls, so preventing loss of blood from the intravascular space, without interrupting blood flow more than is necessary. In order to achieve this, the haemostatic system has a complex set of triggering and controlling mechanisms which result in rapid production of a platelet - fibrin plug at the site of injury, but which at the same time inhibit thrombosis within healthy vessels and initiate repair of the injury and removal of the clot.

The two pivotal events in normal haemostasis at the site of injury are the generation of the enzyme thrombin, which then converts soluble fibrinogen to insoluble fibrin, and the adhesion and activation of platelets. These events are not isolated but interact such that each promotes the other. There are controlling mechanisms which ensure that the process of fibrin and platelet deposition does not extend to intact blood vessels. These events are closely followed by the activation of the enzyme plasmin; a fibrinolytic agent which clears excess thrombus around the area of injury and later promotes recanalization and the restoration of blood flow. Fibrinolysis is an integral part of the haemostatic process and therefore interacts closely with the coagulation system.

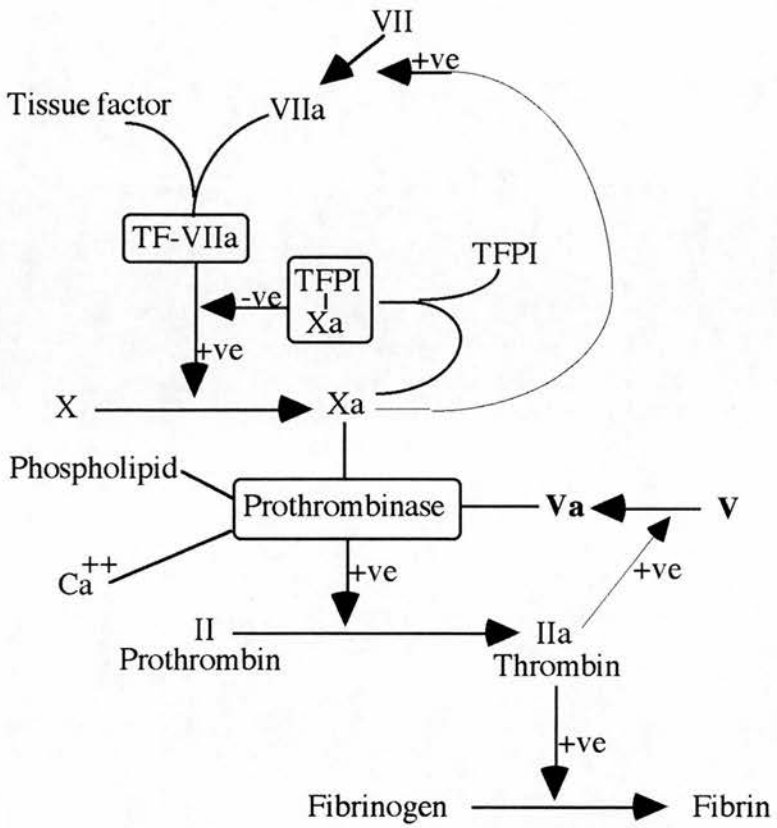
Generation of thrombin

For many years the accepted theory of coagulation was that there were two separate pathways, the intrinsic and extrinsic pathways, that led independently to the generation of thrombin. It was also considered that the intrinsic pathway was more important as deficiencies of factors in the intrinsic pathway, factor VIII and IX, led to well known bleeding diatheses. However recently it has become accepted that *in vivo* it is the extrinsic pathway (also known as the tissue factor pathway) of coagulation which is responsible for the initiation of coagulation and that elements of the intrinsic pathway are vital for amplification of thrombin production.[170] The tissue factor pathway of coagulation which will be described in detail below.

Injury to the vessel wall results in the exposure of collagen and tissue factor (also known as tissue thromboplastin). Tissue factor is a membrane glycoprotein which is present on the surface of many cells, although notably not on unactivated endothelial cells or leucocytes. Tissue factor binds Factor VII which even when not activated can, in combination with tissue factor, activate Factor X, although at a very slow rate. Activated factor X in turn converts prothrombin (Factor II) to thrombin.[171-173] Activation of Factor X and thrombin produces a positive feedback; both these enzymes activate Factor VII which when activated is a more potent activator of Factor X itself (Figure 1).[174] In addition the Factor VII-tissue factor complex can auto-activate by converting VII to VIIa.[175]

There is also a negative feedback pathway. Tissue factor pathway inhibitor, a serine protease inhibitor which is found in the plasma and on the surface of endothelial cells combines with, and inhibits, factor Xa. This complex is a potent inhibitor of the tissue factor-VIIa complex.[176]

Figure 1 *Tissue factor pathway of thrombin activation*



This reaction proceeds quite slowly in isolation, and indeed may do so outside intact vessels as these proenzymes are relatively small and may escape into the extravascular space in undamaged vessels. There are three factors that cause this reaction to be greatly accelerated when the blood vessel is injured. Firstly, disruption of the vessel allows the large coagulation factors, Factor V, VII and VIII, to reach the extravascular space; these co-factors accelerate various reactions in the coagulation cascade. The presence of factor Va speeds up the action of Xa on prothrombin by about 1,000 times. Secondly, all these reactions take place at much greater rates at the surface of membranes, in particular negatively charged membranes. Intact cell membranes are phospholipid bilayers with the negative charges in the centre of the structure. The major source of this phospholipid is from

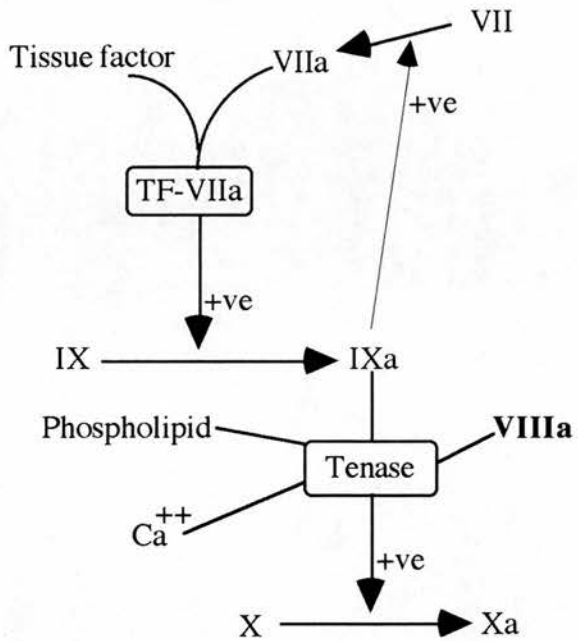
activated platelets. Thirdly, the presence of activated platelets promote thrombin production by releasing prothrombotic factors and by providing a large area of exposed phospholipid.

The combination of factor Xa, factor Va, calcium and phospholipid is known as *prothrombinase*. This complex activates prothrombin at 300,000 times the rate of factor Xa alone.[177]

As stated above factor Xa combines with tissue factor pathway inhibitor and forms a complex that inhibits the activation of factor X. This negative feedback loop prevents the large-scale activation of factor X except when large amounts of tissue factor are present.

Another means exists to overcome this negative feedback loop (Figure 2). The factor VIIa-tissue factor complex not only activates factor X directly, as detailed above, but also indirectly via factor IX, antihaemophilic factor B.[171] Tissue factor-VIIa activates factor IXa which in turn activates factor X. This latter reaction requires calcium ions and is greatly accelerated in the presence of factor VIIIa, a large coagulation co-factor, and negatively charged membrane.[178] The complex of factor IXa and VIIIa on phospholipid is known as *tenase* and is analogous to the prothrombinase complex. The degree of acceleration produced by this complex is such that in the presence of VIIIa and phospholipid, factor IXa can activate as much factor X in one minute as would take 6 months for factor IXa alone to produce. Factor IXa also converts factor VII to VIIa in a positive feedback pathway similar to that of factor Xa.

Figure 2 *Pathway of tenase formation*



This pathway explains why haemophiliacs, either with factor VII deficiency (haemophilia A) or factor IX deficiency (haemophilia B), tend to bleed at sites where tissue factor is in low concentrations e.g. intra-articular haemorrhage but not intracerebral haemorrhage. Where there are large quantities of tissue factor the procoagulant forces may be great enough to overcome the tissue factor pathway inhibitor negative feedback pathway and produce adequate amounts of thrombin without the need for the *tenase* complex.

Thrombin promotes its own production by powerful positive feedback mechanisms at several points. It acts on circulating factor VIII-vWF complex to release and activate factor VIII. It activates factor V which then becomes a potent co-factor in the generation of Xa. Finally thrombin is one of the most potent platelet activators. The influence of platelets on the generation of thrombin will be discussed below.

Thus it can be appreciated that if factor X activation occurs at a great enough rate to overcome the negative feedback of Xa-TFPI complex, the generation of small amounts of thrombin will trigger a series of reactions which result in the explosive generation of large amounts of thrombin.

Platelet adhesion and activation

At the site of vessel injury there are three principle platelet reactions: adhesion, aggregation and release reaction. When the vessel wall is injured circulating von Willebrand factor binds to exposed collagen and elastin fibres. Platelets adhere to von Willebrand factor via the platelet membrane glycoprotein 1b (GP1b).[179,180] This step is of vital importance in initiating normal haemostasis. For example in both von Willebrand's disease, in which there is deficiency of von Willebrand factor, and Bernard-Soulier syndrome, in which platelets lack GP1b receptors, the bleeding time is markedly prolonged.

Platelets, once adherent at the site of injury, undergo a structural change and release factors which both stimulate the aggregation of further platelets and promote coagulation. This reaction is strongly stimulated by thrombin. The platelet changes from a discoid shape to a spherical shape, then forms processes and finally flattens and spreads. During these structural changes, glycoprotein expression on the platelet membrane changes. The most significant of these is the expression of GPIIb/IIIa, a binding site for other platelets and fibrin.

The principle agents which promote platelet aggregation are thromboxane, which is synthesised de novo from membrane phospholipid after platelet adhesion, and platelet activating factor, which is stored in intracellular granules. Platelets release large amounts of factor V which, after being activated by thrombin, accelerates the coagulation system as detailed above.

One of the most important mechanisms by which platelets accelerate coagulation is the "flip-flop" reaction.[181] As platelets undergo the release reaction, there is a conformational change in the platelet membrane which exposes the negatively charged end of phospholipids. This phospholipid is necessary for the formation of

both the prothrombinase and tenase complex and increases the activity of IXa-VIIIa and Xa-Va by about 1,000 times.

The physical presence of a platelet aggregate may be important in the process of coagulation. The reduced blood flow in the region of the platelet aggregate allows adequate concentrations of various coagulation factors to accumulate and remain in close approximation for sufficient time to enable the series of reactions which produce fibrin to take place.

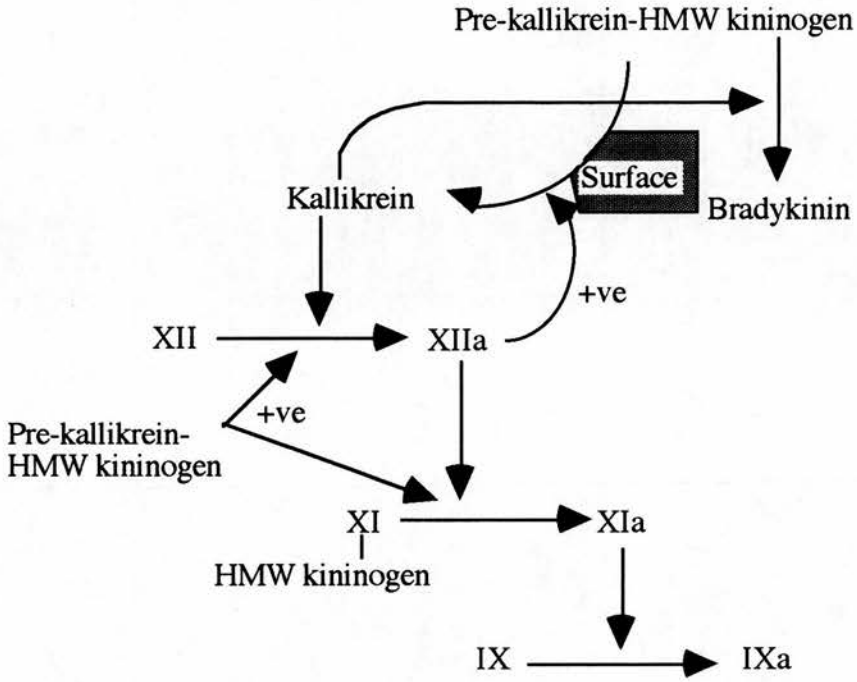
The contact pathway of coagulation.

When blood is placed in contact with foreign surface it clots. This process occurs via the intrinsic pathway of coagulation, so called because all the components required for coagulation are contained within the plasma. This pathway is shown in Figure 3. The later stages from factor IXa are the same as in the tissue factor pathway detailed above. The early steps which lead to factor IX activation are known as the contact phase. This contact phase is also important in other physiological systems such as the complement and kallikrein systems.

Pre-kallikrein activation is the trigger for the contact phase. Pre-kallikrein circulates bound to high molecular weight (HMW) kininogen. When this complex comes into contact with foreign surface the prekallikrein is released as kallikrein, a serine protease. Kallikrein acts on high molecular weight kininogen producing bradykinin. Factor XII, also known as Hageman factor, is activated by a numbers of enzymes, factor XIIa, XIa, plasmin and kallikrein, with kallikrein being the most important of these. There is a positive feedback loop with XIIa increasing the rate of activation of pre-kallikrein. Factor XIIa activates factor XI which in turn acts on factor IX. All these reactions take place at a negatively charged surface. The subsequent events are as detailed above.

The contact system is interesting in that deficiency of any of the components does not lead to a bleeding disorder. It has thus been considered that this pathway is not important in the *in vivo* physiological haemostatic system. However this pathway may be the means by which the coagulation system is activated during disease states.

Figure 3 *The contact pathway of coagulation*



Formation of the platelet - fibrin plug

The principle substrate for thrombin is fibrinogen, one of the most plentiful circulating plasma proteins. Thrombin cleaves two polypeptides from fibrinogen creating fibrin monomer. The fibrin monomer rapidly polymerises to form insoluble fibrin strands. Adjacent strands cross link by covalent bonds in a calcium dependent process which is initiated by factor XIIIa, a circulating enzyme which is activated by thrombin.[182,183] The cross-linked fibrin is mechanically stronger and more resistant to fibrinolysis. Fibrin binds to activated platelets via the platelet membrane protein glycoprotein IIb/IIIa (GPIIb/IIIa) forming a strong platelet-fibrin plug. Identical GPIIb/IIIa receptors are present on endothelial cells and may help to anchor the clot.

It must be considered that cross-linking is an important clinical process as deficiency of factor XIII is associated with a bleeding tendency.[184] Patients have poor wound healing and frequently die from intracranial haemorrhage.

An important feature of the fibrin clot is that it is not a solid gel but is rather an open mesh of long fibres. This structure allows fibroblasts to migrate easily through the fibrin meshwork and also facilitates thrombolysis by plasmin. This is important in the healing process.

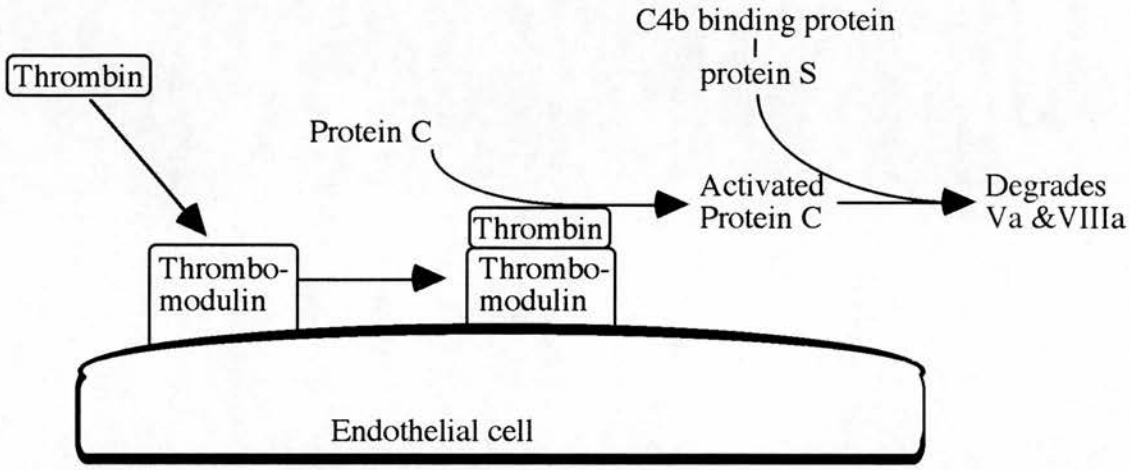
Thrombin inactivation

As detailed above, injury to the vessel wall results in explosive production of thrombin. In order to prevent massive and inappropriate thrombosis there must be an efficient mechanism to inactivate thrombin and to remove excess fibrin. This is mainly mediated by the anticoagulant and fibrinolytic effects of intact endothelial cells

Thrombin is inactivated and cleared from the circulation by a series of antithrombins; principally by antithrombin III (70%), and to a lesser extent by $\alpha 2$ macroglobulin (20%) and $\alpha 1$ antitrypsin (10%). Thrombin binds to these antithrombins and splits them. It remains attached to the split product and is thus no longer active. The thrombin-antithrombin complex is cleared from the circulation by the liver in less than five minutes.[185] The presence of heparin-like molecules on the surface of healthy endothelial cells greatly accelerates this reaction.[186] This process is highly efficient and free thrombin is rapidly removed from the circulation. However the fact that antithrombins are consumed during this process means that the ability to inactivate thrombin is limited and may be overcome if the rate of thrombin generation is high.

Thrombomodulin, a protein which is found on the surface of endothelial cells, is a major inhibitor of thrombotic activity (Figure 4). Thrombin binds to thrombomodulin on the endothelial cell surface. This complex is then able to activate protein C which in turn cleaves factors Va and VIIIa rendering them inactive. This reaction is increased approximately ten-fold in the presence of protein S - a vitamin K dependent cofactor which circulates bound to C4b binding protein. Protein C has a much greater affinity for factor Va and VIIIa than for their precursors factors V and VIII.

Figure 4 *Thrombomodulin, protein C, protein S pathway of inhibition of coagulation*



Fibrinolysis

Fibrinolysis is an essential process in normal haemostasis. It is by this means that excess fibrin is removed and vessels are recanalised after repair of injury. The major fibrinolytic enzyme is plasmin which circulates in the form of an inactive precursor plasminogen synthesised by the liver. The principle activator of plasminogen is tissue plasminogen activator (tPA) which is produced by endothelial cells. Endothelial cells store tPA and thus a large amount can be released upon stimulation of the endothelial cell. Various substances stimulate the release of tPA from endothelial cells, the most potent being thrombin.[187] tPA binds to fibrin and undergoes a conformational change which increases it ability to activate plasminogen. Plasminogen and plasmin also bind to fibrin. These interactions with fibrin mean that plasmin activity is maximal in the area of the clot.[188]

Plasmin is a serine protease whose principle action is to lyse fibrin. This it does by splitting a series of lysyl-arginine bonds to produce a number of fibrin degradation products (FDPs). In addition to its fibrinolytic effects, plasmin also acts in a lytic fashion on various coagulation factors such as V, VIII, fibrinogen and vWF.

As with other physiological processes there is a mechanism for inactivation and inhibition of fibrinolysis and this occurs at two levels. The inactivation of tPA is controlled by endothelial cells which produce plasminogen activator inhibitor 1 (PAI-1) and the inactivation of plasmin is effected by α-1 antiplasmin, a circulating plasmin inhibitor. PAI-1 is normally produced at a greater rate than tPA and thus there is net inhibition of tPA, except when the endothelial cell is stimulated and releases large quantities of stored tPA. When plasmin is bound to fibrin it is inaccessible to α-1 antiplasmin and thus avoids inactivation. However any freely circulating plasmin is quickly inactivated by α-1 antiplasmin.

Endothelial cells

Endothelial cells were at one time thought merely as inert lining cells. However it is now well established that they play a vital role in the control of haemostasis, with effects on coagulation, fibrinolysis, platelets and vessel tone, and have important interactions with the immune system, regulating leucocyte adhesion and migration. Healthy, intact endothelial cells have a strong anticoagulant effect by inhibiting thrombin production, platelet aggregation and by removing thrombin and the activated clotting factors.

Endothelial cells provide a physical barrier preventing the large clotting factors and platelets from coming into contact with tissue factor and collagen which are abundant in the sub-endothelium. The cell membrane contains large amounts of heparans, endogenous heparin-like factors which enhance thrombin inactivation by anti-thrombins. Endothelial cells are the main site of synthesis of tissue factor pathway inhibitor, which is released into the general circulation and is also bound to the cell surface.[189] Endothelial cells produce prostacyclin, a potent inhibitor of platelet activation and a vasodilator.

Endothelial cells are important in the inactivation of thrombin. Thrombomodulin is produced by endothelial cells and expressed on the luminal surface. Thrombin binds to thrombomodulin on the endothelial cell surface forming a complex which activates protein C. Activated protein C in conjunction with protein S, also produced by endothelial cells, inactivates factors Va, VIIIa, tenase and prothrombinase.

Endothelial cells are important in the control of fibrinolysis as they may both promote fibrinolysis, by production of tPA, and inhibit it by production of plasminogen activator inhibitor. The means by which endothelial cells exert control on this process remains obscure. The lack of understanding on this subject is largely

due to the fact that endothelial cells in culture behave in a markedly different fashion to endothelial cells in vivo in the expression of tPA. The difference in cellular activity in this area also casts some doubt on the validity of results from studies of other facets of endothelial cell behaviour which have largely been determined from cell culture models.

Endothelial cells also play a vital role in the regulation of vascular tone through the release of either vasodilator factors such as prostacyclin and endothelial derived relaxing factor, or vasoconstrictor factors such as endothelin.

As well as this anti-coagulant and fibrinolytic role, endothelial cells have important pro-coagulant functions. Endothelial cells produce nearly all of the circulating von Willebrand factor. This released constitutively and is also stored intracellularly in the Weibel-Palade bodies.[190,191] The contents of the Weibel-Palade bodies are released upon stimulation by factors such as thrombin and fibrin.

Tissue factor may be expressed by endothelial cells following stimulation by a variety of means including endotoxin, cytokines and hypoxia. Tissue factor is an integral membrane protein and is therefore released by the shedding of membrane vesicles.[192]

While it was previously thought that denudation of the endothelial cells with exposure of underlying collagen was required for initiation of platelet aggregation and coagulation, it is becoming apparent that such processes may take place on the surface of intact but activated endothelial cells. Animal models have shown that endothelial cell injury, sufficient to activate cells but not destroy them, is followed by platelet aggregation on the surface of endothelial cells.[193,194]

The luminal surface of the endothelial cell has receptors for a number of coagulation factors and may increase the rate of various reactions in the coagulation cascade by

bringing certain components closer to one another. Activation of factor X on the surface of endothelial cell occurs at a slow rate via the intrinsic pathway, facilitated by factor IX and XI binding to the cell surface, in unstimulated cells and at a much greater rate via the extrinsic pathway, promoted by tissue factor expression, on activated cells.[195]

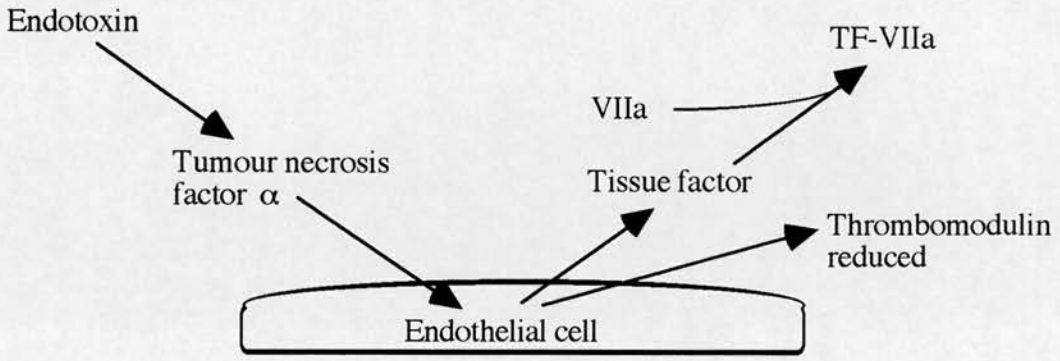
Pathogenesis of DIC

As noted above, DIC is not a disease in its own right but is rather a secondary response to an underlying primary pathology. It may be regarded as a final common pathway for a number of conditions which are highly varied and otherwise completely unconnected. Thus it can be expected that there is not one single all-encompassing pathogenic mechanism but rather a multitude of pathways which lead to a final common event. It is generally considered that this common event is excessive thrombin generation.

The pathways which lead to this thrombin generation are varied. It may be quite direct as with some snake venoms.[169] The venom of *Vipera Russelli* (Russell viper) directly activates factor X and *Echis Carinatus* venom directly activates prothrombin. In other conditions it is considered that activation takes place via the tissue factor pathway. For example in abruptio placenta and severe brain injury large amounts of tissue factor are released into the circulation.

In septicaemia the pathway which leads to thrombin generation more complex (Figure 5).[196] The presence of circulating endotoxin lead to increased expression of various cytokines, most notably tumour necrosis factor α (TNF-α).[197,198] TNF-α activates endothelial cells giving rise to increased tissue factor expression and subsequent activation of coagulation via the extrinsic pathway.[199,200] Monocytes may also be important mediators in this process as these cell also express tissue factor when activated by endotoxin. In addition monocytes can activate endothelial cells.[201]

Figure 5 *Pathway of thrombin activation by endotoxin*



Whatever the cause of its production, the presence of large amounts of thrombin within the circulation leads to a self promoting downward spiral. At this point it is important to reiterate that in normal circumstance these events are prevented by the rapid removal of thrombin from the circulation by antithrombins and the thrombomodulin/protein C/protein S system on intact endothelial cells. Thus in order for the following sequence of events to occur, either thrombin generation must be so great that it overcome the inhibitory process or the inhibitory process itself must be defective.

An example of a defective inhibitory system is found in liver failure. Patients with liver disease have a reduced ability to synthesise clotting factors, including inhibitory factors such as antithrombin III and protein C, and have a reduced ability to clear activated clotting factors from the circulation. These patients are at much greater risk of developing DIC when subjected to a procoagulant stimulus. In animal models the administration of thoratrast, which affects liver function, induces DIC.[202]

In septicaemia increased levels of TNF-a can reduce expression of thrombomodulin by endothelial cells.[203,204] This reduces the capacity to inactivate thrombin.

Inhibition of the protein C-protein S pathway in primate experiments results in the production of DIC with 10 times lower doses of E Coli.[205]

The sequence of events that follows excessive thrombin production is as follows. Thrombin via a number of positive feedback loops promotes its own production. Circulating thrombin activates platelets, producing circulating platelet-fibrin microthrombi which themselves are potent stimulants of thrombin production. Activated platelets release procoagulant substances and provide a source of phospholipid which increases the rate of production of thrombin. This process leads to the consumption of fibrinogen and clotting factors which is exacerbated by the widespread activation of plasmin.

Thrombin stimulates endothelial cells to produce tPA which in turn promotes plasmin production. Plasmin performs its physiological role and lyses fibrin leading to the production of fibrin degradation products. Fibrin degradation products have an anticoagulant effect; they bind to fibrin monomers preventing polymerisation to fibrin and producing soluble fibrin monomer.

Plasmin is a relatively non-specific serine protease, unlike thrombin, and has effects on a number of proteins. It lyses fibrinogen and various clotting factors, most notably factors V, VIII, IX and XI, and a number of other plasma proteins. This further reduces the amount of circulating fibrinogen and clotting factors and increases fibrinogen degradation products which have a similar anticoagulant effect to fibrin degradation products.[206] Plasmin also has effects on platelets causing reduced GP1b expression either by lysis or redistribution within the platelet.

This increased plasmin activity has been described as inappropriate but it should be remembered that in DIC there is widespread deposition of fibrin in the microvasculature and the increased plasmin activity required to remove this is appropriate. The inhibition of plasmin, using drugs such as aprotinin and tranexamic

acid may therefore be detrimental to the patient.

Whether increased plasmin activity is the primary agent in producing DIC, rather than a secondary event to thrombin activity, has been much debated. In some cases, such as the administration of large amounts of tPA, it may be the primary event but it is most often secondary to excess thrombin production. Fibrinolysis as a primary cause of bleeding is uncommon, although documented,[207] and the bleeding disorder in DIC is thought to be due to reduced levels of clotting factors and platelets rather than direct fibrinolysis of clot at the site of injury.

By this combination of events the end state of DIC is reached: There is widespread fibrinolysis with elevated fibrin degradation products and increased thrombin activity as shown by increased fibrinopeptide A and thrombin-antithrombin -complexes. Levels of fibrinogen, clotting factors and platelets are markedly reduced such that normal haemostasis is severely impaired.

Management of DIC

A great deal of controversy surrounds the management of DIC. There is much opinion and dogma but little hard evidence available from clinical trials. Much of the problem lies in the fact that DIC is not a single clinical entity but varies in clinical presentation and has a large number of underlying causes. Thus it is unlikely that a single management strategy will be adequate for all patients. This diversity also produces problems in clinical trials. In some studies patients with DIC from a variety of causes are grouped together and a benefit from treatment in some patients may be obscured by there being no benefit, or a detrimental effect, in patients with DIC of a different aetiology.

The mainstay of therapy for DIC is to treat the underlying disorder and thus remove the stimulus for thrombin production. The patient should be generally supported to maintain adequate oxygenation and perfusion of vital tissues. In many patients, for example those with sepsis, who are not actively bleeding, this may be all that is necessary. In patients who are actively bleeding further intervention may be required. Treatment options include the replacement of clotting factors and platelets, blocking thrombin production with heparin, inhibiting thrombin with the serine protease inhibitor gabexate or hirudin, increasing clearance of thrombin by administering anti-thrombin III and inhibiting plasmin with a serine protease inhibitor such as tranexamic acid or aprotinin. All of these treatments are controversial.

Clotting factors are replaced in the form of fresh frozen plasma and cryoprecipitate. Fresh frozen plasma contains all clotting factors in approximately physiological concentrations. Cryoprecipitate is mainly used to provide fibrinogen, containing 0.15-0.3g fibrinogen in 50ml, and does not contain significant amounts of other clotting factors. Some authors advocate that fibrinogen should not be given in

ongoing DIC as it will only "stoke the fire" and lead to greater consumption of clotting factors, increased fibrin degradation products and increased fibrin deposition in vital tissues.[208]

The transfusion of platelet in patients with DIC and a bleeding problem has few drawbacks although platelet life span is likely to be severely limited and massive quantities may be required to keep the platelet count at near normal levels. Platelets are normally supplied as a suspension of platelet in plasma. The plasma may contain significant amounts of clotting factors and fibrinogen. Each unit of platelets contains about 30×10^{10} platelets, however the proportion of these which are fully functional depends on the age of the platelets.

Heparin is a well established treatment for DIC.[209] The rationale behind heparin therapy is that it reduces thrombin production and activity and thus breaks the cycle that causes DIC. In addition it should reduce fibrin deposition in the microvasculature of vital organs. It seems to be particularly effective in patients with the chronic thrombotic form of DIC that is commonly associated with malignancy. Heparin has been used successfully to correct the DIC associated with a non-ruptured aortic aneurysm prior to elective surgery.[210,211]

However the use of heparin in situations where bleeding is the predominant problem is controversial.[208] Some authors advocate an aggressive approach administering heparin even in the presence of severe bleeding. However others express concern that heparin may exacerbate the bleeding tendency. In a randomised trial of heparin use in patients with DIC following trauma excessive bleeding was reported in those patients treated with heparin.[212] In addition there are practical problems in establishing the correct dosage. The dosage of heparin is normally adjusted according to two parameters - the APPT and the presence of bleeding. Both these are unreliable in a patients with DIC who is bleeding excessively.

While some of authors who advocate heparin therapy state that it should be given alone, others recommend that clotting factor replacement and platelet transfusion should be given concomitantly as there will be some delay between inhibiting the DIC process and the manufacture of clotting factors and platelets which are required to stop the bleeding.[213]

Antithrombin III has several features which indicate that it might be useful in DIC: antithrombin III is the principle physiological means by which thrombin is removed from the circulation and levels of antithrombin III are low in DIC. The first report of the use of antithrombin III in DIC was published in 1974.[214] There have been many subsequent reports, including some randomised trials and animal studies, which have suggested a benefit from antithrombin III.[212,215,216] A recent review recommended the use of antithrombin III in acute DIC,[208,217] but it has not yet become established as first line treatment.[213,218]

Activated protein C has been suggested as a possible therapeutic agent for much the same reasons as antithrombin III. Protein C is an important inhibitor of coagulation and levels are reduced in DIC.[196] Its use has been reported in 3 patients with DIC and was said to be effective.[219] Clinical trials of protein C in patients with DIC are now underway.[220]

There are a number of case reports and small studies concerning the use of gabexate mesilate in DIC. Gabexate mesilate is a serine protease inhibitor with particular affinity for thrombin. Published reports have been favourable, and it appears to be well established in Japan, but larger clinical trials will be required to establish its use elsewhere.[221-224]

Tissue factor pathway inhibitor has been shown to be effective in preventing DIC in a rabbit model of DIC induced by tissue thromboplastin infusion.[225] However further evidence of efficacy, for example from primate models of septicaemia, will

be required before clinical trials could be contemplated. Another substance at an early stage of development is hirudin, a specific thrombin inhibitor produced by medicinal leeches.[226] It can be produced using recombinant DNA technology. Some animal studies have indicated that it may be useful in DIC but clinical studies are awaited.

In summary there are a number of management strategies in acute DIC with severe haemorrhage and all combinations of heparin, antithrombin III and clotting factor replacement have been recommended. Unfortunately there are few well designed and conducted trials of these modalities on which to base recommendations and there is little consensus at present.

Coagulopathy and abdominal aortic aneurysms

Relatively little detailed research has been carried out concerning the coagulation system in patients with aortic aneurysms. Most publications on this subject have been clinical in nature, describing the epidemiology, clinical features and outcome for patients with coagulopathy in association with aortic aneurysms. In addition there is no suitable animal model. However some investigation has been carried out as will be detailed below.

Some patients with asymptomatic aortic aneurysms have deranged coagulation systems on laboratory testing[77] and may rarely present with symptoms of coagulopathy such as gingival bleeding and excessive bruising.[71-75] In the study by Fisher *et al* about 10% of patients with non-ruptured aneurysm had elevated levels of fibrin degradation products and a mild thrombocytopenia.[77,227] It has been suggested that this is due to consumption of clotting factors and platelets during turnover of the clot within the aneurysm sac. This is supported by studies using radiolabelled platelets and fibrinogen which have demonstrated platelet and fibrin deposition within the aneurysm sac.[228,229] A more recent study using radiolabelled monoclonal antibodies to tissue plasminogen activator has demonstrated fibrinolytic activity within the aneurysm sac which was not present after aneurysm repair.[230]

The development of coagulopathy during elective aneurysm repair is a well recognised, although uncommon, complication of elective aortic surgery. The pathological process which leads to the development of this coagulopathy remains obscure and very little is known of the changes which occur in the coagulation system during operation. In one study of the coagulation system during aorto-femoral bypass grafting for occlusive disease it was shown that levels of fibrinogen dropped throughout operation, most markedly after aortic cross-clamping.[231] A more recent study has examined the coagulation system in patients undergoing aorto-

femoral grafting for a mixture of occlusive and aneurysmal disease.[232] This indicated that fibrinolytic activity, as determined by B-Beta 15-42 and fibrin degradation products, increased during operation. Platelet count decreased steadily through operation. There was a slight drop in levels of inhibitory factors such as α2-antiplasmin and anti-thrombin III. However a policy of haemodilution during the early stages of operation and the administration of large amounts of fresh frozen plasma during operation mean that the validity of these findings are doubtful. No examination of thrombin activation was carried out.

The coagulopathy reported in association with ruptured aortic aneurysm may be present on admission or may rise intra-operatively. The laboratory findings are of DIC although some patients on admission have thrombocytopenia without prolongation of clotting times or elevated fibrin degradation products.[77,162] Most studies have presented findings in post-operative patients with well established DIC and there is a lack of information about the early changes in the coagulation as found on admission and the changes which occur during operation.

On the basis of on this knowledge, and the understanding of the haemostatic system derived from in vitro studies, a hypothesis can be formulated to explain the pathogenesis of the coagulopathy associated with aortic aneurysms.

Hypothesis

Patients with intact abdominal aortic aneurysms have constant turnover of clotting factors and platelets in the clot within the aneurysm sac. This is usually compensated but in the case of very large aneurysms or reduced ability to synthesise clotting factors there may be DIC evident on laboratory studies or even clinically apparent abnormal bleeding. Thus the haemostatic system is "primed" and thus more easily perturbed by the effects of rupture and operation.

Rupture has two effects: firstly there is active bleeding and a large blood clot within the abdominal cavity, secondly there is hypovolaemic shock. Blood loss, active bleeding and fibrinolysis within the blood clot produce large amounts of thrombin and consume platelets and clotting factors. Hypovolaemic shock leads to tissue hypoperfusion and hypoxia. Endothelial cells and leucocytes are directly and indirectly activated by hypoxia and in turn trigger the coagulation and fibrinolytic system.

During operation, tissue trauma, continued blood loss and blood transfusion contribute to increasing production of thrombin and consumption of clotting factors and platelets. Aortic cross-clamping leads to hypoperfusion and hypoxia in tissue distal to the clamp with effects as described above. In particular there is bowel ischaemia resulting from a combination of clamping the inferior mesenteric artery, hypovolaemic shock and the effects of retracting the small bowel from the abdominal cavity.[233,234] This leads to bacterial translocation across the gut mucosa resulting in endotoxaemia. Endotoxaemia alone can produce DIC and may well contribute in this situation.

Finally reperfusion of ischaemic tissues on release of the aortic clamp washes out noxious metabolites and activated leucocytes with further adverse effects on the

haemostatic system.[235]

At some stage in this sequence of events, the forces driving activation of the haemostatic system may so consume vital components of the haemostatic system and overcome the normal homeostatic mechanism that disseminated intravascular coagulation occurs.

It is on this hypothesis that the studies in this thesis have been designed and most of the elements of this hypothesis will be examined, in the hope of proving, disproving or refining this hypothesis.

Chapter 2

Post-operative Haemorrhage Following Aortic Aneurysm Repair: A Retrospective Case-control Study

Introduction

Haemorrhage both during and after operation used to account for the majority of deaths after aneurysm repair. The incidence of this complication was much reduced by the introduction of the "inlay" technique, which avoids the need for extensive dissection and reduces the length of operation.[26] However haemorrhage remains one of the more common complications of aneurysm repair.[122,143,236,237] Most authors have concentrated on technical operative errors as the cause of these complications - e.g. bleeding from anastomoses or unrecognised lumbar arteries.[238] The primary aim of this study was to determine whether technical errors are to blame for most of these bleeding episodes or whether they could be attributed to the presence of a coagulopathy. The secondary aims were to determine the incidence of post-operative haemorrhage after elective and emergency aortic surgery and what factors (e.g. operation length, blood loss, transfusion) are associated with post-operative haemorrhage.

Methods and materials

Patients undergoing laparotomy for control of haemorrhage following aortic aneurysm repair during the period 31st July 1988 - 1st August 1994 in the Vascular Surgery Unit of the Royal Infirmary of Edinburgh were identified by means of the Vascular Audit system. Nineteen patients were identified, 11 male and 8 female, with a mean age of 73 ± 5 years. In 7 cases the first operation had been elective and in 12 the operation had been performed for rupture. During this period 378 elective operations and 408 emergency operations were carried out for abdominal aortic aneurysm in this unit giving an incidence of operation for post-operative bleeding of 1.9% after elective operations and 2.7% after emergency operations. Case-controls, matched for sex and indication for operation, were selected from operation records during the same period by the following method. For each study patient undergoing laparotomy for the control of haemorrhage the next patient of the same sex undergoing aortic aneurysm repair for the same indication was selected. The mean age of the control group was 71 ± 8 years.

Operations were carried out either by a Consultant vascular surgeon or by a Senior Registrar with considerable experience in vascular surgery. All patients undergoing elective operation were given systemic heparin (5,000 iu) prior to cross-clamping. Those undergoing operation for rupture were not given systemic heparin but heparinised saline (5,000iu / 500ml 0.9% saline) was used for irrigation.

Statistical analysis was carried out using Systat for windows v5.0®. Mann-Whitney U-test, Chi-squared and Fisher exact tests were used where appropriate. Data are given as median and range unless otherwise stated.

Results

Operative findings

Mean operating time, cross clamp time, blood loss and transfusion requirements were all greater in patients who bled post-operatively than in the controls, although these differences were not statistically significant except for blood loss - $p< 0.05$ by Mann-Whitney test (Table 3 & 4). The study group had a lower mean temperature at the end of operation - $p < 0.05$ by Mann-Whitney test. These data suggest that patients who bled post-operatively had more complicated primary operations.

At re-exploration a single bleeding point was found in only 5 patients. Of these three were bleeding from the proximal anastomosis, one from a lumbar artery and one from the median sacral artery. In 2 patients no active bleeding point was found although there was a considerable volume of blood in the peritoneal cavity. In the remaining 12 multiple minor bleeding points and/or diffuse oozing were found.

Clinical Outcome

In total there were 11 deaths (58%) within 30 days in the study group compared with 4 deaths (21%) in the control group - $p < 0.05$ Pearson chi-squared (Table 5). The mortality rate for the patients who had undergone an elective primary operation (5/7) was greater than for those who had undergone operation for rupture (6/12).

Eight of those who died in the study group followed a clinical course of progressive deterioration and death from continuing haemorrhage and multi-organ failure. Ten patients made a good initial recovery and were discharged from the intensive care unit back to the general ward. In this group there were three late deaths; two from cerebro-vascular accident (CVA), both at ten days post-operatively and one from

cardiac arrest on the fourteenth post-operative day, subsequent to a myocardial infarction diagnosed on electrocardiogram findings earlier that day. In addition to these deaths a further patient suffered a non-fatal CVA 19 days after operation. In two of the three CVA cases the diagnosis was made on clinical grounds and in the other the diagnosis was confirmed by CT scanning. There were no late deaths in the control group but one patient suffered a non-fatal MI on the second post-operative day.

Coagulation studies

All but two patients who required re-operation had abnormal results on coagulation screen following the first operation (Tables 3 & 4). The two patients who did not have a coagulopathy both bled because of a technical error - failure to under-run a lumbar artery and bleeding from the proximal anastomosis. Of the seven elective patients who required re-laparotomy for haemorrhage, 4 had platelet counts of less than 100×10^9 /l and a further patient had a platelet count less than 150×10^9 /l following the first operation (normal range $150\text{-}450 \times 10^9$ /l). Only one of the elective patients in the control group developed post-operative thrombocytopenia with a platelet count of 93×10^9 /l.

Table 3 *Clinical and operative details of patients requiring re-operation after primary operation for ruptured aneurysm. (Figures are median and range)*

	Study Group		Control Group		
Pre-operative platelet count ($x10^9$ /l)	103	81-385	253	244-336	NS
Platelet count after first operation ($x10^9$ /l)	92	40-140	112	64-175	NS
Fibrinogen after first operation (g/l)	1.12	0.6-2.9	1.30	0.6-3.8	NS
Prothrombin ratio after first operation	1.6	1.1-3.3	1.4	1.0-2.2	NS
Temperature at the end of operation(^oC)	34	31.6-36	35	32.5-37	$p<0.05$
Operation length (mins)	180	85-320	112	80-300	NS
Cross-clamp time (mins)	65	44-100	45	40-70	NS
Blood Loss(ml)	5150	2000-12500	2035	840-10700	$p<0.05$
Transfusion RCC (units) during first operation	12	6-18	6	1-16	NS

Table 4 *Clinical and operative details of patients requiring re-operation after elective primary operation for aortic aneurysm. (Figures are median and range)*

	Study Group		Control Group		
Pre-operative platelet count ($x10^9$ /l)	163	120-304	201	124-395	NS
Platelet count after first operation ($x10^9$ /l)	81	41-181	173	93-220	NS
Fibrinogen after first operation (g/l)	1.59	1.1-2.2	3.28	1.7-4.8	NS
Prothrombin ratio after first operation	1.6	1.0-1.8	1.2	1.0-1.4	NS
Temperature at the end of operation (^oC)	35.2	35-36.1	36.5	36-37	NS
Operation length (mins)	160	160-240	120	110-220	NS
Cross-clamp time (mins)	80	44-100	45	40-70	NS
Blood Loss (ml)	2125	390-3665	1200	450-2000	NS
Transfusion RCC (units) during first operation	3	0-8	2.0	0-3	NS

Table 5 *Outcome of patients requiring re-operation after aortic aneurysm repair*

	Type	Outcome		Operative findings
1	Elective	Died	Early Death	Proximal anastomosis
2	Elective	Died	MI day 12	Diffuse bleeding
3	Elective	Survived		Lumbar artery
4	Elective	Died	Early Death	Diffuse bleeding
5	Elective	Died	CVA day 10	Proximal anastomosis
6	Elective	Survived		Proximal anastomosis
7	Elective	Died	Multi-organ failure	Diffuse bleeding
8	Rupture	Died	CVA day 10	Diffuse bleeding
9	Rupture	Survived	Renal Failure	Diffuse bleeding
10	Rupture	Survived		Median sacral artery
11	Rupture	Died	Early Death	Diffuse bleeding
12	Rupture	Died	Early Death	Diffuse bleeding
13	Rupture	Survived	Renal Failure	Diffuse bleeding
14	Rupture	Survived		Diffuse bleeding
15	Rupture	Survived	CVA day 19	Diffuse bleeding
16	Rupture	Survived		Diffuse bleeding
17	Rupture	Died	Early Death	No active bleeding
18	Rupture	Died	Renal Failure and MI	No active bleeding
19	Rupture	Died	Early Death	Diffuse bleeding

Discussion

This study confirms that operation for control of haemorrhage after aortic aneurysm repair is an uncommon but serious complication which is nearly always associated with a coagulopathy and thrombocytopenia. The mortality rate of 58% in this study was lower than that reported elsewhere. Slootmans *et al* and Gloviczki *et al* reported mortality rates of 85% and 90% respectively for series of patients operated on for haemorrhage after repair of ruptured aneurysm.[153,239] However a remarkably low mortality rate of 11% was reported by Fielding in a study of 9 patients undergoing laparotomy for post-operative haemorrhage.[144]

The importance of coagulopathy has not been emphasised in previous reviews on this subject.[238,239] From the data available in this study it is impossible to determine whether the coagulopathy is the cause of the haemorrhage or vice-versa. However, the fact that patients who bled had lower platelet counts prior to the first operation and that very often no significant bleeding point could be found suggests that coagulopathy is an important causal factor in most patients. It should also be noted that many of the patients with ruptured aneurysms received platelet transfusions (median 5 units, range 0-20 units) and it may be that without them the severity of post-operative thrombocytopenia would have been greater.

The role of coagulopathy as a causal factor of haemorrhage is easily understood in patients with ruptured aneurysms, as it is well established that many of them have coagulopathy on admission.[162] It may also be the case that coagulopathy is a causal factor in patients who undergo elective repairs. Coagulopathy is an established complication of elective aortic aneurysm repair. It was first reported in 1955 and subsequent studies have revealed that laboratory evidence of abnormal coagulation following aortic surgery is common.[15,66,227,231] In the present study 5 out of 7 patients who required re-operation after elective operations had thrombocytopenia

following the primary operation. Examination of the records of 20 consecutive patients undergoing elective aneurysm repair revealed that only one had post-operative thrombocytopenia. This is a significantly lower incidence of post-operative thrombocytopenia than in the study group - $p < 0.02$ by Fisher exact test.

It is notable that the two patients who had normal platelets counts both bled because of a technical error and both had a good outcome. Thus it appears that there are two distinct causes of haemorrhage after elective aneurysm repair:- one group of patients bleed because of an operative error. These patient have normal coagulation and a good outcome - 100% survival in this study. A second group of patients bleed secondary to a coagulopathy which has developed intra-operatively. These patients have a poor outcome - 100% mortality in this study.

It is interesting to note that many patients were hypothermic at the end of operation. The hypothermia in the patients who bled may simply reflect the fact that they had undergone longer and more complicated operations but it should be noted that hypothermia itself is associated with a bleeding tendency. Hypothermia may cause a bleeding tendency due to a direct effect on the coagulation system; the coagulation cascades are enzymatic processes which function optimally at 37^{o}C and are less efficient at lower temperatures. This phenomenon is not detected by laboratory testing as laboratory tests, such as prothrombin time and activated partial thromboplastin time, are performed at a standard temperature and not at the temperature of the patient. Hypothermia causes reversible platelet dysfunction, possibly due to effects on thromboxane activity, and potentiates the activation of platelets by plasmin in vitro.[240]

These theoretical effects of hypothermia on the coagulation system have been bourne out in clinical studies. In one study it was found that bleeding times during aortic aneurysm surgery become more prolonged as core temperature falls.[241] It may be

advisable that greater efforts are made to avoid heat loss in these patients through precautions such as heated water blankets, polythene bags around the lower legs, the use of an efficient blood warmer and placing the small intestine in a "gut-bag".

There was a high incidence of stroke in this study. In one case it is known that the stroke was thrombo-embolic in nature rather than haemorrhagic but in the other two cases this information is not available. If these events and the post-operative MI were thrombotic it might reasonably be thought that these patients with a perioperative coagulopathy are developing a "rebound" pro-thrombotic state some days later. Further evidence for this hypothesis is the fact that at the time of these events the platelet count was rising; in two cases the platelet count had more than doubled in the preceding 48 hours.

The presence of a pro-thrombotic state after ruptured aneurysm has been suggested in a recent publication.[163] In a clinical study of patients presenting with ruptured aortic aneurysm it was found that 15/35 patients who survived operation developed a thrombocytosis (platelet count > 400 x 10^9/l) and 10 of these 15 suffered a deep vein thrombosis, 8 also suffered a pulmonary embolus.

An association between coagulopathy and post-operative thrombotic events is also suggested by data published in another study of ruptured aortic aneurysms. Davies *et al* reported that in a group of 12 patients who developed a coagulopathy in association with a ruptured aneurysm there were 3 post-operative deaths which might be attributed to thrombotic events; two from MI on days 1 and 9 and one from stroke on day 14. In contrast, in the group of 23 patients who had no evidence of coagulopathy there were no deaths from MI or stroke ($p = 0.05$ by Fischer exact test).[162] The presence of a prothrombotic state may account for the high incidence of MI in the early post-operative period found in most published series of aortic aneurysm surgery.

In conclusion the evidence from this study indicates that

1. Patients requiring re-operation for control of haemorrhage following aortic aneurysm repair usually have a coagulopathy.

2. Haemorrhage following aortic surgery is uncommonly due to an operative error.

3. Patients requiring re-operation for control of haemorrhage following operation for ruptured aneurysm had greater blood loss during primary operation and had a lower core temperature at the end of the primary operation

4. The development of a coagulopathy is probably responsible for the majority of episodes of post-operative haemorrhage following aortic surgery.

Chapter 3

Methodology for Coagulation Studies

Except where indicated in the text all coagulation assays, sample handling, processing and storage were performed by the staff of the coagulation laboratory of the South-East Scotland Blood Transfusion Service in the Royal Infirmary of Edinburgh. The tests were carried out using the laboratory's standard methods which have been satisfactorily assessed by the National External Quality Control Assurance Scheme (NEQAS).

Blood sampling

In patients with asymptomatic aortic aneurysm venous blood samples were taken from an antecubital vein using a 19 gauge needle with no tourniquet occlusion. In patients undergoing elective and emergency aortic aneurysm repair blood samples were taken from an indwelling intra-arterial line placed in the radial artery. The first 5ml were discarded. After sampling the line was flushed with heparinised saline - 10,000iu in 500ml 0.9% NaCl. All samples were separated within 20 minutes of sampling

A 2.7ml sample was taken into an EDTA (ethylene diamine tetra-acetic acid) anticoagulant - Monovette®, Sarstedt.

A 9ml blood sample was taken into sodium citrate anticoagulant on ice. The anticoagulant comprised of 3.12% trisodium citrate, 5% HEPES in 1,000ml distilled water. 1ml of anticoagulant was placed in a Monoject Z/10 tube. The sample was spun at 2,500 rpm for 10 minutes, the serum was separated and stored at -40°C for later analysis.

For fibrinopeptide A assay a 4.5 ml blood sample was taken into aprotinin/heparin anticoagulant on ice. The anticoagulant comprised of 5mls aprotinin (20,000

KIU/ml), 4mls heparin (25,0000 iu/ml) 9mls 0.9% sodium chloride and 82mls distilled water. 0.5 ml of this anticoagulant was placed in a 10ml siliconised glass tube. The tubes were stored at -70°C until required. The sample was spun at 4°C at 2,500rpm for 30 minutes. The middle third of the plasma was separated and stored at -40°C for later analysis.

For B-thromboglobulin assay a 2.7ml blood sample was taken into EDTA/PGE_1/theophylline anticoagulant on ice. The anticoagulant comprised of 0.1 ml EDTA (10g/dl disodium ethylene diamine tetra-acetic acid adjusted to pH 7.4 with NaOH), 0.1 ml PGE_1 (1μg/ml of prostaglandin E_1 in aqueous solution of 20mg/dl of sodium carbonate) and 0.1 ml theophylline (5.4 mg/ml theophylline in distilled water adjusted to pH 7.4) which were added to a siliconised glass tube. The tubes were stored at -70°C until required. The sample was spun at 4°C at 2,500rpm for 30 minutes. The middle third of the plasma was separated and stored at -40°C for later analysis.

Assay methods

Platelet count

The platelet count was determined on an EDTA sample using a Coulter® T-890 counter.

Fibrinogen

The time to coagulation in a plasma sample activated with thrombin is dependent on the concentration of fibrinogen. A standard thrombin reagent is added to known concentrations of fibrinogen, the time to coagulation is measured and a standard curve is drawn. Plasma samples from patients are activated with the standard thrombin reagent, coagulation times are measured and the fibrinogen concentration is interpolated from the standard curve. Fibrinogen assay was performed on a citrated plasma sample using a Coag-A-mate®-X2, General Diagnostics and Fibriquik® reagent kit , General Diagnostics.

Fibrin degradation products

The assay was performed on a citrated plasma sample using a Diagnostica Stago kit for fibrinogen degradation products.

Pro-thrombin ratio (PTR)

In this test coagulation is activated via the extrinsic pathway by adding thromboplastin and calcium to citrated plasma. The time to coagulation is measured and expressed as a ratio using a control plasma sample. The time is prolonged by low levels of Factors V, VII or X and by low levels of fibrinogen. The test is

performed on a citrated plasma sample. 100µl of brain thromboplastin is added to each of two wells. 100µl of control plasma is added to the first well and 100µl of patient plasma is added to the second sample. The samples are warmed to 37°C and then 100µl of $CaCl_2$ is added to start the reaction. Reagents were obtained from General Diagnostics and the test was carried out on a Coag-A-mate®-X2, General Diagnostics.

Activated partial thromboplastin time (APPT)

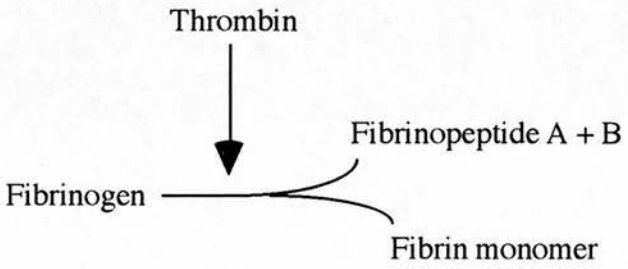
The principle is similar to that of the prothrombin time but the coagulation pathway is activated via the intrinsic pathway using an activator of the contact system such as kaolin. This results in activation of Factor XII which initiates activation of other contact phase factors. After these reaction are established dilute thromboplastin is added, followed by calcium and the time to coagulation is measured. The APPT is expressed as a ration with normal plasma. The test is prolonged by low levels of factors II, V, VIII, IX, X, XI, XII, kallikrein high molecular weight kininogen or fibrinogen. The test was performed on a citrated plasma sample. Reagents were obtained from General Diagnostics and the test was carried out on a Coag-A-mate®-X2, General Diagnostics.

D-dimers

D-dimer is a sensitive marker of fibrinolytic activity.[242] D-dimer consists of two cross-linked D domains cleaved from cross-linked fibrin by the action of plasmin.[243] Analysis was performed using a commercially available enzyme linked immunosorbant assay (AGEN) using a monoclonal antibody to D-dimer (DD3B6).[244]

Fibrinopeptide A

Fibrinopeptide A (FPA) is a sensitive marker of thrombin activity.[247, 248] When thrombin acts on fibrinogen to produce fibrin monomers, two small polypeptides are cleaved, FPA and fibrinopeptide B.



FPA is a 16 amino acid polypeptide with an *in vivo* half-life of 3-5 minutes.[249] FPA is useful for demonstrating changes occurring within the coagulation system over short periods of time because the conversion of fibrinogen to fibrin occurs rapidly early during the course of thrombosis and because it has a short half-life.

Assay

Assays for fibrinopeptide A were performed by Dr Drummond at the National Science Laboratory, Scottish National Blood Transfusion Service, Edinburgh. The assay was performed by a double antibody radioimmunoassay. The plasma sample is first treated with bentonite (Sigma B-3378) to remove any fibrinogen from the sample. The sample is incubated overnight with the primary antibody, rabbit anti-human FPA (IMCO, Stockholm), in tris buffer. The amount of primary antibody added is in excess of the amount of FPA and thus all the FPA is bound to the primary antibody. To this is added a radiolabelled secondary antibody - Donkey anti-rabbit IgG (SAPU) coupled with Sephacryl S1000. Sufficient secondary antibody is added

to react with all the primary antibody. The sample is mixed thoroughly and left to stand for 15 minutes at room temperature. The supernatant, containing unbound primary and secondary antibody, is aspirated from the tube leaving the solid phase, with FPA-primary antibody-secondary antibody complexes, undisturbed at the bottom of the tube. The amount of radioactivity in each tube is determined using a counter. A standard curve is drawn using samples of known concentration and the test samples are read against this curve.

ß-thromboglobulin

ß-Thromboglobulin (ß-TG) is a specific marker of platelet activation.[250-252] It is a protein, molecular weight 35,800, specific to platelets. It is the most abundant platelet specific protein, is contained within α-granules and is released during platelet activation.[253] It is cleared from plasma with a half time of 100 minutes at 37°C.[254]

Assay

Assays for ß-TG were performed by staff at the coagulation laboratory, South-West Blood Transfusion Service, Southmead Hospital , Bristol. Analysis was performed using an enzyme linked immunosorbant assay (ELISA), Asserachrom® ß-TG, (Diagnostica Stago, Asnieres-sur-Seine, France). This is a sandwich type ELISA. The well is coated with rabbit anti-human B-thromboglobulin antibodies. The test sample is added to this and the B-TG in the sample binds to the rabbit anti-BTG antibodies. The well is washed leaving the BTG bound. Next rabbit anti-BTG antibody coupled with peroxidase is added. This binds to the BTG. The excess is washed off. Ortho-phenylenediamine is reacted upon by the peroxidase in the presence of hydrogen peroxide undergoing a colour change. The speed of reaction, and hence the intensity of colour change is determined by the amount of peroxidase presence, which is dependent on the amount of BTG within the test sample. The absorbance is measured at 492nm by a spectrometer. A calibration curve of absorbance against BTG level is drawn for samples of known concentration. The test samples are read against this curve.

Glycocalicin

Glycocalicin is a marker of platelet activity and turnover.[245] Glycocalicin is soluble glycoprotein 1b (GP1b). GP1b is the platelet receptor which binds to von Willebrand factor, initiating platelet adhesion and activation. GP1b can be cleaved from the cell surface by the action of plasmin, thrombin, calpain and elastase. The presence of increased serum glycocalicin indicates platelet activation and turnover.

The half-life of glycocalicin is relatively long - in the region of 2-3 days. It is therefore unsuitable for the identification of rapid changes in platelet activation. However it is useful in determining platelet turnover and whether there is a state of chronic or repeated activation.

Glycocalicin activity in the plasma is found in two forms: free plasma glycocalicin and platelet microvesicle associated glycocalicin. Microvesicles can be removed either by centrifugation at high g-force or by filtration. About 75% of glycocalicin activity is free glycocalicin. Glycocalicin level is directly correlated to platelet count and is therefore usually expressed as u/platelet x 10^8.

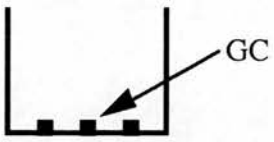
Principles of competitive enzyme-linked immunosorbant assay

The concentration of glycocalicin in the plasma was determined using a competitive binding enzyme-linked immunosorbent assay (ELISA).[246] The principles of the ELISA method are outlined in Figure 6. The well is first coated with a known amount of the antigen of interest. The sample to be assayed is added to the well followed by a monoclonal antibody to the antigen of interest - the primary antibody. There is competition for the monoclonal antibody between the antigen coating the well and the antigen contained within the sample being tested. The more antigen

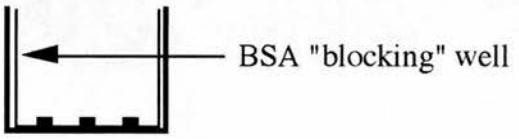
there is in the sample the less the monoclonal antibody will bind to the antigen coating the bottom of the well. The well is then washed leaving the antigen coating the bottom of the well with the primary antibody bound to it. A secondary antibody is added which is specifically active against the primary monoclonal antibody. This secondary antibody is also bound covalently to an enzyme such as peroxidase. The well is then washed leaving a complex of antigen-primary monoclonal antibody-secondary monoclonal antibody bound to the bottom of the well. Horseradish peroxide developing agent is added which reacts with peroxidase enzyme bound to the secondary antibody producing a colour change which varies in intensity according the amount of secondary antibody present. The colour intensity is determined using a absorbance spectrometer. Thus the greater the intensity, the more antibody which was bound to the coating antigen and thus the less antigen in the sample. The actual concentration is determined by putting known concentrations of antigen in some wells of every plate then drawing a curve of intensity against the known concentration. The concentration of the antigen in the test samples can be determined from this curve.

Figure 6 *Competitive binding enzyme linked immunosorbant assay*

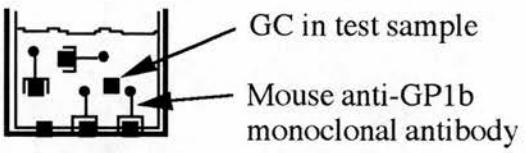
Well coated with glyococalicin (GC) after overnight incubation at 4°C



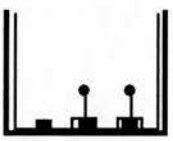
Well "blocked" with bovine serum albumen (BSA)



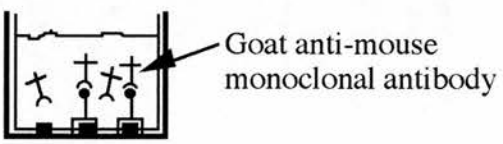
Test sample and mouse anti-GP1b antibody added to well. Mouse anti-GP1b bind to both GC in sample and GC coating well.



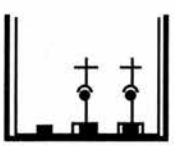
Well washed removing the mouse anti-GP1b bound to GC in sample. The mouse anti-GP1b which bound to GC coating well is left.



Goat anti-mouse (GAM) monoclonal antibody added which binds to mouse anti-GP1b



Well washed to remove excess GAM. Then developed using horseradish-peroxidae method



Method

The plate was coated with 100μl/well 10% glycocalicin in coating buffer (50mM bicarbonate adjusted to pH9.6). The plate was covered and left overnight at room temperature. The glycocalicin/coating buffer was decanted and the well were washed with ELISA buffer (137mM NaCl, 8.1mM $Na_2HPO_412H_2O$, 1.5 mM KH_2PO_4, 2.7mM KCl, 0.05% Tween 20) 300 μl/well three times. The wells were blocked with 3% bovine serum albumin in ELISA buffer for 1 hour at room temperature. The plate was washed 3 times with ELISA buffer 300 μl/well. Standard dilutions of glycocalicin were prepared. In wells 1-3 A,B,C ELISA buffer 100μl/well was added. The standard were dilutions 50μl/well were added. Samples 50μl/well were added to remaining wells. Anti GP1b monoclonal antibody, 25 ng/ml, 50μl/well was added to all wells except 1-3 A,B,C. The plate was covered and left for 1 hour at room temperature and then washed 3 times with ELISA buffer 300 μl/well. Goat anti-mouse antibody was prepared at 1:400 dilution and 100 μl/well was added to all wells. The plate was covered and left for 1 hour at room temperature and then washed 3 times with ELISA buffer 300 μl/well. The colour developing agent was prepared. Solution (a) 10 mg TMB was added to 1ml DMSO. Solution (b) 100 μl 30% hydrogen peroxide in 10ml 0.1M acetate buffer. 50 μl of solution (a) and 50 μl of solution (b) were added to 10 ml 0.1M acetate buffer. The colour developing agent 100 μl/well was added to each well and left for 5 minutes to develop. The reaction was stopped with H_2SO_4 50 μl/well. The plate was read in a absorbance spectrometer producing a measure of absorbance in each well. The absorbance in for the standard dilution were plotted against the dilution and the best fit curve was drawn. The glycocalicin level for the test samples was determined by reading off the standard curve.

Reagents

Glycocalicin was produced by Dr H Bessos, SNBTS by DEAE Sepharose fast flow. Mouse anti-GP1b monoclonal antibody was purchased from Dakopatts a/s Denmark No M719. Goat anti-mouse monoclonal antibody was purchased from Bio-Rad laboratories, Richmond Ca. Coating buffer and ELISA buffer were prepared freshly for each assay.

The effect of aspirin on plasma glycocalicin levels

Glycocalicin is a relatively new test of platelet activity and while much is known of the factors which influence plasma levels knowledge is as yet incomplete. Studies concerning factors such as tourniquet application prior to venepuncture, various types of anticoagulant and the influence of age and sex have been carried out.[245, 246] However the effect of aspirin on glycocalicin levels is unknown. As many of the patients in the present studies might be expected to be taking aspirin it is important to determine the effect of aspirin on glycocalicin levels and therefore the following study was performed.

Methods and materials

Eight healthy adult males were recruited, mean age 33 years. Five were assigned to take aspirin and three to acts as controls. No subjects had taken aspirin or any non-steroidal anti-inflammatory in the two weeks before the study started. In the group assigned to take aspirin a venous blood samples was taken two days before the start of the study. On the first day of the study a venous blood sample was taken, from the subjects who then took aspirin 300mg orally. Further blood samples were taken 1 hour and 6 hours after taking aspirin. On the following four days a venous blood sample was taken in the morning and each subject took aspirin 300mg after venesection. In the control group daily venous blood samples were taken.

At each time of venesection a 3ml EDTA sample was taken to determine platelet count and a 5ml citrated sample was taken and immediately spun, separated and frozen at -40^{o}C for glycocalicin assay in one batch at a later date.

Results

Glycocalicin levels did not alter significantly in those taking aspirin during the time course of the study (Figure 7). There was no significant difference in absolute values or day-to-day variability in the subjects taking aspirin compared with controls (Figure 8).

Figure 7 *Glycocalicin levels in normal subjects commenced on aspirin 300mg daily on day 1*

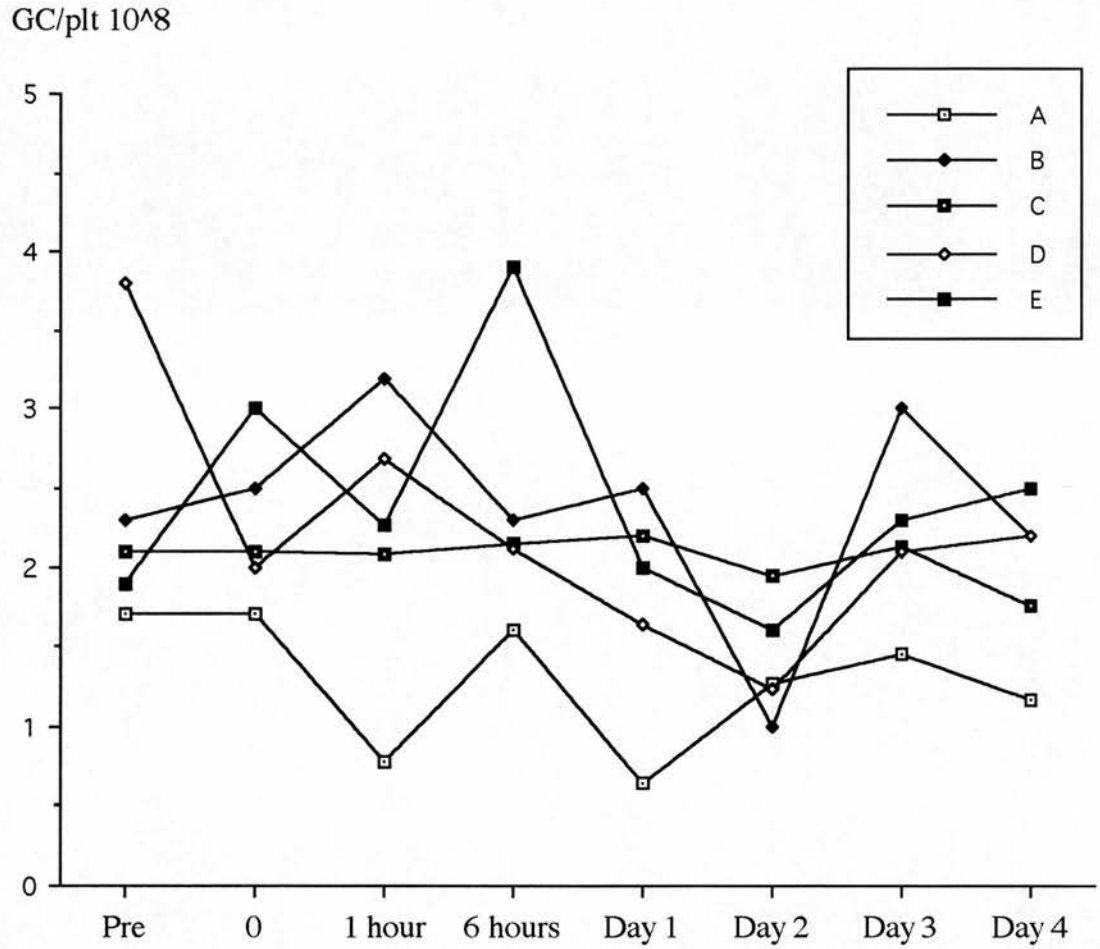
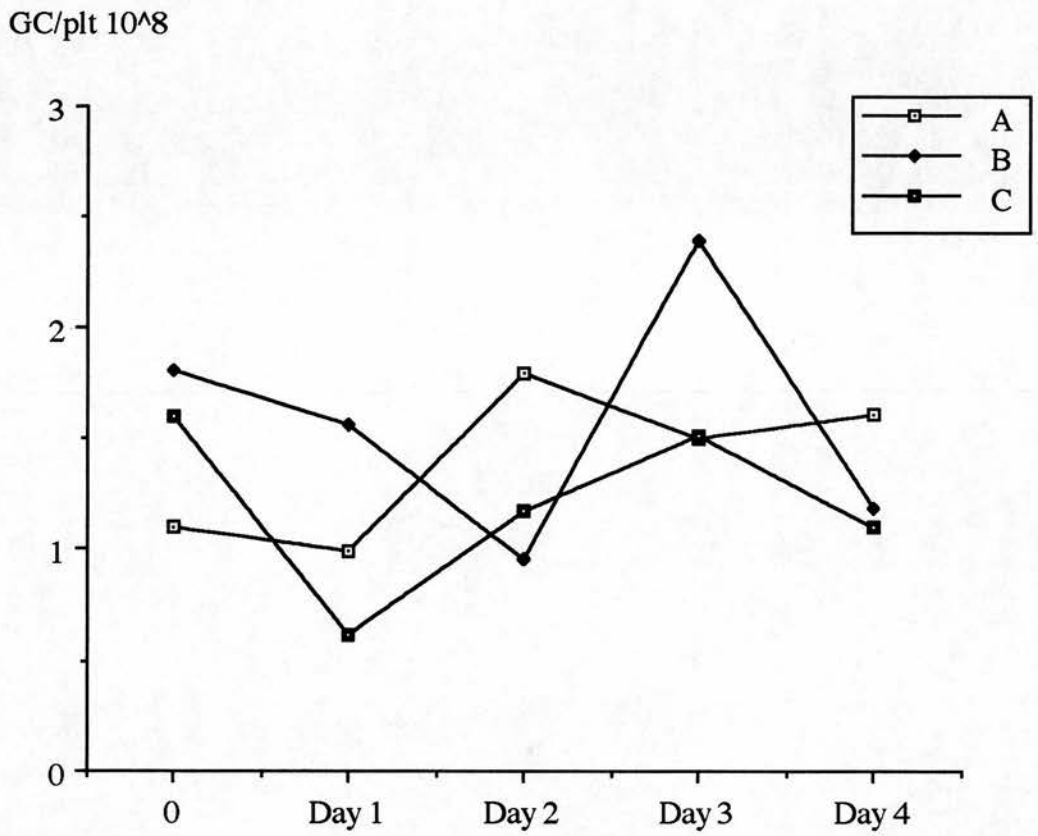


Figure 8 *Glycocalicin levels in control subjects over a 4 day period*



Discussion

This study does not demonstrate any effect of aspirin on levels of glycocalicin. The lack of change at one hour and 6 hours indicates that recent ingestion of aspirin does not affect either plasma levels of glycocalicin and does not have an ex-vivo effect on the assay. The time course of this study was relatively short and it may be that a longer period of study would demonstrate more subtle effects of aspirin on glycocalicin levels.

An important finding of this study which is of some concern is that there is considerable day-to-day variation in glycocalicin levels. This may be due to genuine fluctuations in the level of glycocalicin or it may be due to inaccuracy in the assay method. Previous studies of the reliability of this assay have shown it to have good reproducibility and it may well be that this day-to-day variation is a genuine finding whose cause and significance remains obscure.

It should be remembered that this study was carried out on healthy subjects. It therefore does not provide data on the effect of aspirin on glycocalicin levels in patients with increased platelet activation. Such patient would have elevated glycocalicin levels. It may be that aspirin would reduce platelet activation and thus glycocalicin levels in these patients.

Chapter 4

The Haemostatic System in Patients with Asymptomatic Abdominal Aortic Aneurysms

Introduction

As noted above some patients with asymptomatic aortic aneurysms have been shown to have derangement of the coagulation systems on laboratory testing[77] and may rarely present with symptoms of coagulopathy such as gingival bleeding and excessive bruising.[71-75] In one study about 10% of patients with non-ruptured aneurysm had elevated levels of fibrin degradation products and a mild thrombocytopenia.[77,227] This is thought to be due to consumption of clotting factors and platelets during turnover of the clot within the aneurysm sac.[228-230]

On close examination of case reports of coagulopathy with non-ruptured aortic aneurysms, it is notable that most patients are elderly, >80 years of age, and have large aneurysms. Furthermore coagulopathy is much more common in patients with thoraco-abdominal aneurysms. It has been suggested that in patients with aneurysms there is a balance between destruction of clotting factors and platelets within the aneurysm and synthesis in the liver and bone marrow and that DIC occurs when consumption is greater than synthesis.[77] Patients with larger aneurysm volumes may have greater destruction of clotting factors and elderly patients, or patients with liver disease, are less able to synthesise new factors and platelets. This may be of relevance to the coagulopathy related with rupture as these patients are more elderly and have larger aneurysms than patients undergoing elective operation.[122,148,237] Thus these patients may be more likely to have a coagulopathy prior to rupture.

The aims of this study were to determine the degree of activation of platelets and the coagulation and fibrinolytic systems in a population of patients with non-ruptured aneurysms and secondarily to determine whether factors such as age of patient and size of aneurysm were correlated to changes in the haemostatic system..

Patients and methods

Patients with a diagnosis of abdominal aortic aneurysm were identified in the out-patient department. In all cases the diagnosis had been confirmed by ultrasonography. The antero-posterior diameter, as measured by ultrasonography, was recorded as were clinical details such as age, past medical history and drug history.

Twenty-four patients were recruited. The mean age of patients was 72 ± 7 years. The male:female ratio was 4.5:1. The mean aneurysm diameter was 48 mm range 32-65 mm. Blood samples for glycocalicin levels were also taken from sixteen patients referred to the vascular clinic with carotid artery disease as part of a separate study. The mean age of these patients was 65± 9 years and the male to female ration was 1.3:1.

In addition to the above patient on whom complete coagulation studies were performed there are data on platelet count in further 33 patients with aortic aneurysm and 16 patients with carotid artery stenoses. Data on these patients are available as they were recruited to other studies - i.e. the fibrin sealant trial.

Results

Platelet count

Platelets counts were in the lower part of the normal range and 3/44 were below the normal range (Figure 9). Platelet counts were significantly lower in patients with aortic aneurysm, mean 214 ± 44 x 10^9/l, than those with carotid stenoses, mean 269 ± 57 x 10^9/l ($p = < 0.001$ by T-test) (Figure 10). The platelet count did not correlate with size of aneurysm or age.

Fibrinogen

All patients had plasma fibrinogen levels within the normal range, although these tended to be at the upper end of the normal range (Figure 11). The fibrinogen levels in patients with carotid artery disease were similar, tending to be at the upper limit of the normal range.

Fibrin degradation products

Only one patient had an elevated level of fibrin degradation products. This was not associated with any other abnormality on coagulation screen

Prothrombin ratio

All patients had a prothrombin ratio within the normal range.

Activated partial thromboplastin time

All patients had an APTT within the normal range.

Glycocalicin

The level of glycocalicin per platelet, was elevated in 7/24 patients (Figure 12). There was no correlation between glycocalicin level and size of aneurysm or age. The glycocalicin levels, both corrected and uncorrected for platelet count, were significantly higher in the group of patients with aneurysm than the group of patients with carotid artery disease ($p = 0.0001$ by Mann Whitney U test).

Figure 9 *Platelet counts in patients with asymptomatic infrarenal aortic aneurysm*

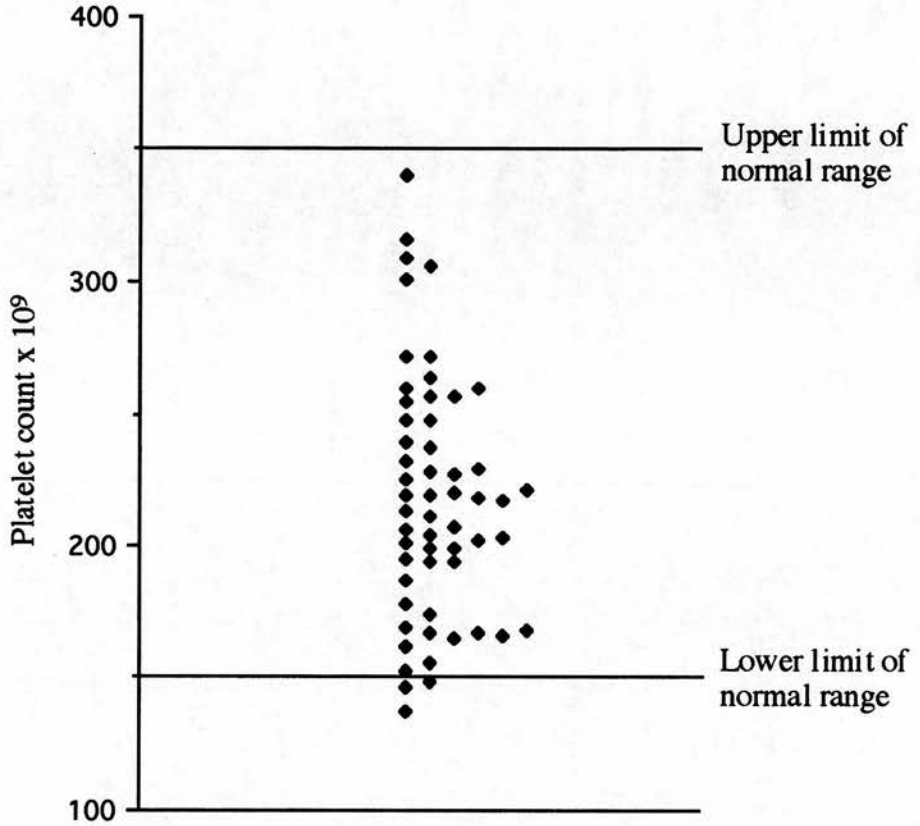


Figure 10 *Platelet counts in patients with asymptomatic infrarenal aortic aneurysms compared to patients with carotid stenoses*

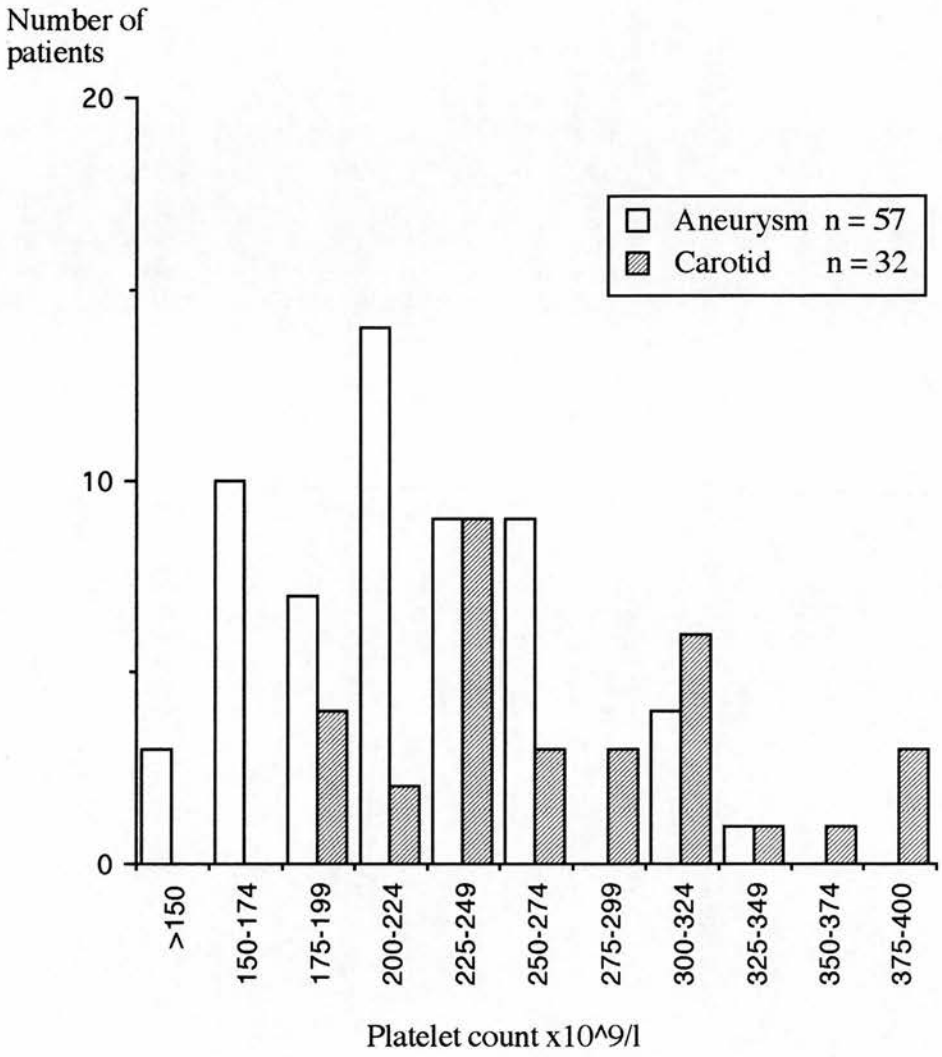


Figure 11 *Fibrinogen levels in patients with asymptomatic infrarenal aortic aneurysm and patients with carotid stenoses*

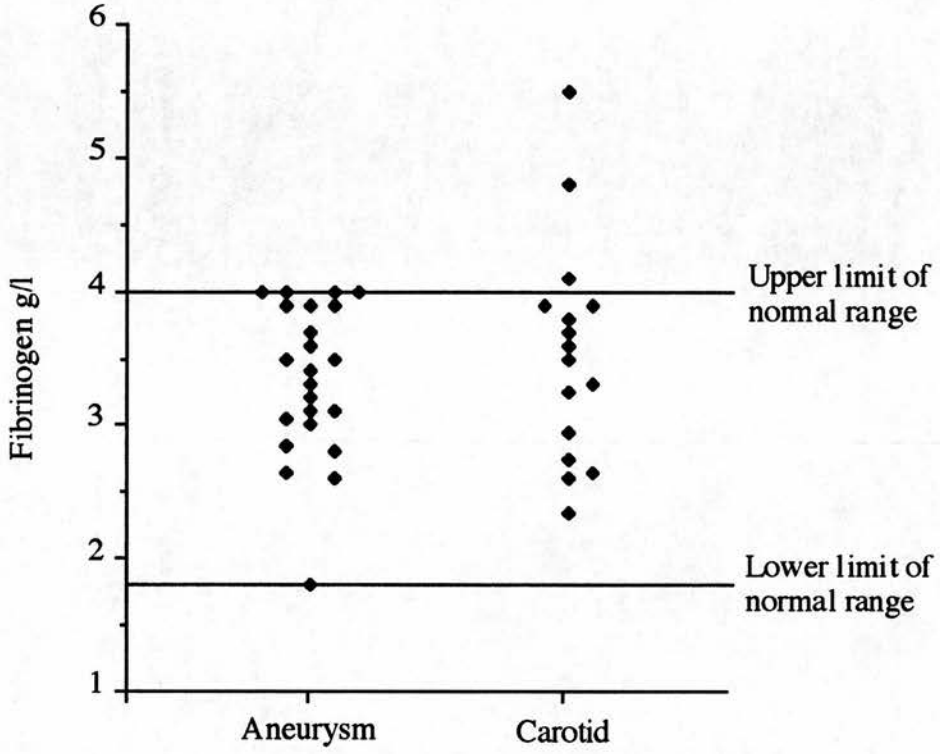
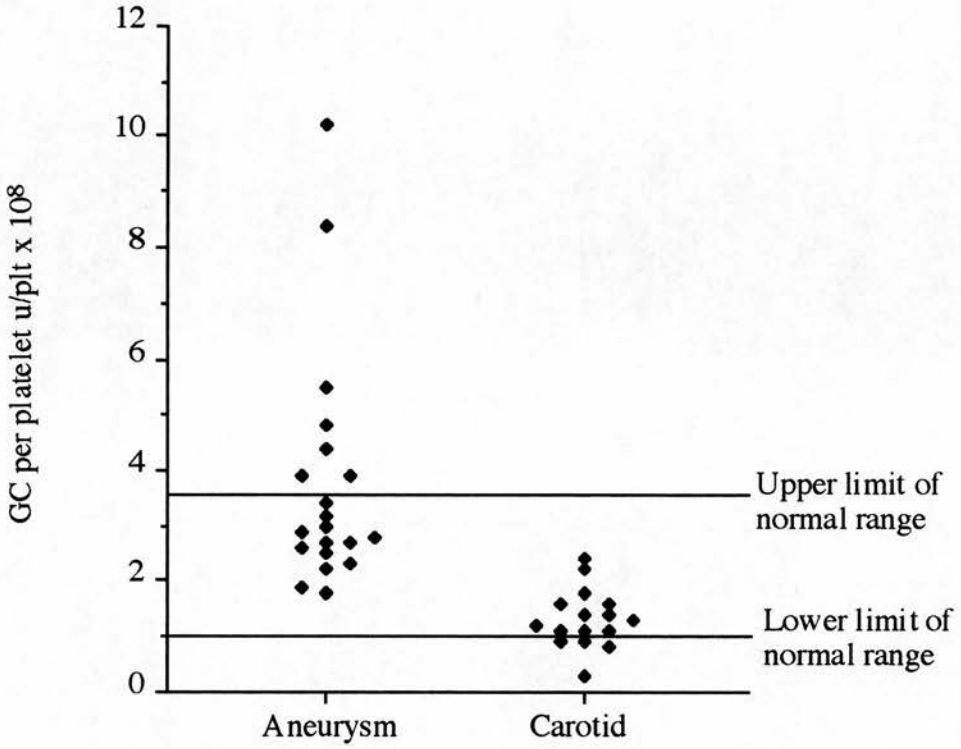


Figure 12 *Glycocalicin levels in patients with asymptomatic infrarenal aortic aneurysms and carotid stenoses*



Discussion

This study of patients with asymptomatic aneurysms has confirmed that there is some activation of the soluble coagulation system in patients with abdominal aortic aneurysms. However the degree of activation is quite slight and is only found in a small number of patients.

Platelet counts were low, although generally within the normal range. The difference is quite slight when compared to a normal population but it must be remembered that patients with aortic aneurysms generally have widespread atherosclerosis and thus may be expected to have the abnormalities of the coagulation system associated with vascular disease. Thus a more useful group for comparison would be patients of a similar age, with vascular disease but without an aneurysm - for example patients with carotid stenoses as used in this study. Both groups of patients may be expected to be quite closely matched in terms of having widespread atherosclerosis, and having similar risk factors for atherosclerosis such as smoking, age, male sex, hyperlipidaemia, hypertension and genetic predisposition. The difference in age between the two groups was statistically significant but the actual difference was relatively small, about 6 years. Thus the major difference between the two groups was that one group had aortic aneurysms while the other did not. The differences in platelet count between the group of patients with aortic aneurysms and those with carotid stenoses were quite marked. It would therefore appear that the presence of an aortic aneurysm has a significant effect on platelet numbers.

There was a difference in glycocalicin levels in patients with aneurysms compared to patients who had carotid disease with elevated levels being found in patients with aneurysms. Using the same argument as applied to the difference in platelet numbers between these groups it can be concluded that the elevated glycocalicin level is related to the presence of an aneurysm rather than atherosclerosis. These data are

supported by a previous study of glycocalicin levels in various groups of patients and normal subjects which reported that patients with peripheral vascular and cardiovascular disease had significantly lower levels of glycocalicin.[245]

Fibrinogen levels in all patients were within normal limits but at the upper end of the normal range. This is to be expected in a group of patients with atherosclerosis: peripheral vascular disease, coronary heart disease and cerebrovascular disease are all associated with elevated plasma fibrinogen.[255] Thus there is no evidence that the presence of a aortic aneurysm has an independent effect on fibrinogen levels.

There are four mechanisms of thrombocytopenia: (1) decreased rate of production (2) increased rate of destruction (3) platelet sequestration, e.g. in the spleen (4) dilution. The data on glycocalicin levels give some indication as to which of these mechanisms is the likely cause for the low platelet counts in the patients in this study. It should be remembered that glycocalicin is produced when GP1b, the platelet membrane glycoprotein receptor for vWF, is lysed and released from the platelet membrane.[373] Therefore the elevated levels of glycocalicin suggest that the most likely explanation is an increased rate of destruction.

The simplest explanation is that platelets are activated by the clot in the aneurysm sac, adhere and become incorporated into the clot. GP1b is released at the time of activation accounting for the elevated glycocalicin. This process might be termed consumption within the sac. If this is this case then the remaining circulation platelets would have normal expression of GP1b.

However the explanation may be more complex. Platelets have a normal life span of about ten days but the ageing process which marks them for destruction is poorly understood. It is known that coming into contact with a foreign surface, such as subendothelium, can activate platelets and such platelets are cleared from the circulation regardless of their age.[379] Such activation of the platelet without

adhesion may occur within the sac producing changes to the circulating platelet which mark it for early destruction.

The elevated glycocalicin may then be accounted for by one of two explanations. It may simply be that GP1b is released at the time of platelet destruction in the reticuloendothelial system. The other explanation concerns the mechanism by which platelets are marked for early clearance from the circulation. It has been proposed that platelets undergo gradual loss of GP1b throughout their life span and that this is the means by which senescent platelets are identified by the reticuloendothelial system.[373] It has been demonstrated in vitro that activation of platelets can lead to the generation of a protease which cleaves glycoprotein 1b (GP1b) close to the site of its insertion onto the platelet membrane. Thus it may be that activation within the sac causes release of GP1b marking the platelet for early clearance. If this is the case then there may be circulating platelets which are deficient in GP1b receptors.

GP1b is central to the process of platelet adhesion at the site of vessel wall injury and thus vital for clot formation and haemostasis. It is therefore easy to envisage that platelets deficient in GP1b would be dysfunctional. Such loss of GP1b occurs during platelet storage in blood banks and has been proposed as the reason for the decreased functional ability of stored platelets.[373] Thus in these patients there may be a combination of dysfunctional platelets and a mild degree of thrombocytopenia which could significantly compromise the coagulation system. Haemostatic function in the resting state might be near normal but the threshold at which clinical bleeding problems occur would be much lower and there would be little reserve in the face of a major challenge to the haemostatic system such as aneurysm rupture or aortic operation.

In order to resolve these questions more detailed studies of expression of cell surface receptors are required using techniques such as flow cytometry. Such studies would

not only provide information about platelet function in these patients but may also help to explain the mechanism by which senescent platelets are identified and destroyed by the reticuloendothelial system.

Chapter 5

Changes in the Haemostatic System during Elective Infrarenal Aortic Aneurysm Repair

Introduction

The development of coagulopathy during elective aneurysm repair is a well recognised although uncommon complication. As shown in Chapter 3, it may have a causal relationship to post-operative haemorrhage. The pathological process which leads to the development of this coagulopathy remains obscure and very little is known of the changes which occur in the coagulation system during operation.

In one study of the coagulation system during aorto-femoral bypass grafting for occlusive disease it was shown that levels of fibrinogen dropped throughout operation, most markedly after aortic cross-clamping.[231] A study which included assays of thromboxane A_2, a marker of platelet activation which correlates well with B-thromboglobulin,[256] indicated that there was marked platelet activation during cross-clamping which then reduced on reperfusion.[257] However in neither of these studies were patients given systemic heparin prior to cross-clamping. This is now common practice and may be expected to have considerable effects on the haemostatic system.

A more recent study has been carried out in patients undergoing aortobifemoral grafting for occlusive disease (8/10) and aneurysmal disease (2/10).[232] This study included more sophisticated investigation of the haemostatic system but a policy of deliberate haemodilution during the early stages of operation and the administration of large amounts of fresh frozen plasma during operation produce some difficulty in the interpretation of the findings. The significant findings of this study were that there was increasing fibrinolytic activity during operation in association with falling platelet counts and that these events were not related to aortic cross-clamping. No examination of thrombin activation was made.

The primary aim of this study was to determine the timing and degree of activation

of the various components of the haemostatic system - thrombin activation, platelet activation and plasmin activation. The secondary aims were to determine whether these changes were related to the operative factors identified in Chapter 3, such as length of operation, length of cross clamping, blood loss and transfusion, which appeared to be related to post-operative haemorrhage.

Methods and materials

A series of 6 consecutive patients undergoing elective aortic surgery for abdominal aortic aneurysm were studied. Age, size of aneurysm, past medical history and drug history were recorded and a venous blood sample was taken pre-operatively for haemoglobin estimation, white cell count, platelet count, prothrombin time and activated partial thromboplastin time. Patients with abnormal coagulation or who had taken aspirin within the previous ten days were excluded.

Blood samples were taken from an indwelling arterial cannula at four time points during surgery. The first sample was taken after the induction of anaesthesia but prior to the start of operation, the second was taken immediately prior to aortic cross-clamping, the third was taken immediately prior to the release of the aortic cross-clamp and the fourth was taken ten minutes after the release of the cross-clamp. The first 5ml was discarded. Thereafter samples were prepared as described above for estimation of APTT and assays of FPA, B-TG, and D-dimers. The time at which the samples were taken, relative to the start of operation, was recorded. Operative details for each patient are shown in Table 6.

Similar blood sampling was carried out during operation for ruptured aortic aneurysm in one patient (R). This patient presented with contained rupture and was not shocked on admission. Intra operative heparin was not used. Operative details for this patient are shown in Table 6 and FPA and BTG levels are shown in Figure 20.

Table 6 *Operative details in patients undergoing elective operation for aortic aneurysm*

	Age	Heparin dose	Operation length	Cross-clamp time	Transfusion requirements	Intraoperative IV Fluids	Blood loss
A	73	2,000 iu	77 mins	50 mins	None	3000 ml	2,000 ml
B	73	3,000 iu	134 mins	49 mins	None	2000 ml	1,000 ml
C	78	5,000 iu	110 mins	63 mins	None	2500 ml	1,500 ml
D	78	3,000 iu	171 mins	80 mins	4 units RCC	2000 ml	2,500 ml
E	74	5,000 iu	125 mins	65 mins	4 units RCC	3000 ml	3,000 ml
F	65	5,000 iu	110 mins	50 mins	1 unit RCC	2500 ml	1,700 ml
R	58	None	110 mins	63 mins	2 unit RCC	2500ml	3990 ml

Results

Activated partial thromboplastin time

All patients had a normal APTT at the start of operation and until heparin was administered. The APTT for each patient, as a ratio to control sample, at time of release of cross-clamp and after 10 minutes of reperfusion are shown in table 7. Patient A who had the smallest dose of heparin, 2,000 iu, had a much shorter APTT than the other patients. There was no relationship between the length of cross-clamp, and therefore the time between administration of heparin and blood sampling, and the APTT.

Table 7 *APTT and heparin dose in patients undergoing elective operation*

	Age	Heparin dose	Cross-clamp time	APTT at release of cross-clamp	APTT after 10 minutes reperfusion
A	73	2,000 iu	50 mins	2:1	1.6:1
B	73	3,000 iu	49 mins	not coagulable	5.5:1
C	78	5,000 iu	63 mins	not coagulable	not coagulable
D	78	3,000 iu	80 mins	3.5:1	3.5:1
E	74	5,000 iu	65 mins	4:1	3.5:1
F	65	5,000 iu	50 mins	6.5:1	5.5:1

Fibrinopeptide A

The level of fibrinopeptide A (FPA) rose significantly during the period of initial dissection (Figure 13). This rise was proportional to the duration of this period of dissection $r^2 = 0.75$ $p = 0.025$ (Figure 14). Following cross-clamping and administration of heparin the levels of FPA dropped except in patient A. This patient was only given 2,000 iu heparin compared with 3,000 or 5,000iu given to the other patients in this study and had a much shorter APTT. Following reperfusion there was no significant change in levels of FPA except in patient A who had a marked increase in level of FPA.

Figure 13 *Fibrinopeptide A levels at 4 time points during elective aortic aneurysm repair; after induction, before cross-clamping, after removal of cross-clamp and after 10 minutes reperfusion.*

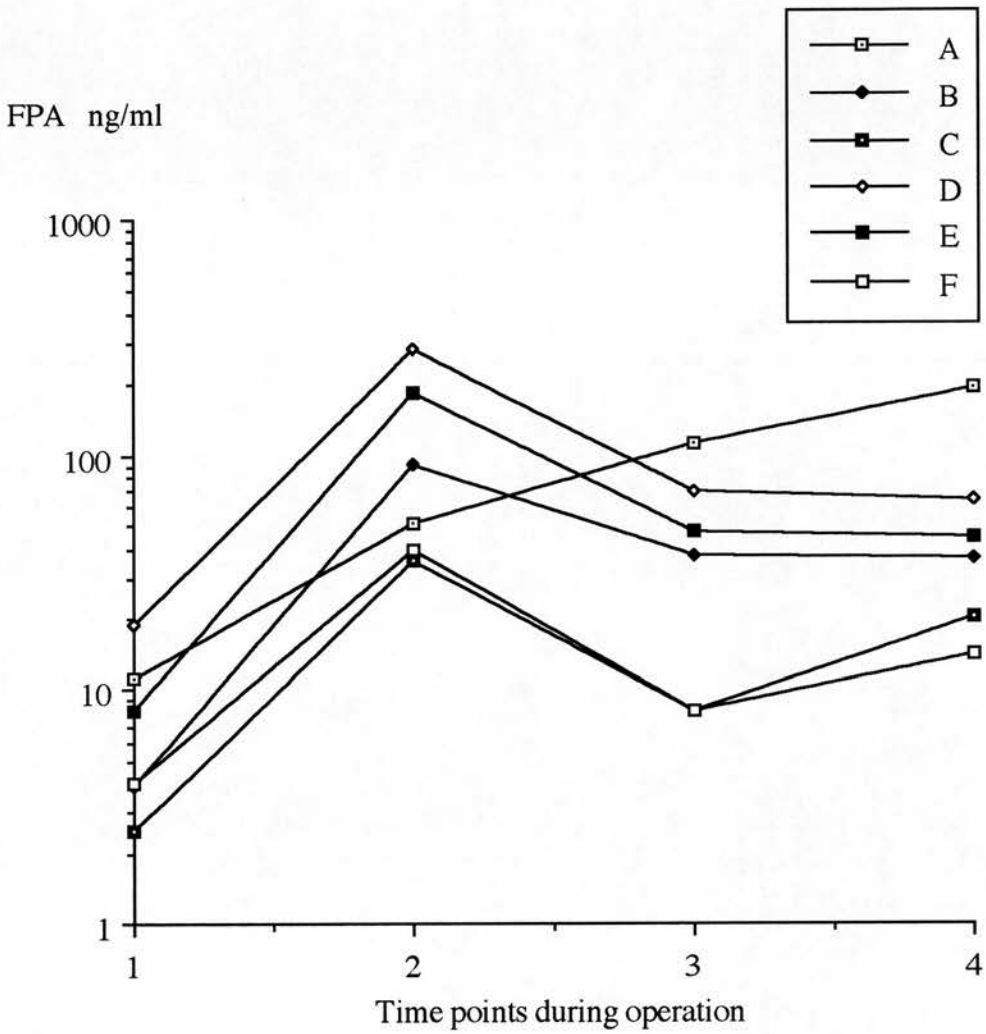
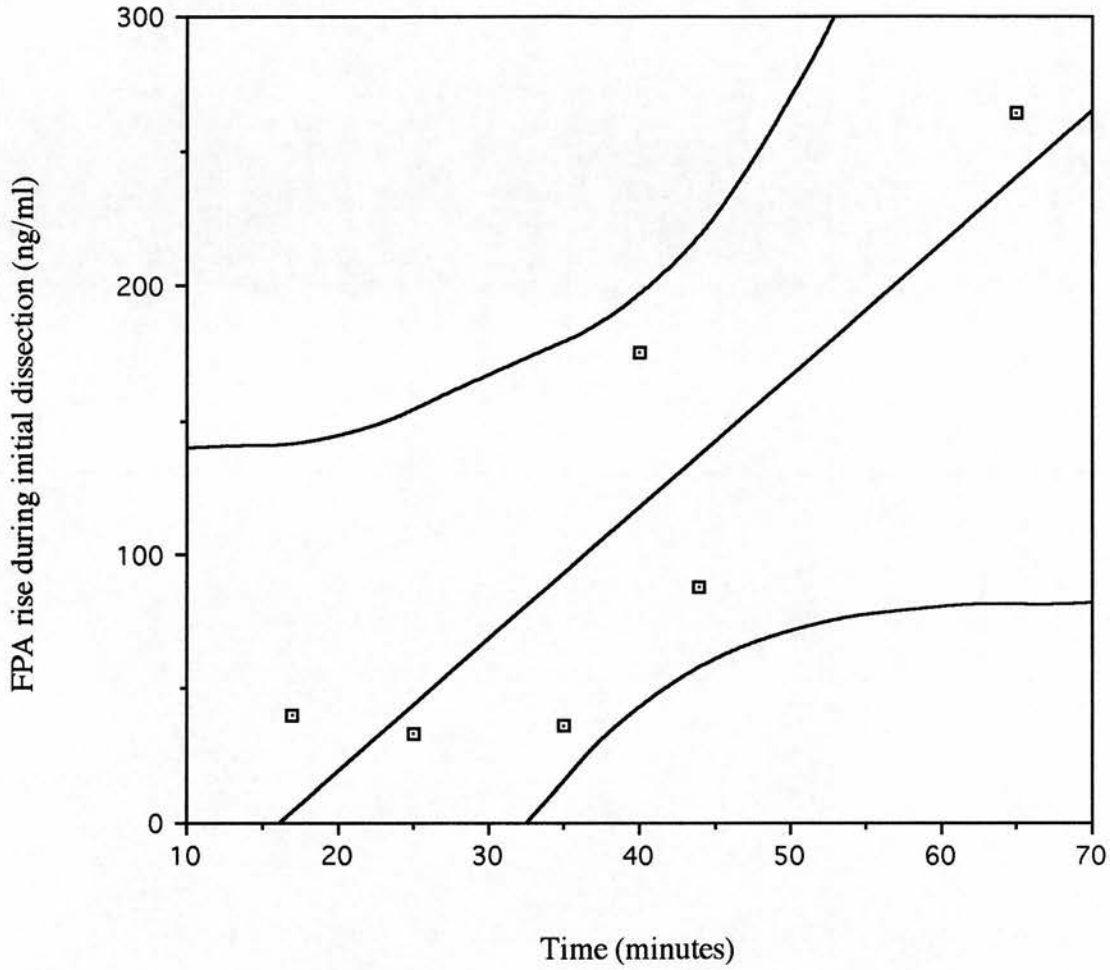


Figure 14 *Fibrinopeptide A increase from induction to sampling prior to cross-clamping against time from induction to cross-clamping*



B-Thromboglobulin

The level of beta-thromboglobulin (BTG) rose significantly during the period of initial dissection $p = 0.046$ (Figure 15). This rise was proportional to the duration of this period of dissection $r^2 = 0.91$ $p = 0.003$ (Figure 16). Following cross-clamping and administration of heparin the levels of BTG dropped significantly $p = 0.027$. Patient A who had received a smaller dose of heparin than the other patients had only a small drop in BTG level, within the limits of the error of the assay. After ten minutes of reperfusion levels of BTG rose in four patients and dropped in two patients. When this change is related to the length of cross-clamping there is good correlation, with a greater BTG rise being observed in those with longer cross-clamp times (Figure 17). One patient did not fit in well with this pattern and this was patient A who received a smaller dose of heparin. If this patient is excluded the correlation is excellent $r^2 = 0.98$ $p < 0.05$.

Figure 15 *Beta thromboglobulin levels at 4 time points during elective aortic aneurysm repair; after induction, before cross-clamping, after removal of cross-clamp and after 10 minutes reperfusion.*

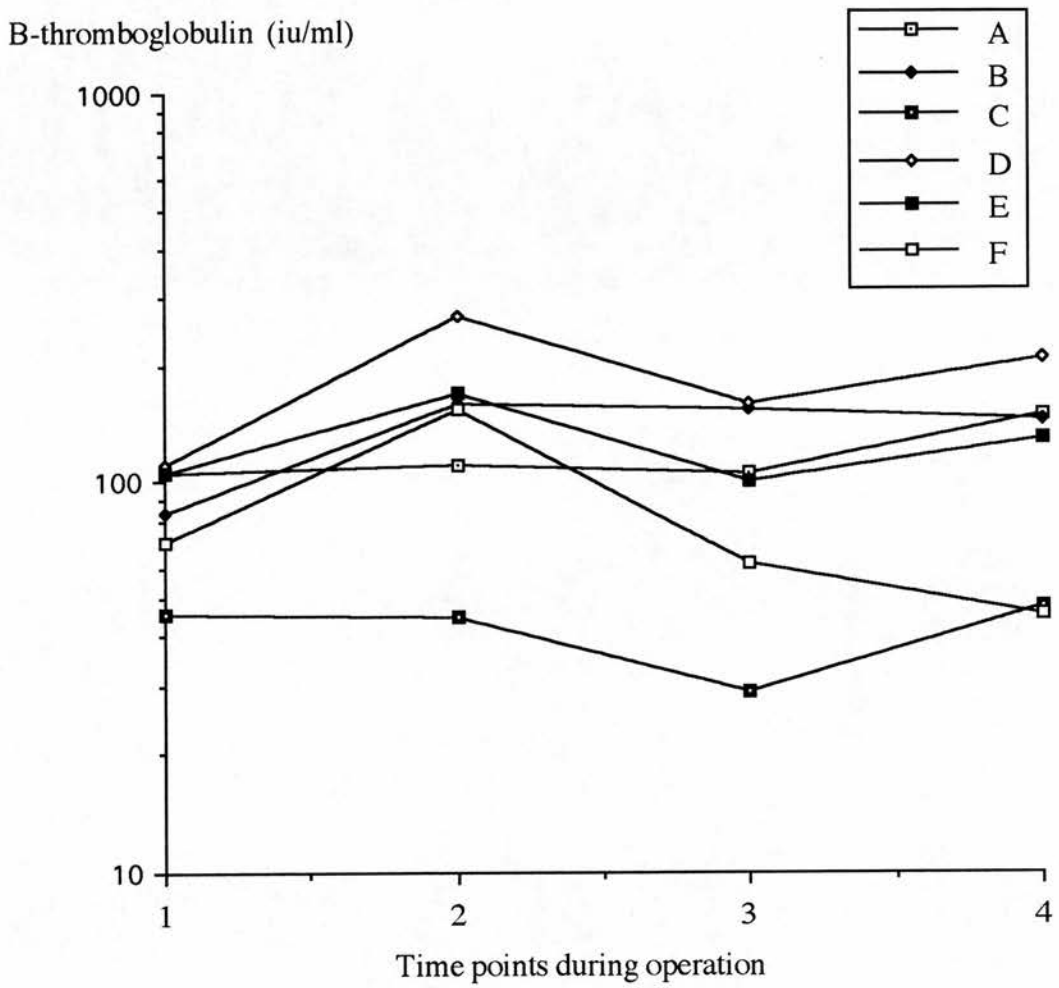


Figure 16 *Beta thromboglobulin increase from induction to sampling prior to cross-clamping against time from induction to cross-clamping*

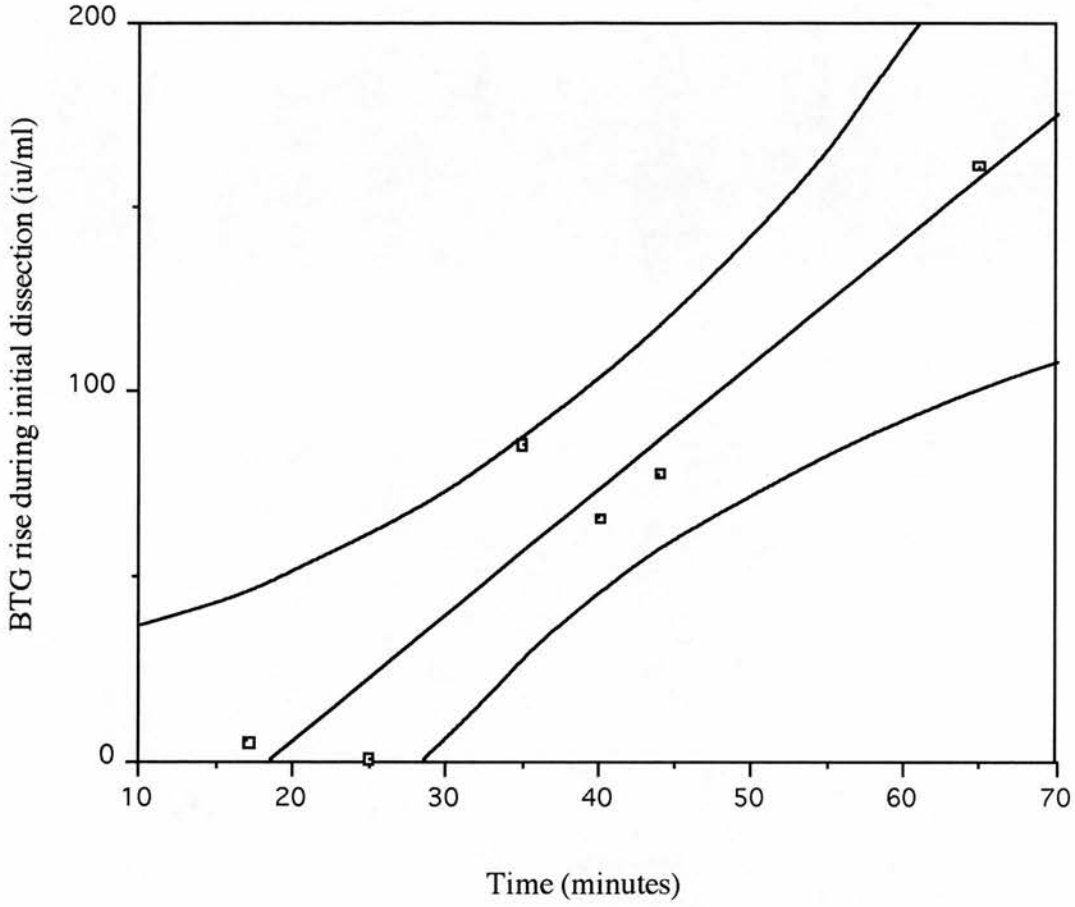
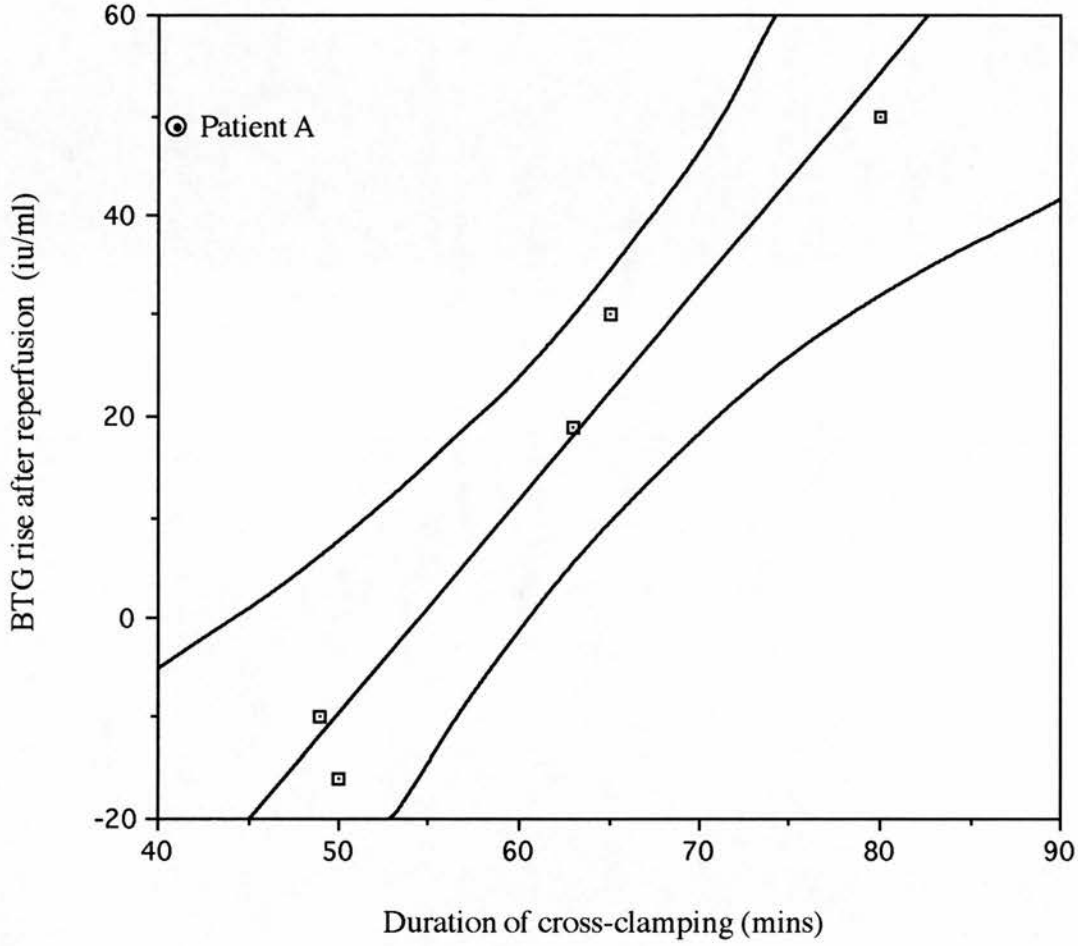


Figure 17 *Beta thromboglobulin increase from release of cross-clamp to 10 minutes after reperfusion against duration of cross-clamping (excluding patient A)*



D-dimer

The level of D-dimer rose significantly during the period of initial dissection $p = 0.0022$ (Figure 18). The rise was proportional to the duration of this period of dissection $r^2 = 0.845$ $p = 0.018$ (Figure 19). Following cross-clamping and administration of heparin the levels of D-dimer dropped in 4/6 patients. The two patients who had a rise in D-dimer, patients A and D, had received the smallest doses of heparin, 2,000 iu and 3,000 iu heparin respectively, and who had the lowest APTT at 2.11 and 3.4 respectively. After ten minutes of reperfusion levels of D-dimer rose in four patients and dropped in two patients. The rise or fall in D-dimer after reperfusion was not related to the length of cross-clamping

Figure 18 *D-Dimer levels at 4 time points during elective aortic aneurysm repair; after induction, before cross-clamping, after removal of cross-clamp and after 10 minutes reperfusion.*

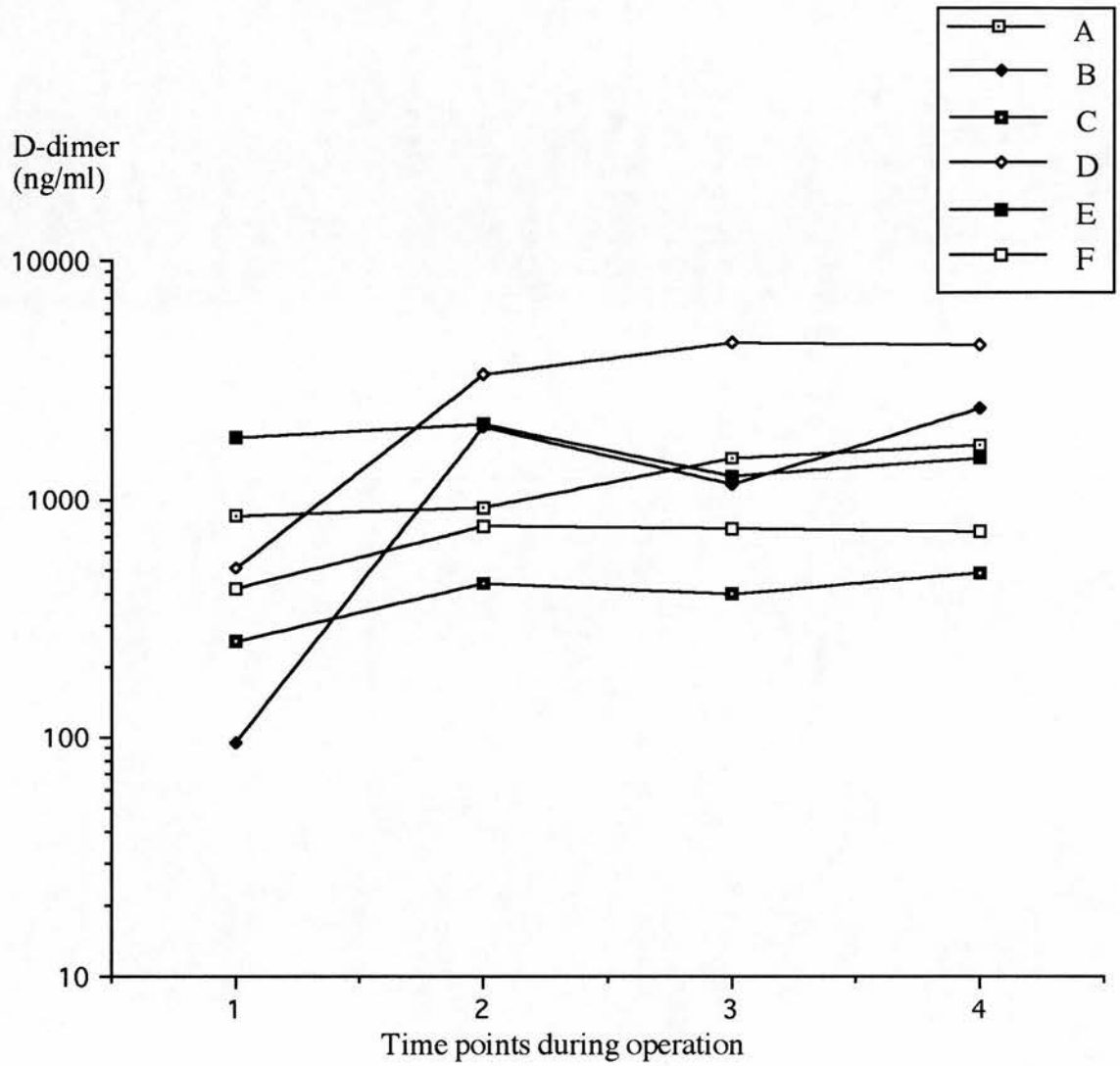


Figure 19 *D-dimer increase from induction to sampling prior to cross-clamping against time from induction to cross-clamping*

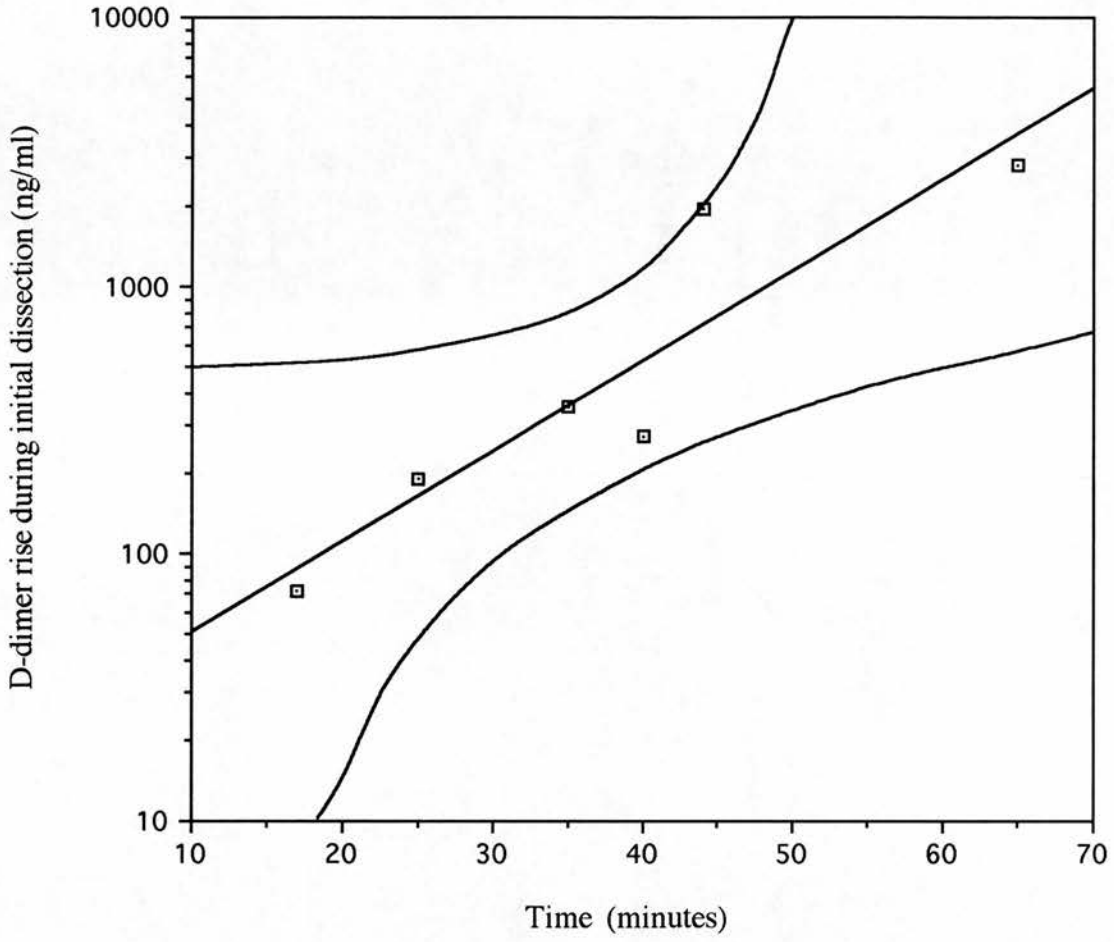
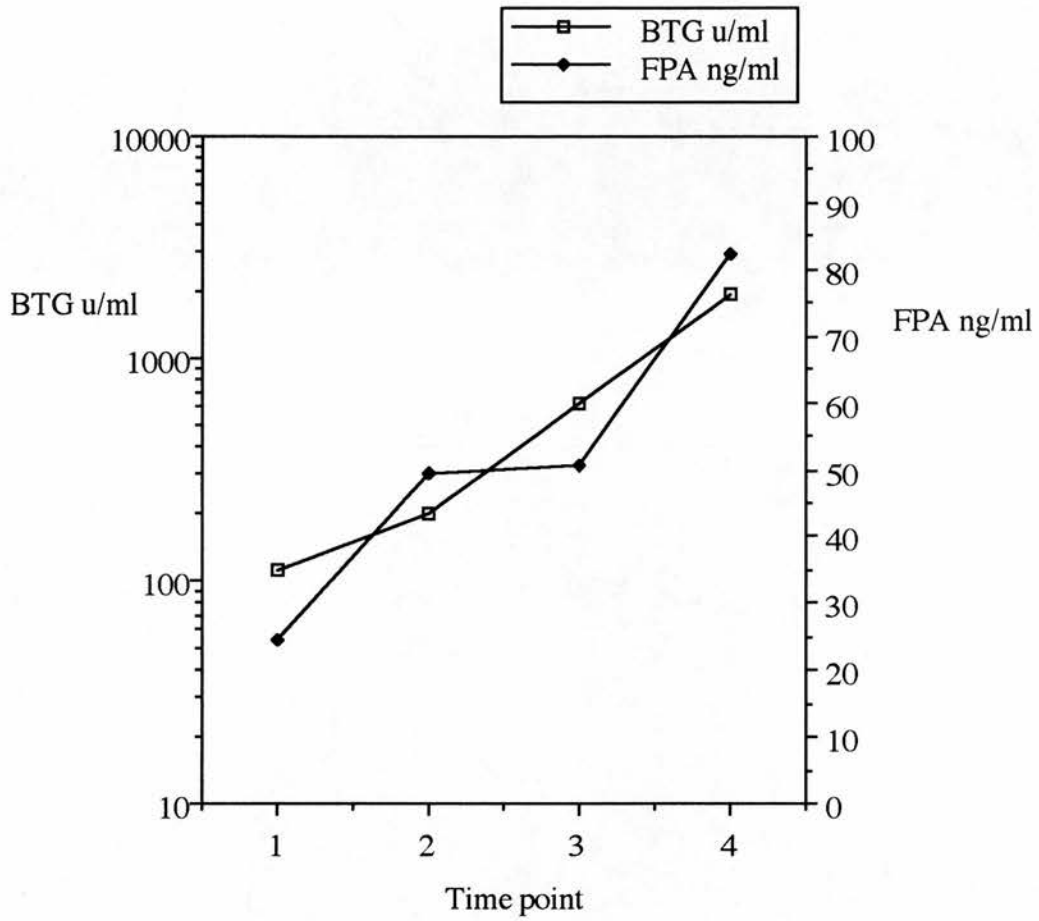


Figure 20 *Intraoperative levels of BTG and FPA in patient R who presented with contained rupture at 4 time points; after induction, before cross-clamping, after removal of cross-clamp and after 10 minutes reperfusion.*



Discussion

The results from this study were to a large extent quite unexpected. The limited amount of information from the two previous studies on this subject suggested that the major changes in the haemostatic system would take place during aortic cross-clamping and/or reperfusion. In contrast it was found that thrombin and platelet activation actually reduced markedly during the period of cross-clamping. Plasmin activation, as demonstrated by level of D-dimer, reduced during cross clamping in 4/6 patients.

These changes during cross-clamping could either be due to the effects of cross-clamping or as a result of heparin administration. It seems most likely that heparin was the cause and it may be thrombin, platelet and fibrinolytic activation would increase during cross-clamping and reperfusion if heparin were not given prior to cross-clamping. Increasing thrombin activation during cross-clamping was found in one patient who received a lower dose of heparin and who had the shortest APTT. In addition this patient also had a marked increase in FPA after reperfusion. The two patients who had a rise in D-dimer after cross-clamping received the smallest doses of heparin and had the shortest APTT. In the study by Utsunomiya *et al* platelet activation, as determined by level of thromboxane A_2, markedly increased during cross-clamping in a group of patients who were not given systemic heparin.[257] This effect was not found in patients who were given aspirin 650mg ten hours prior to operation.

Further evidence that heparin is the cause of the drop in FPA and BTG during cross-clamping is provided by the data on patient R who underwent operation without the administration of heparin. In this patient there was a marked rise in BTG and a small rise in FPA during cross-clamping. Of course there are other possible confounding factors in this patient as he had suffered a ruptured aneurysm but there was no

evidence of hypovolaemic shock prior to surgery and the length of operation, cross-clamping, blood loss and transfusion requirements were similar to those undergoing elective repair.

It would be interesting to repeat these studies without administering heparin. Unfortunately there are ethical difficulties with such a study as it has recently been shown that intra-operative heparin confers a significant benefit by reducing the rate of post-operative myocardial infarction.[258] The mechanism by which heparin produces this effect is unknown but it is presumably due to some effect on the haemostatic system during operation. In this study a marked reduction in platelet and thrombin activation was shown after heparin administration. If this is important in the mechanism by which heparin reduces peri-operative MI, it could be proposed that a greater benefit would be gained by administering heparin at an earlier stage during operation since thrombin and platelet activation increases during a time dependent manner during the period of initial dissection. However the relevant effect of heparin may be elsewhere.[259] Heparin has many effects apart from those on the coagulation cascades; for example heparin has been shown to reduce endothelial permeability and hydrogen ion accumulation in muscle ischaemia-reperfusion injury.[260]

The effects of reperfusion are of some interest. Platelet activation occurred following reperfusion in a manner that was dependent on the length of cross-clamping. There was no corresponding increase in thrombin or fibrinolytic activity. This suggests that platelet activation is occurring via a mechanism which does not involve activation of the coagulation cascade. Similar findings have been reported in studies of patients undergoing knee surgery with tourniquet ischaemia.[256] An increase in B-thromboglobulin was found after 30 minutes of tourniquet ischaemia and this markedly increased after 5 minutes of reperfusion. No increased fibrinolytic activity was found either during ischaemia or reperfusion.

This effect on platelets might be mediated directly by endothelial cells. It has been shown in animal studies of myocardial ischaemia and reperfusion that ischaemia of as little as 30 minutes causes alteration in endothelial function and that the degree of dysfunction increases with length of ischaemia and markedly increases on reperfusion.[261-263] One of the effects of ischaemia on endothelial cells is to reduce the production of endothelial derived relaxing factor (EDRF) and prostacyclin which are potent inhibitors of platelet adhesion and aggregation.[264-266] It may be that lower limb ischaemia during cross-clamping causes similar dysfunction in the microvasculature of skeletal muscle resulting in platelet adhesion and aggregation during reperfusion.

From the data presented in Figure 17 it would appear that about 45-60 minutes of lower limb ischaemia are required before significant activation of platelets will take place after reperfusion. This is somewhat longer than the 30 minutes of ischaemia required to produce platelet activation in studies of tourniquet ischaemia.[256] However due to collateral circulation the degree of lower limb ischaemia produced by aortic clamping is much less than that produced by tourniquet inflation above systolic pressure.

This apparent time dependent effect of cross-clamping on platelet activation may be an artefact due to a reduction in heparin effect with time. In patients undergoing elective aorto-iliac surgery it has been shown that an intravenous bolus of heparin results in maximal heparin effect, as determined by anti-Factor Xa activity, at 5-20 minutes with a fall in activity up to 120 minutes.[267] The rate of fall in activity is greatest between 30 and 60 minutes after administration. In the present study the 2/3 patients with the longer cross-clamp times did have shorter APTTs although these were still greater than 3:1. One patient with a cross-clamp time of 63 minutes had an APTT which was not coagulable and still had a significant rise in BTG on reperfusion.

In conclusion this study indicates that:-

1. Thrombin, platelet and fibrinolytic activation increase during the period of initial dissection.

2. The increase in activation of platelets and fibrinolysis is related to the length of time of initial dissection.

3. Thrombin, platelet and fibrinolytic activation decreases after the administration of heparin and cross-clamping.

4. Platelet activation increases during reperfusion and this increase in activation is related to the duration of cross-clamping.

5. The administration of systemic heparin appears to markedly inhibit platelet, thrombin and fibrinolytic activity during cross-clamping.

Chapter 6

The Haemostatic System in Patients with Ruptured Abdominal Aortic Aneurysm

Introduction

There have been no detailed studies of the haemostatic system in patients with ruptured aortic aneurysms. Some information is available from clinical studies which have described relatively unsophisticated markers of disruption of the haemostatic system such as platelet count, fibrinogen levels, PTR, APPT and fibrin degradation products.[66,162,163,268] There are some more sophisticated studies of disseminated intravascular coagulation in which patients with DIC secondary to ruptured aortic aneurysm were included but in these studies the timing of blood sampling was not stated, several patient groups were combined and specific data on those patients with ruptured aneurysms were not presented.[206,269] In addition there are no data concerning the changes which occur during operation.

The primary aims of this study were to determine the degree of activation of thrombin, plasmin and platelets at the time of admission in patients with ruptured abdominal aortic aneurysms and to determine the changes which occur in these factors during operation. The secondary aims were to determine whether any of the factors at time of admission were related to outcome.

Methods and materials

Patients

Twenty-two patients undergoing operation for ruptured aortic aneurysm in the Royal Infirmary of Edinburgh between 1/4/93 - 1/8/94 were included in this study. In all cases the diagnosis of ruptured aneurysm was confirmed at operation. The mean age of patients was 70 years (range 56-84 years). The male:female ratio was 3.4:1.

Blood sampling

Blood samples were obtained from 22 patients. Blood samples were taken from an indwelling cannula placed in the radial artery. The first 5ml was discarded. Thereafter samples were prepared as described above for platelet count PTR, APPT and assays of fibrinogen, FPA and B-TG. Samples were taken immediately prior to operation, at the end of operation and 12-24 hours after the end of operation.

Results

Clinical outcome

The overall 30 day mortality rate was 55%. Five patients died "on-the-table" from uncontrolled haemorrhage. Five patients survived operation but died within 48 hours of operation due to multiple organ failure. Two patients subsequently died from multiple organ failure at 10 and 12 days post-operatively. One patient who survived developed acute renal failure which required prolonged dialysis. The remaining 9 patients had uncomplicated post-operative courses.

The patients can therefore be divided into three groups; those who died on-the-table, those who died post-operatively from multi-organ failure and those who survived.

In the first group who died on-the-table, two patients with free intraperitoneal rupture arrested at the start of operation and were not successfully resuscitated. Two patients, one with extensive intraperitoneal adhesions from previous surgery and another with a suprarenal aneurysm were never successfully cross-clamped and died from exsanguination. None of these four patients had a coagulopathy at the start of operation. The fifth patient had a severe coagulopathy and although a graft was inserted there was continuing haemorrhage and the patient died from cardiac failure.

The cause of death in the group who died in the early post-operative period was predominately multi-organ failure with varying degrees of respiratory, cardiac and renal failure. Four of the seven patients had significant lower limb ischaemia with two undergoing an embolectomy and one undergoing an above knee amputation.

There was only one significant complication in the group of patients who survived; one patient developed acute renal failure requiring prolonged haemodialysis.

The operative details of patients in each group are shown in Table 7. Patients who survived had shorter operations, shorter cross-clamp times, lower blood loss and lower red cell transfusion requirements than those patient who died in the early post-operative period. Fresh frozen plasma and platelet transfusion were administered to 4/7 patients whoi died post-operatively compared with 2/10 in those who survived. There was no difference in core temperature at the end of the procedure.

The results of the coagulation test taken at admission and at the end of operation are shown in Table 8 . The patients who died in the early post-operative period had lower platelet count, lower fibrinogen levels, higher FPA and BTG and more prolonged PTR and APPT on admission and on post-operative testing than the patients who subsequently survived. These differences did not attain statistical significance apart from the post-operative BTG which was lower in those who survived - $p > 0.05$ by Mann-Whitney test. The patients who died on the table showed no difference on coagulation screen on admission compared with the survivors.

During operation there were significant changes in coagulation studies. There was a drop on platelet count in all but 2 patients, a drop in fibrinogen in all but 2 patients and a rise in levels of FPA in all patients and BTG in all but one patient

Table 7 *Operative details in patients with ruptured aneurysm Figures are median (range)*

	Survivors n = 10	Died post-op n = 7
Age	69.7 (62-83) years	69.3 (56-77) years
Length of operation	113 (70-180) mins	148 (60-255)mins
Length of cross clamp	55 (45-75) mins	92 (40-210) mins
Blood loss	1780 (490-3990) mls	5985 (550-18000) mls
Red cell transfusion	5.4 (0-11) units	11 (3-26) units
Fresh frozen plasma transfusion	2 patients given 2 units	2 patients given 2 units 2 patients given 4 units
Platelet transfusion	2 patients given 5 units	2 patients given 5 units 2 patients given 10 units
Temperature at end of procedure	35.5 (34-36.5) ^{o}C	35.2 (32-37) ^{o}C

Table 8 *Results of coagulation testing on admission and immediately post-operatively in patients with ruptured aneurysms in each clinical group. Figures are median (range)*

	Survivors n = 10	**Died post-op n = 7**	**Died "on-the-table" n = 5**
On admission			
Platelet count ($x10^9$/l)	289 (160-445)	138 (27-249)	238 (140-297)
Fibrinogen (g/l)	3.8 (1.7-5.13)	2.9 (0.5-7.3)	3.8 (0.6-6.2)
PTR	1.06 (1-1.3)	1.92 (1.2-1.5)	1.5 (1-2.6)
APPT	1.1 (1-1.3)	2.15 (1-5)	2.4 (1-5)
FPA (ng/ml)	27 (10.9-94)	67 (21.4-121)	98 (53.5-164)
BTG (u/l)	249 (105-1000)	370 (176-1375)	650 (136-925)
Post op			
Platelet count ($x10^9$/l)	185 (107-481)	123 (92-167)	
Fibrinogen (g/l)	3.1 (1.2-4.6)	2.6 (0.6-4.9)	
PTR	1.26 (1-1.5)	1.83 (1.2-2)	
APPT	1.3 (1-1.8)	2.5 (1-6)	
FPA (ng/ml)	110 (54.3-146)	208 (89.5-555)	
BTG (u/l)	270 (300-1950)	970 (280-2275)	

Figure 21 *Platelet counts pre-operatively and post-operatively in the three clinical groups (survivors, post-op deaths and died-on-table)*

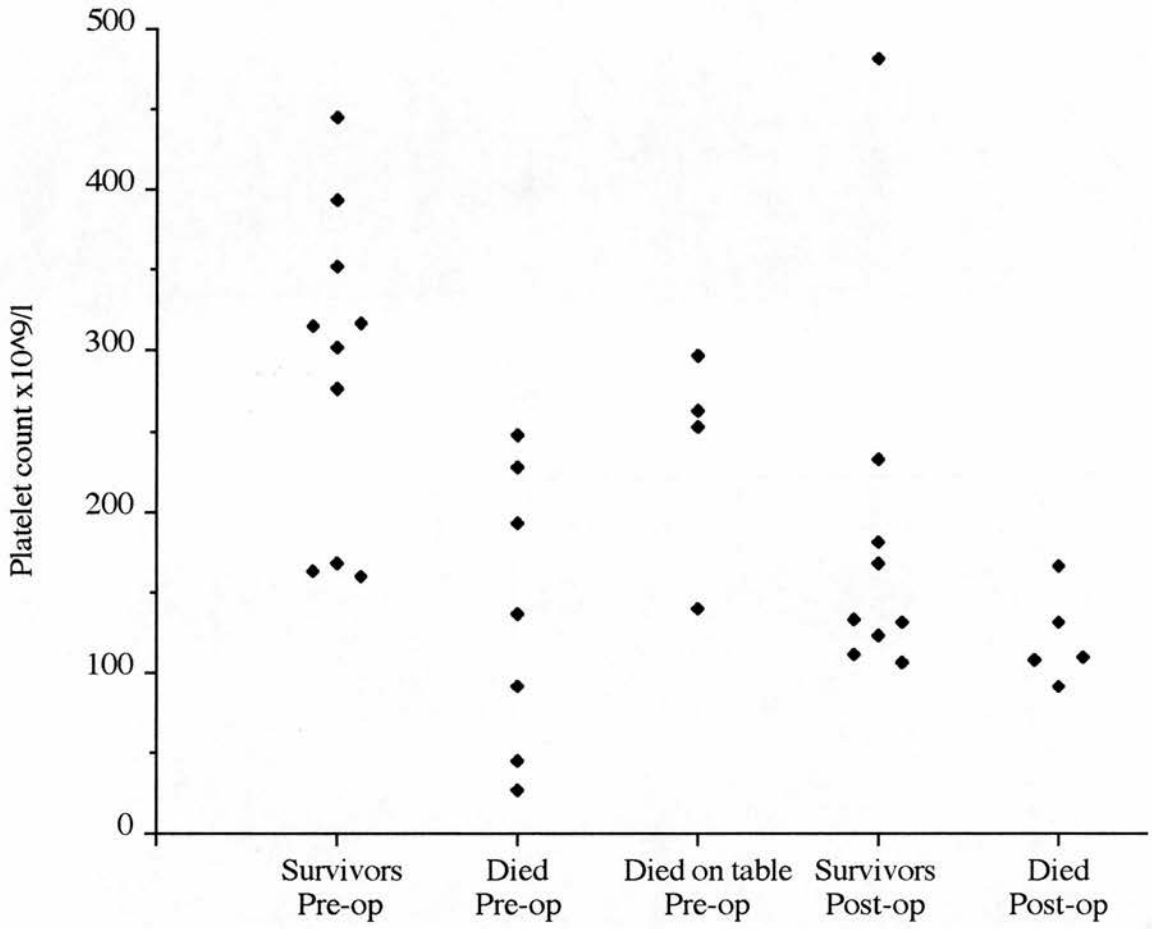


Figure 22 *FPA levels pre-operatively and post-operatively in the three clinical groups. (survivors, post-op deaths and died-on-table) Normal range >10 ng/ml*

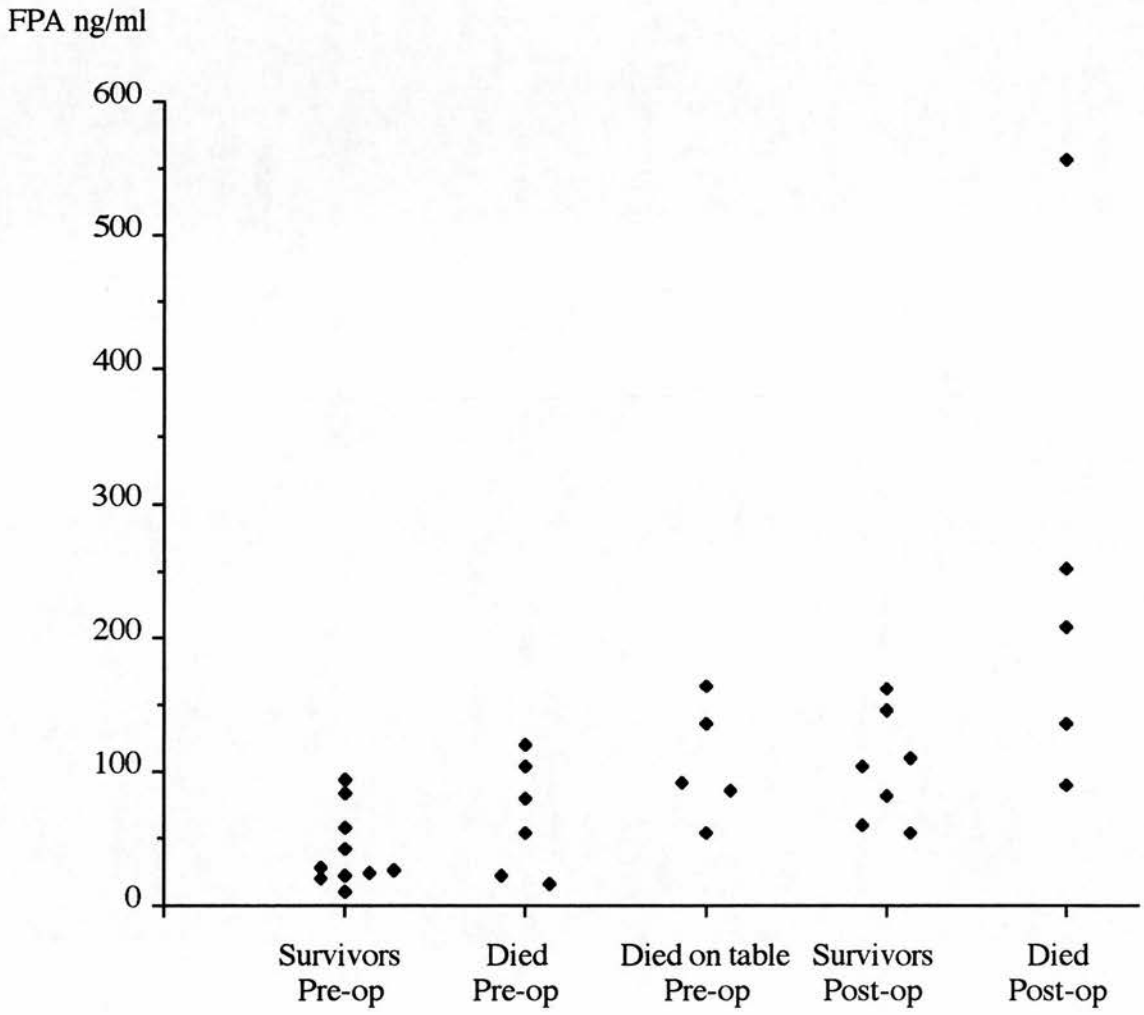
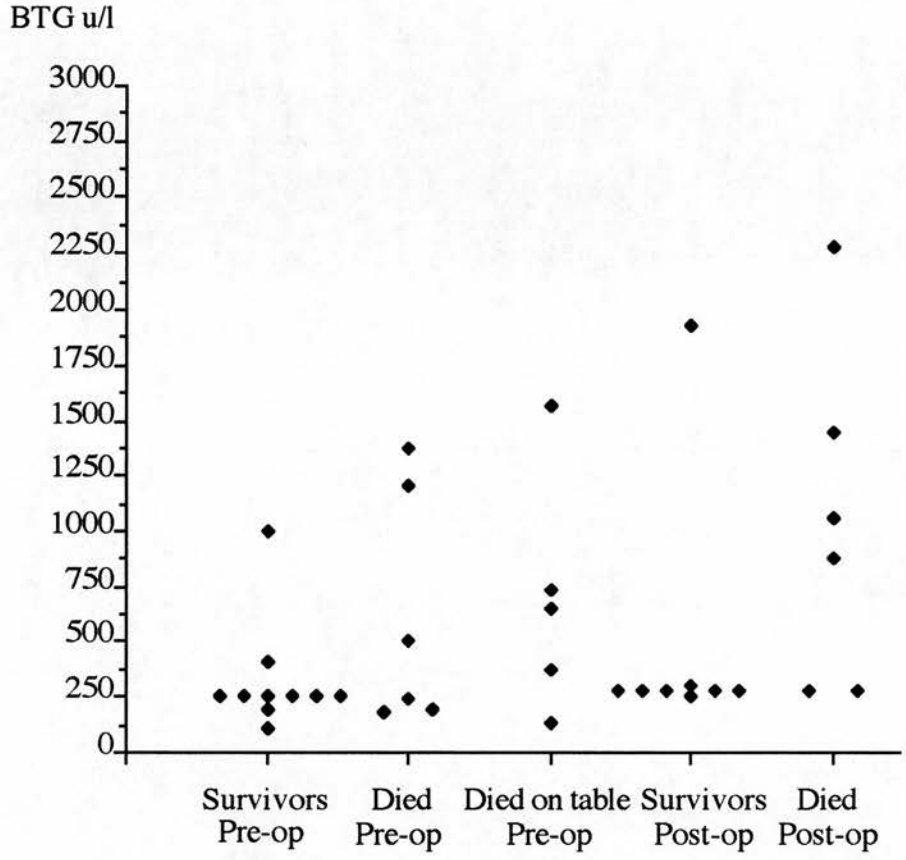


Figure 23 *BTG levels pre-operatively and post-operatively in the three clinical groups (survivors, post-op deaths and died-on-table)*



Discussion

The clinical findings in this study are similar to those described elsewhere; patients who died in the early post-operative period underwent longer operations with long cross-clamp times, greater blood loss and transfusion requirements.[145-147,149,151,270]

As previously described a low platelet count on admission was a predictor of death; all of the patients with a platelet count less than 150 $x10^9/l$ at time of admission died.[162,163] However a normal platelet count was not a good predictor of survival as a significant number of patients with normal platelet counts died.

The level of BTG on admission was not a good predictor of outcome. It is notable that levels of BTG were elevated in all patients at time of admission even those who had a normal platelet count. Levels of BTG at time of admission were significantly higher than found pre-operatively in patients undergoing elective aneurysm repair suggesting that the elevated BTG was related to the rupture rather than due to the presence of an aneurysm. Levels of BTG at the end of operation were greater than in patients who had undergone an elective repair. This may be due in part to the fact that operation for rupture was carried out without the administration of heparin, although it seems more likely that it is due to a combination of active pre-operative bleeding, hypovolaemic shock and the greater intra-operative blood loss during emergency repair.

FPA was a poor predictor of outcome with elevated levels being found in all patients at the time of admission. Levels of FPA rose further at the end of operation and attained levels much higher than in patients undergoing elective operations.

It is unfortunate that the numbers of patients in this study are so small. It may be that with larger numbers some of the differences would have achieved statistical significance, although this must not be assumed. Overall the most significant

differences between the patients who survived and those who did not were in terms of platelet number and markers of platelet activity.

In conclusion this study indicates that:

1. Patients presenting with ruptured aneurysms have elevated levels of FPA and BTG at the time of admission

2. During operation levels of FPA and BTG rise and platelet count drops

3. Patients who survive have significantly lower levels of BTG at the end of operation than those who die in the early post-operative period

Chapter 7

Endothelial Cell Activation in Patients with Ruptured Abdominal Aortic Aneurysm

Introduction

Endothelial cells may play an important role in the pathogenesis of coagulopathy. Recent animal studies of septic shock have shown that the activation of endothelial cells is an important early event in the pathogenesis of the coagulopathy associated with septicaemia.[271] It is well known that endothelial cell have wide-ranging and complex interactions with platelets, leucocytes and the coagulation and fibrinolytic systems as detailed above. It may be that endothelial cells, activated by hypoxia secondary to hypovolaemic shock, could play a similar role in the early pathogenesis of coagulopathy associated with ruptured aortic aneurysm.

A well established method for the investigation of endothelial cell function in vivo does not exist. The majority of previous research on endothelial cell function has been carried out in cell culture models using immunohistochemical or flow cytometric techniques. Some *in vivo* work has been carried out using soluble markers of endothelial cell activation, such as von Willebrand factor and soluble E-selectin. These techniques provide little information as to the site at which endothelial are activated and what is happening at a cellular level.

A number of studies using immunohistochemistry have been carried out on tissue biopsy specimens but the degree of magnification gives little indication as to whether the antigens are present extracellularly, intracellularly, on the luminal membrane or within the lumen of the vessel. Recent animal studies have suggested that immunoelectron microscopy is a useful technique for this purpose.

Immunoelectron microscopy would be the ideal technique but this technique has not previously been used for the examination of endothelial cells in biopsy material from human subjects. It was therefore decided that the initial studies should be carried out using plain transmission electron microscopy of biopsy material obtained at the start

of operation.

The aims of these studies were to determine whether biopsy material obtained at the start of operation from patients undergoing aortic surgery would yield sufficient numbers of undamaged and well fixed endothelial cells and to establish whether this would be a feasible method for further studies of endothelial cell function following rupture. In addition it was hoped that these studies might reveal evidence of endothelial activation at an ultrastructural level at an early stage following ruptured aneurysm.

Pilot Study

Aims

The aims of this study were establish the best type of tissue and fixative for an electron microscopic study of endothelial cells in patients with ruptured aneurysms.

The ideal tissue would:-

1. Be easily obtained very early during operation at no risk to the patient

2. Yield good numbers of small capillaries

3. Fix well and be easy to prepare for electron microscopy

4. Not be affected during the induction of anaesthesia

Methods and materials

Tissue was obtained from patients undergoing elective aortic aneurysm surgery. Tissue was taken during abdominal incision as this obviated the need for a second skin incision. Three types of tissue were easily accessible within a few minutes of incision: skin, adipose tissue and skeletal muscle. Three different fixatives were used:-

1. Glutaraldehyde in phosphate buffer
2. Millonig's EM fixative - paraformaldehyde and glutaraldehyde in phosphate buffer
3. 2.5% glutaraldehyde in cacodylate buffer

Tissue was cut into specimens of about 2mm cubed and immediately placed into fixative. The specimens remained in fixative for 12-24 hours, then washed in 5% sucrose and placed in 1% osmium tetroxide in cacodylate buffer, washed in 10% ethanol, and dehydrated by immersion in absolute alcohol for 30 minutes, repeated three times, then cleared by immersion in propylene oxide B.D.H. for 30 minutes. The tissue was then embedded in Araldite mixture for 12-24 hours at room temperature and then hardened in fresh Araldite placed in an oven at 60^{o}C for 48 hours. Blocks were cut from the Araldite and mounted on a dowel rod with sealing wax. Ultrathin sections were cut from this and mounted on Athene 483 grids without a supporting membrane. The sections were stained by floating on lead citrate for 2 minutes, washed in 0.02 N NaOH for 20 seconds, washed in distilled water for 10 seconds, immersed in Uranyl acetate for 10 minutes and washed in 50% ethanol for 20 seconds. (Table 24)

The processed specimens were viewed on a Phillips 301 electron microscope. Electron micrographs of capillaries were taken for later detailed examination.

Figure 24 *Processing of samples for electron microscopy*

Process	Solution	Time	
Fixation	2.5% glutaraldehyde in cacodylate buffer	1-24 hours	
Washing	5% sucrose	30 mins	
Postosmication	1% osmium tetroxide in cacodylate buffer	1-2 hours	
Washing	10% ethanol	1-24 hours	Continuous rotation
Dehydration	Absolute ethanol	3 x 30 mins	
Clearing	Propylene oxide	30 minutes	
Embedding	Araldite mixture	12-24 hours	Room temperature
Hardening	Araldite mixture	48 hours	60°C oven
Sectioning			
Mounting			Athene 483 grids
Staining	Lead citrate	2 mins	
	0.02 N NaOH	20 seconds	
	Distilled water	10 seconds	
	Uranyl acetate	10 minutes	
	50% ethanol	20 seconds	

Results

Skin

Skin could be obtained immediately at the start of operation and it fixed easily with all three fixatives. However preparation for electron microscopy was difficult as the epidermis became extremely hard during fixation and was difficult to cut. It was more difficult to identify small capillaries than in other tissues, with often only one or two vessels in a high power field.

Adipose tissue

Adipose tissue could be obtained immediately at the start of operation from the subcutaneous layer of fat just as quickly as skin. It was difficult to fix, giving optimal results with glutaraldehyde/cacodylate fixative. Processing for electron microscopy was easy and large numbers of small capillaries were readily identifiable at the junctions between cells. (Figures **25** & **26**)

Skeletal muscle

Skeletal muscle was obtained from rectus abdominis. This could only be achieved a few minutes after the start of the procedure. The tissue fixed well and was easy to prepare for electron microscopy. Large numbers of small capillaries were easily found. (Figure **27**)

In all three tissues the endothelial cells examined appeared normal and intracellular

detail was good when compared with textbook examples. Mitochondria were well preserved and Weibel-Palade were present in endothelial cells of larger capillaries and venules.

Figure 25 *Endothelial cell (EC) from adipose tissue fixed in phosphate buffer. A red blood cell (RBC) is present within the lumen. Scale bar indicates 5μm*

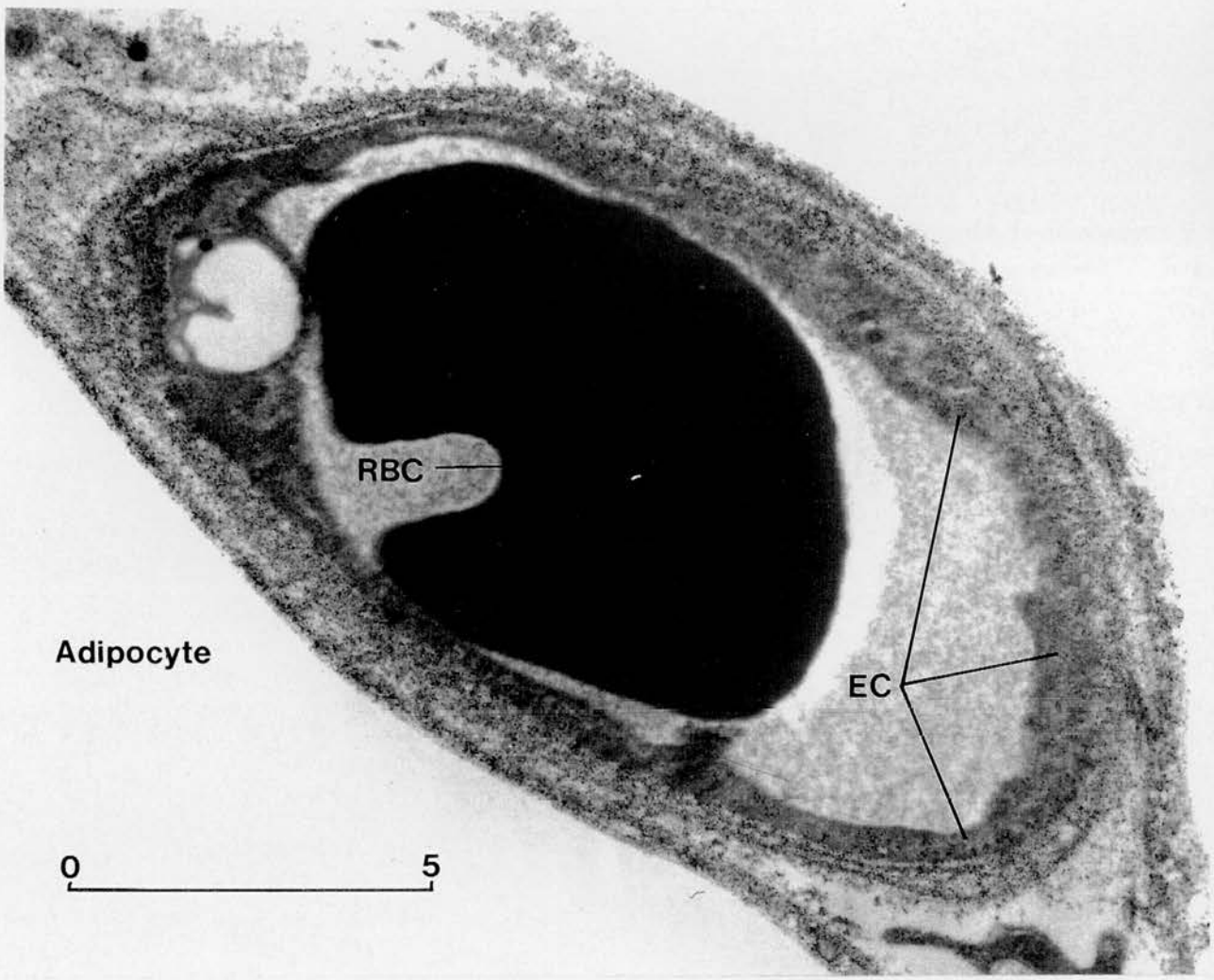


Figure 26 *Endothelial cell (EC) from adipose tissue fixed in glutaraldehyde/cacodylate buffer. A red blood cell (RBC) is present within the lumen. Scale bar indicates 5μm*

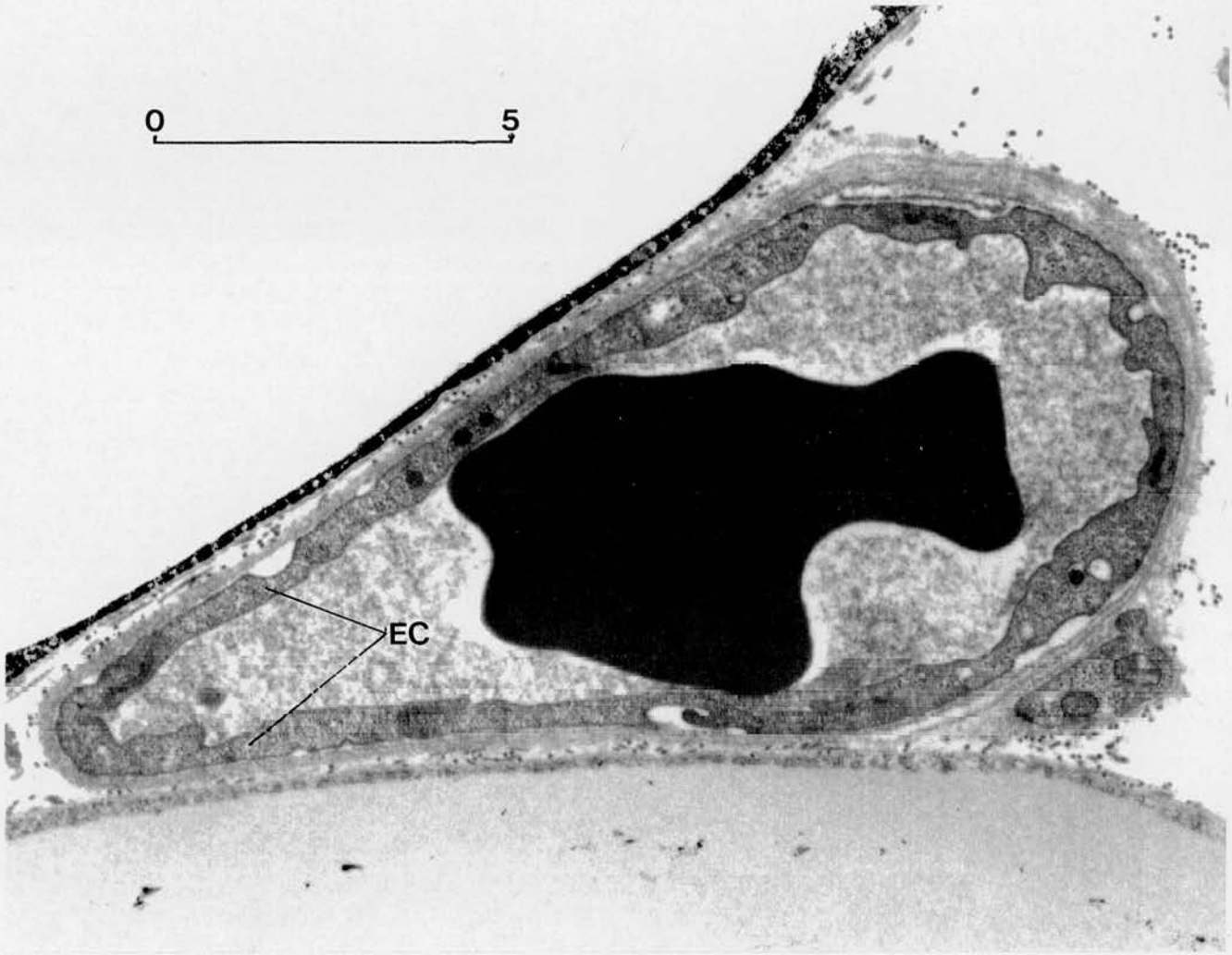
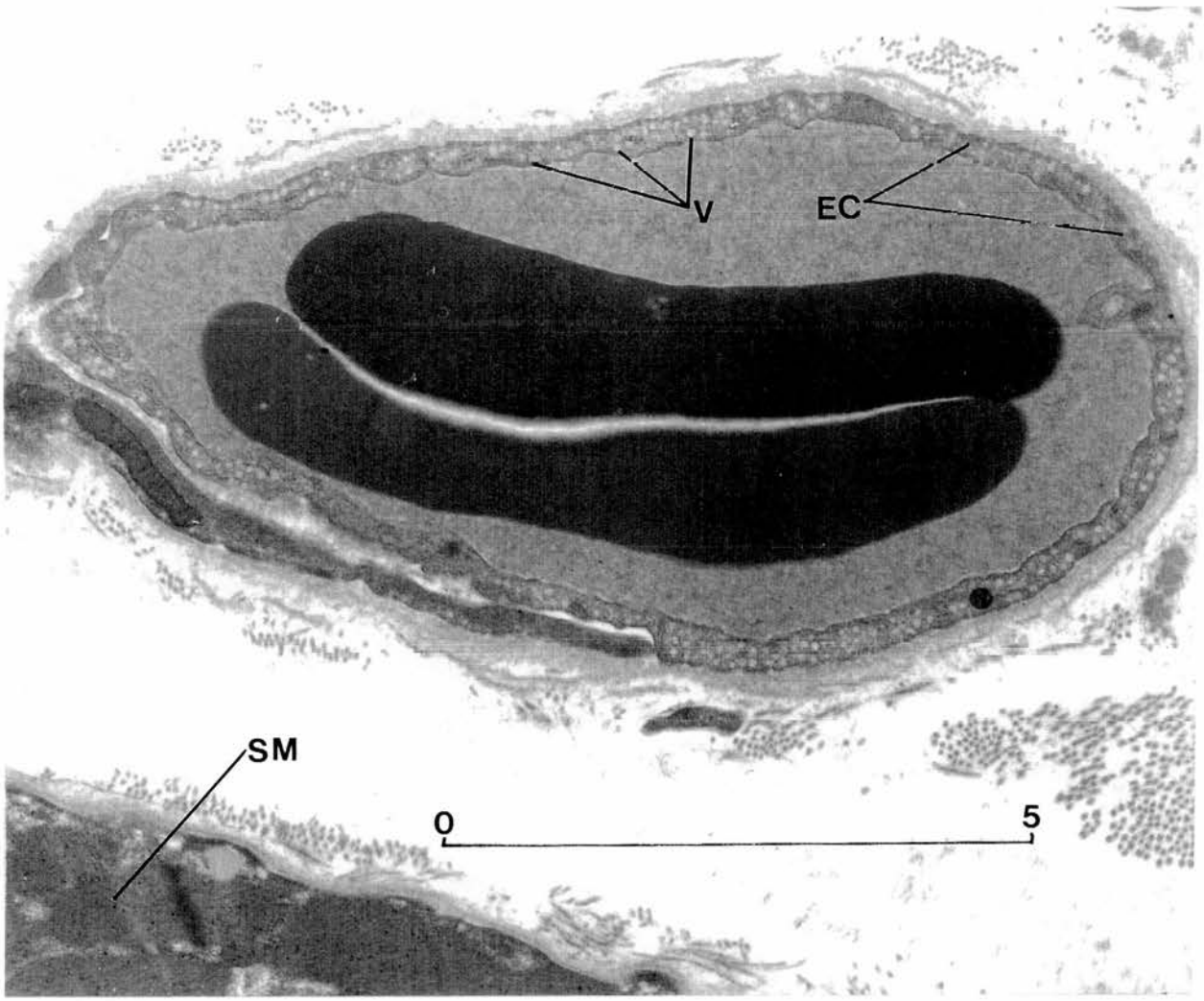


Figure 27 *Endothelial cell (EC) from skeletal muscle (SM). Large numbers of intracellular vesicles (V) are present. Scale bar indicates 5μm.*



Conclusions

Electron microscopy of biopsy material obtained at the start of operation appeared to be a feasible technique for studying endothelial cells in patients undergoing aortic surgery. Skeletal muscle was easy to prepare and provided the most abundant and best fixed capillaries but there were some drawbacks. It could not be obtained until some minutes after the start of operation and might therefore be affected by trauma inflicted during incision. In addition suxamethonium is given at induction of anaesthesia causing intense muscle contraction which may result in tissue injury.

Skin was difficult to work with and provided unreliable numbers of small capillaries. In addition skin preparation for operation involves washing which cools the skin possibly affecting blood flow.

Despite the fact that adipose tissue was difficult to fix, it could be obtained immediately at the start of operation and was not likely to be affected by the induction of anaesthesia and preparation for operation. There were other favourable features. For example large samples of fat could reasonably be taken which would be useful for future immunolabelling studies.

It was therefore decided that adipose tissue should be used for the initial study in patients with ruptured aneurysms and that skeletal muscle should be examined in a second study.

Endothelial cell structure in adipose tissue capillaries following ruptured aortic aneurysm

Methods and materials

Fat samples were taken from the anterior abdominal wall immediately after skin incision at the start of operation in 5 patients undergoing operation for ruptured abdominal aortic aneurysm and from 5 control patients undergoing elective surgery for abdominal aortic aneurysm. The diagnosis of ruptured aneurysm was confirmed in all cases at the time of operation. The fat samples were cut into 2mm cubes and were immediately fixed in 2.5% glutaraldehyde / cacodylate fixative. The specimens were then processed for electron microscopy as detailed above.

Two blocks of tissue were cut from each sample. Electron micrographs were taken of all capillaries present in each section. The number of capillaries examined in each patient ranged from 6-12, mean 8.9 capillaries.

All patients with rupture had suffered a period of hypotension and one patient had an established coagulopathy at the start of operation. No patient had received any blood products prior to operation.

Results

Normal endothelial cells

Examples of a normal endothelial cells are shown in figures 26 & 28. The vessel diameter ranged from 6-38 μm which is in the expected range for capillaries and post-capillary venules. The cell may have numerous intracellular vesicles. The luminal surface is smooth and regular. Endothelial cells are joined by tight junctions. There is commonly a process on the luminal surface where two endothelial cells join. The basal cell membrane is smooth with no gap between cell membrane and basement membrane.

Endothelial cells from patients with rupture

On examination of endothelial cells from the patients with rupture there were a number of subjective differences compared with the endothelial cells from the control group. Firstly the luminal surface appeared more convoluted and the there appeared to be more frequent luminal processes (Figure 29) The cell membrane on the tips of these processes was thicker than elsewhere on the cell surface and had a fuzzy appearance. Intracellular vacuoles were seen frequently. These were lucent, circular structures about 0.5 - 1 micron in diameter commonly found near the cell nucleus. In some cells the basal membrane was irregular and convoluted.

The most striking abnormality found in endothelial cells from patients with rupture was the presence of small spherical processes, about 0.1 μm in diameter, on or adjacent to the luminal surface (Figure 30 & 31). These resemble the budding of small membrane bound vesicles into the lumen of the vessel. However must be remembered that the electron micrographs are two dimensional cross sections and it may be that these process are thin cylindrical processes which have been cut in cross section.

In order to quantify these differences, each photomicrograph was assessed by three independent, blinded observers who graded each capillary in the presence or absence of luminal processes, budding, intracellular vesicles, intracellular vacuoles and irregularity of the basement membrane. The numbers of capillaries in the control and study patients with each feature are shown in Table 9. Process formation on the luminal surface was significantly more common in the patients who had suffered rupture than in the control group. Budding of the luminal surface was found in 11/45 of the capillaries from patients with rupture but in none of the capillaries from control patients $p < 0.001$. Budding was found in at least one capillary from all the patients with rupture but in none of the control patients $p < 0.05$.

The vessels in which these budding processes were seen were larger than those in which no budding was found. The mean diameter of vessel with budding was 26μm (range 11-68μm) compared with a mean diameter of 17 μm (range 7-37μm) in vessels without budding. It may be that the vessels demonstrating budding are post-capillary venules rather than capillaries. In one vessel which demonstrated budding a leucocyte can be seen adhering to the endothelial cell (Figure 32). This supports the hypothesis that these vessels are post-capillary venules as leucocyte adhesion and migration takes place at this site.

Figure 28 *Endothelial cell (EC) from control patient undergoing elective operation. Scale bar indicates 5μm.*

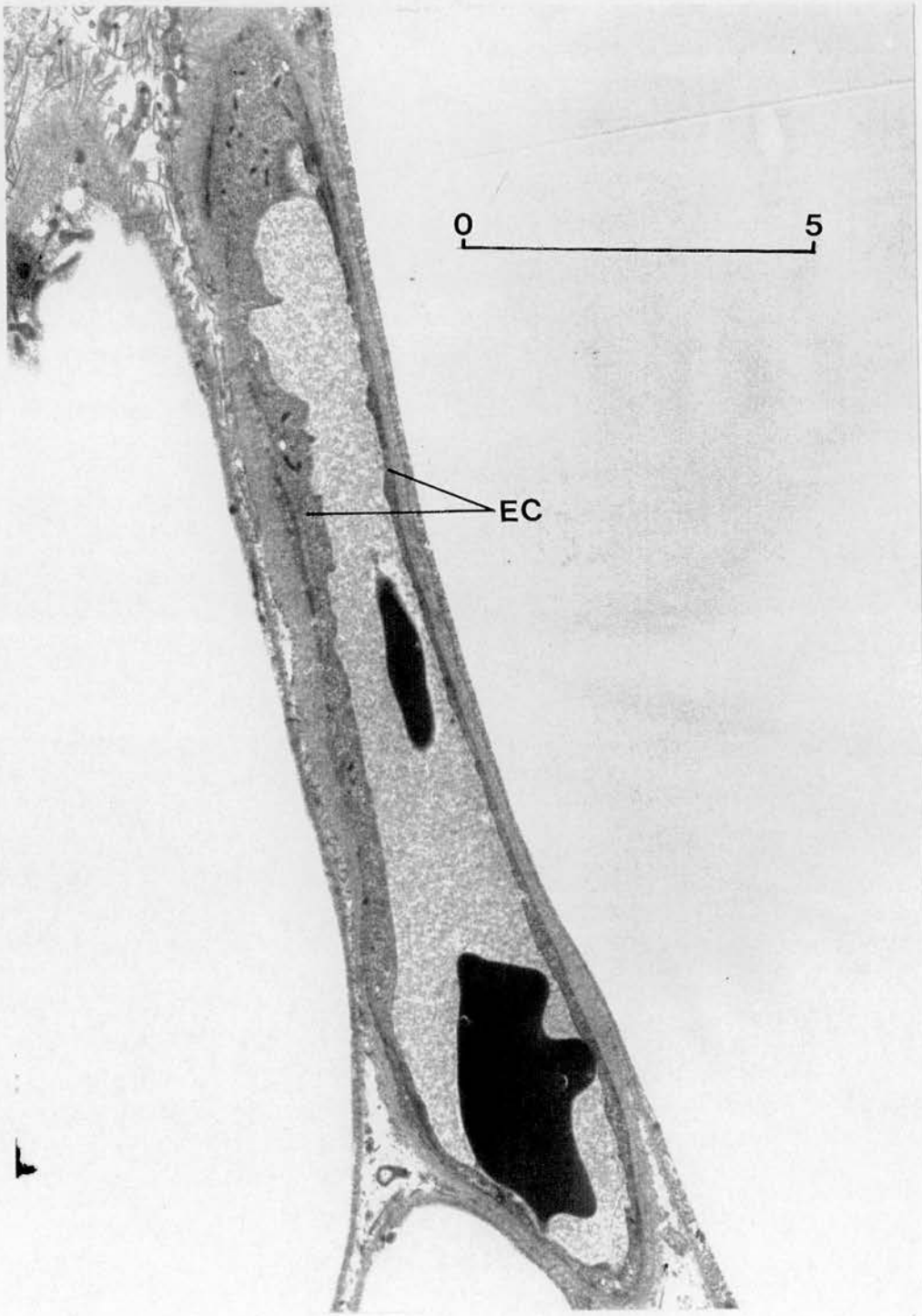


Figure 29 *Endothelial cell from patient with rupture demonstrating processes (P) on luminal surface. Scale bar indicates 5μm.*

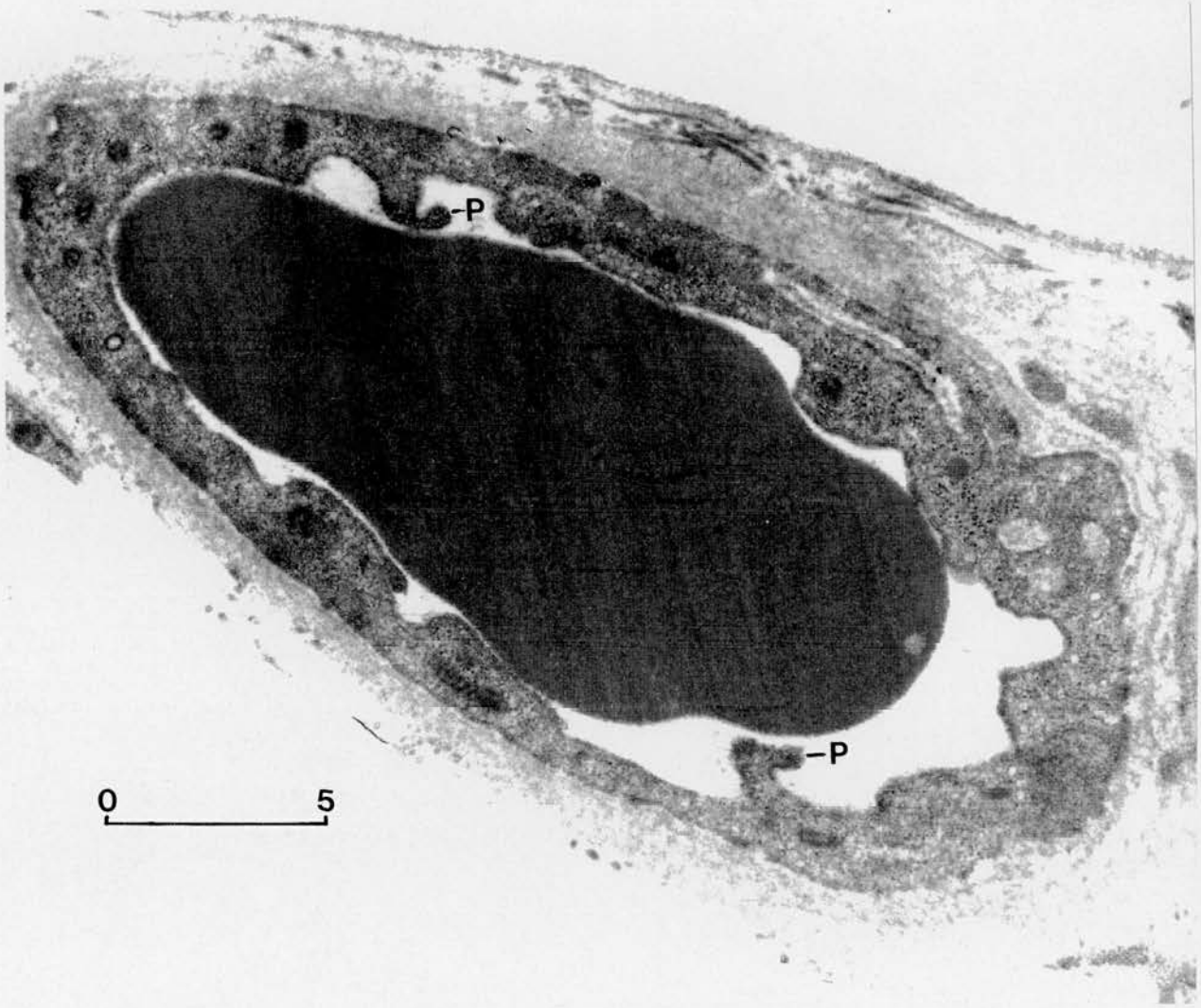


Figure 30 *Endothelial cell from adipose tissue in patient with rupture demonstrating budding (B) on luminal surface. Scale bar indicates 5μm.*

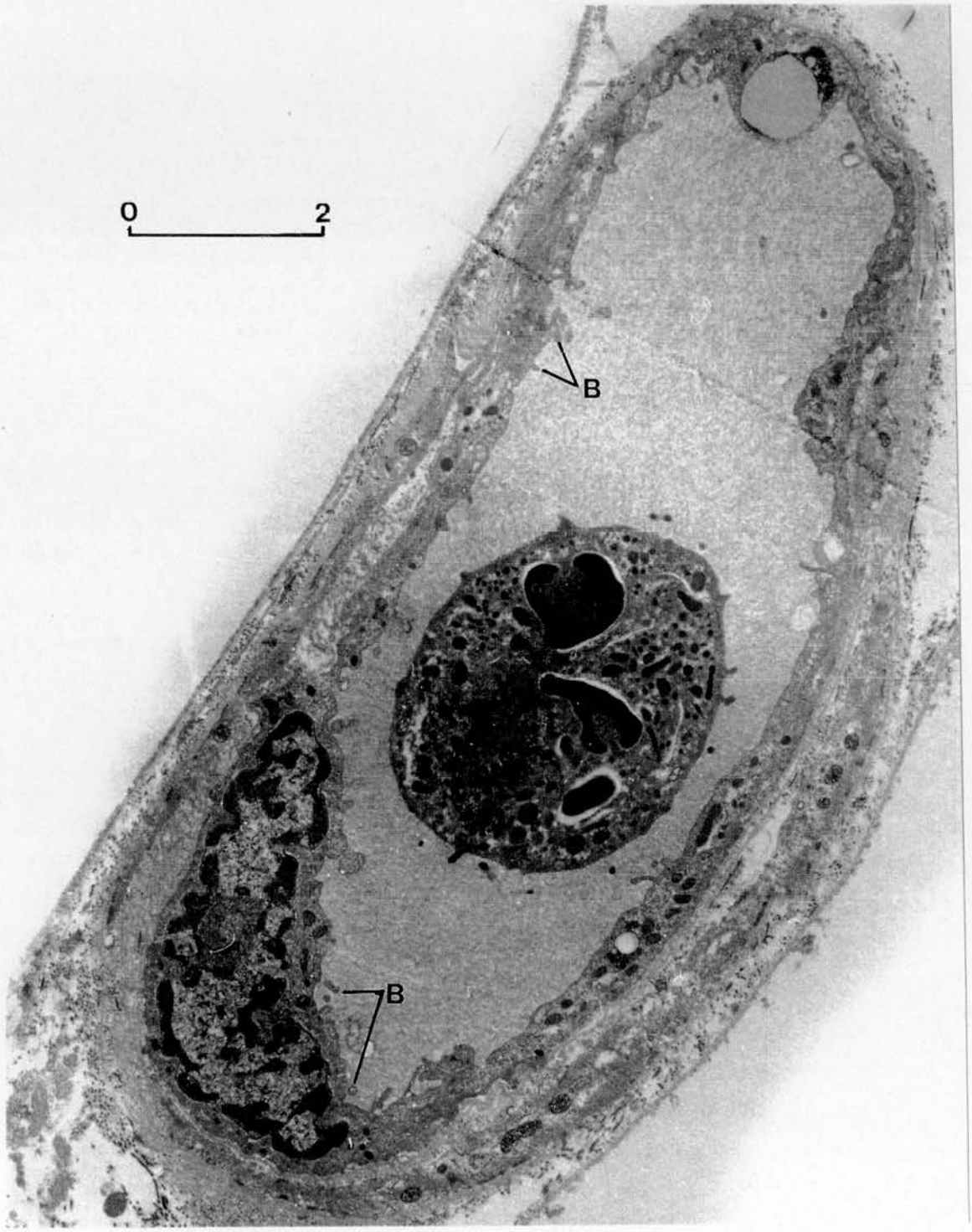


Figure 31 *Endothelial cell from adipose tissue in patient with rupture demonstrating budding (B) on luminal surface. Scale bar indicates 5μm.*

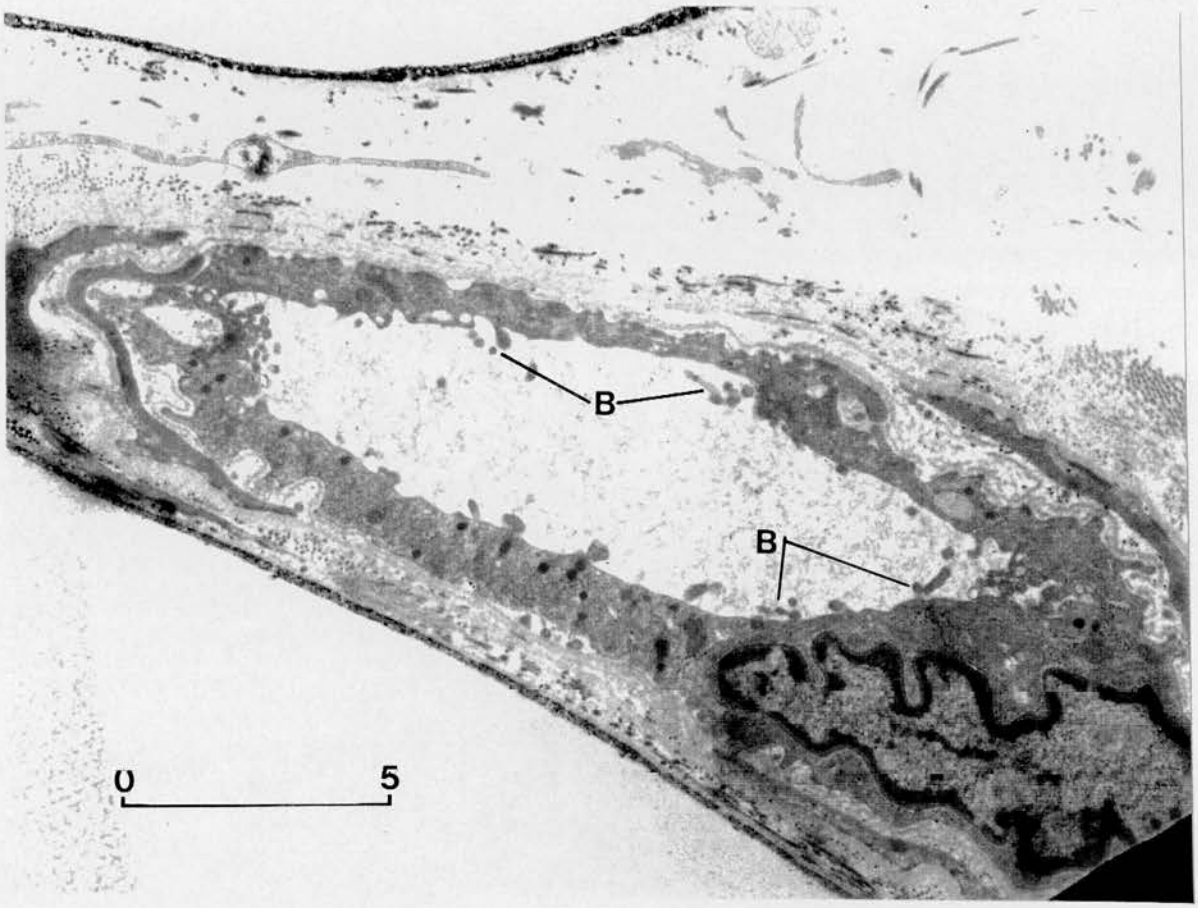


Figure 32 *Endothelial cell from adipose tissue in patient with rupture demonstrating budding (B) and adhesion of leucocyte (L). Scale bar indicates 10μm.*

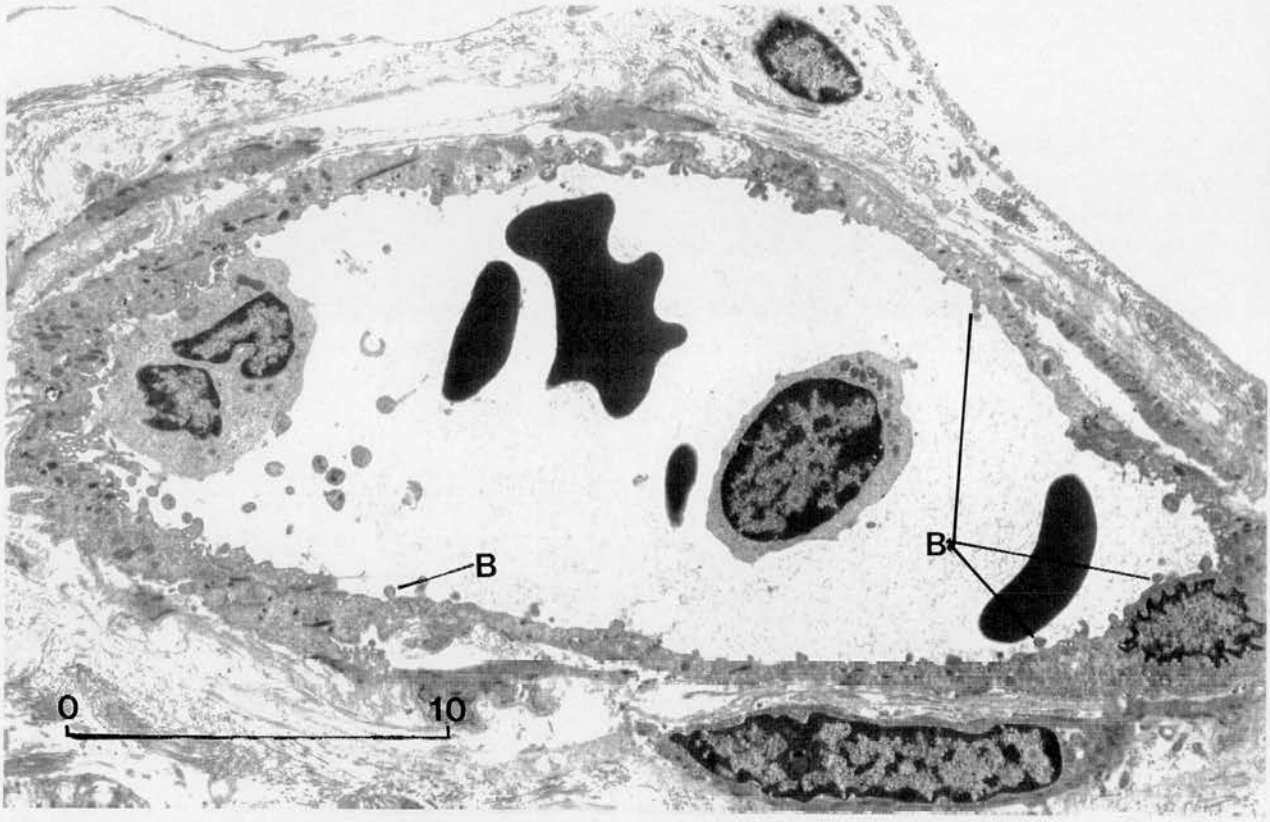


Figure 32b *Enlargement of right corner showing budding in greater detail.*

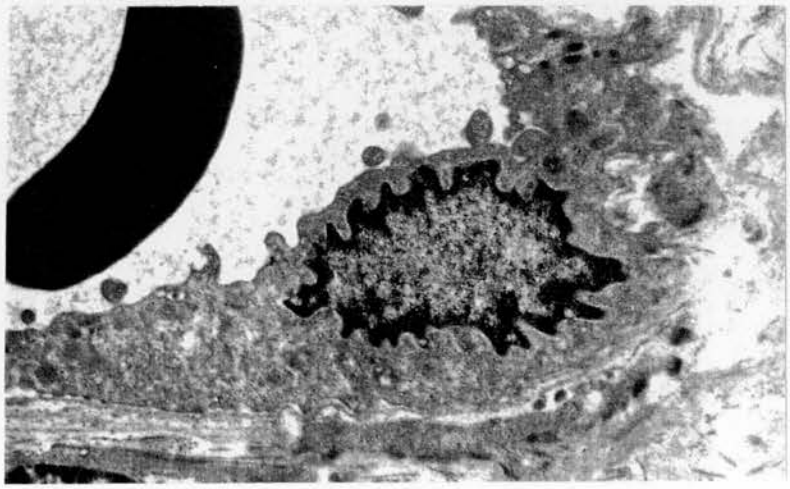


Table 9 *Ultrastructural findings in endothelial cell in adipose tissue*

	Rupture n = 45	**Control n = 44**	
Processes	37	26	$p < 0.05$
Irregularity of basal membrane	12	11	NS
Budding	11	0	$p < 0.001$
Vacuoles	19	8	$p < 0.02$
Intra-cellular vesicles	9	15	NS

Endothelial cell structure in skeletal muscle capillaries following ruptured aortic aneurysm

Methods and materials

Skeletal muscle samples were taken from rectus abdominis at the earliest opportunity during operation in 3 patients undergoing operation for ruptured abdominal aortic aneurysm and in 2 control patients undergoing elective operation for aortic aneurysm. The diagnosis of ruptured aneurysm was confirmed in all cases at the time of operation. The muscle samples were cut into a 2mm cubes and were immediately fixed in 2.5% glutaraldehyde / cacodylate fixative. The specimens were then processed for electron microscopy as detailed above.

All patients with rupture had suffered a period of hypotension, no patient had a coagulopathy at the start of operation and one patient developed a coagulopathy by the end of operation. No patient had received any blood products prior to operation.

Results

The appearances of endothelial cells in capillaries from control patients were similar to those in the adipose tissue samples from control patients although intracellular vesicles were more often found, being present in nearly all endothelial cells including those from ruptures (Figure 33). Budding on the luminal surface of the endothelial, similar to that found in adipose tissue, was found in endothelial cells in 2/3 patients with rupture (Figures 35 & 34). No endothelial cells demonstrated budding in the control group. As with the adipose tissue samples the budding was more commonly found in larger vessels with diameters in the range 22-45 μm. Vacuoles were present in 4/9 endothelial cells of one patient with rupture but were not seen in any of the other patients.

The number of capillaries demonstrating processes, budding, vacuoles and intracellular vesicles are shown in Table 10.

Table 10 *Ultrastructural findings in endothelial cell in skeletal muscle. (n = number of capillaries identified)*

	Control		Rupture		
	1 n = 8	**2** n = 9	**3** n = 7	**4** n = 10	**5** n = 9
Vesicles	8	9	7	6	7
Vacuoles	0	0	0	0	4
Processes	3	2	5	2	5
Budding	0	0	0	2	3

Figure 33 *Endothelial cell from skeletal muscle in patient with rupture demonstrating budding (B). Scale bar indicates 10μm.*

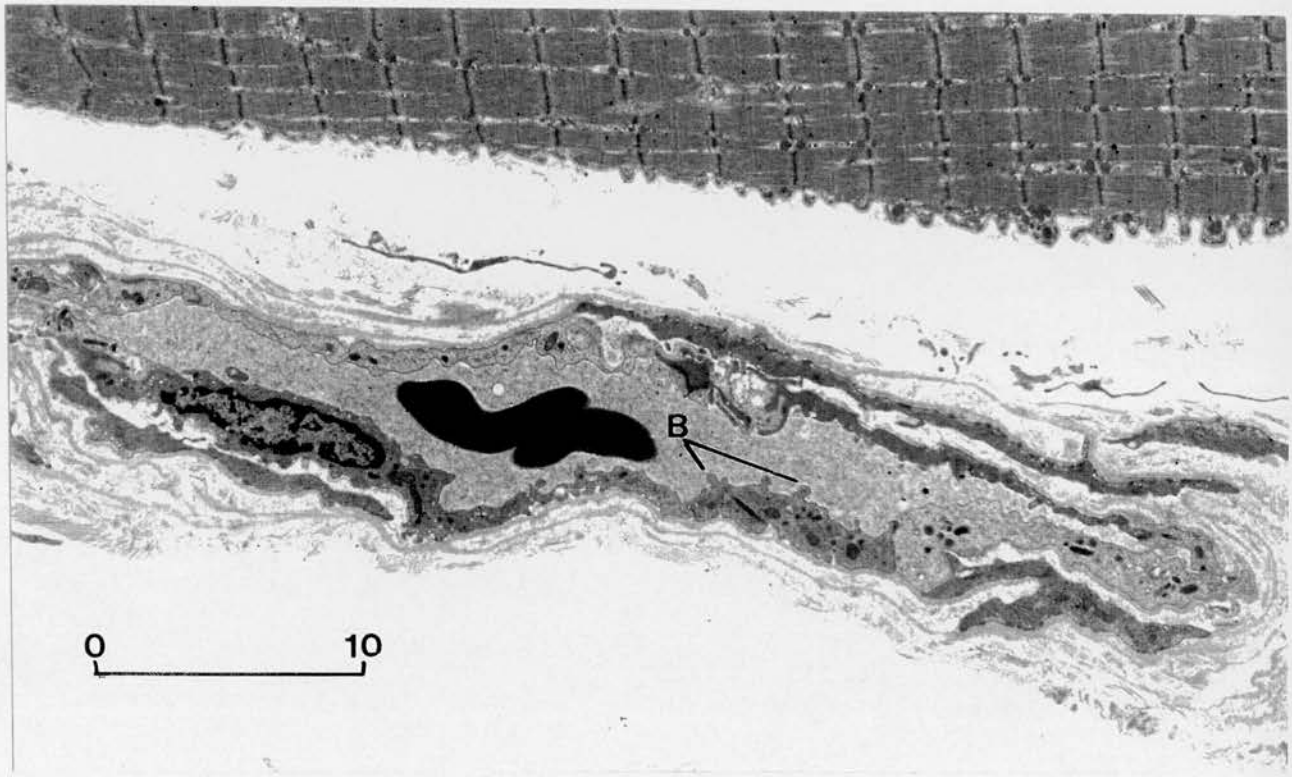


Figure 33b *Enlargement of central area of picture showing budding in greater detail.*

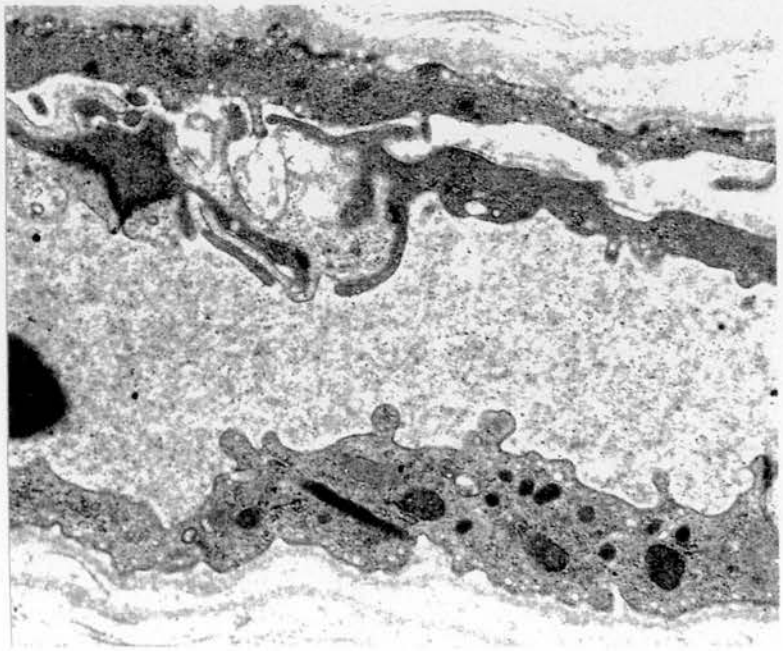


Figure 34 *Endothelial cell from skeletal muscle in patient with rupture demonstrating intracellular vacuoles (V).*

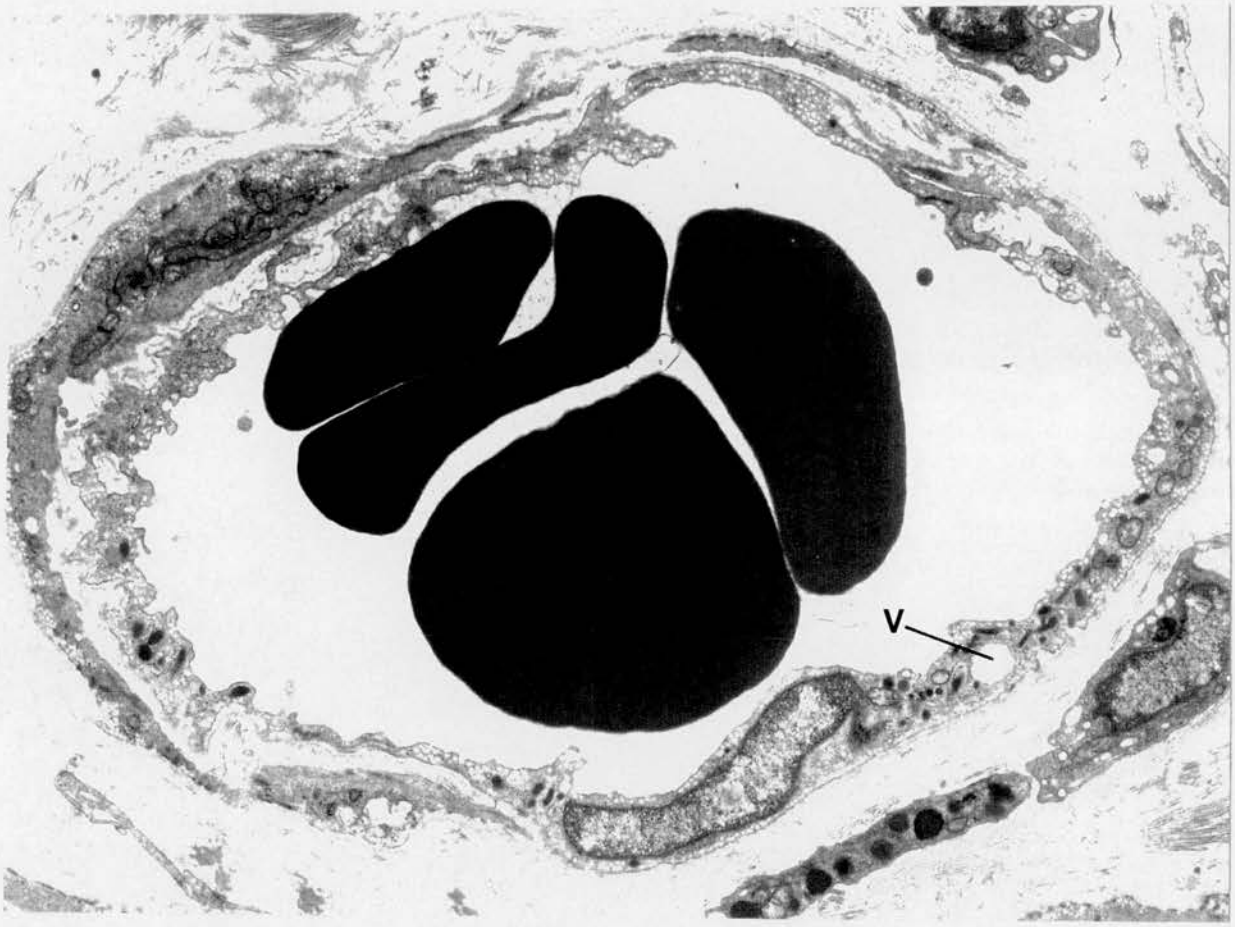
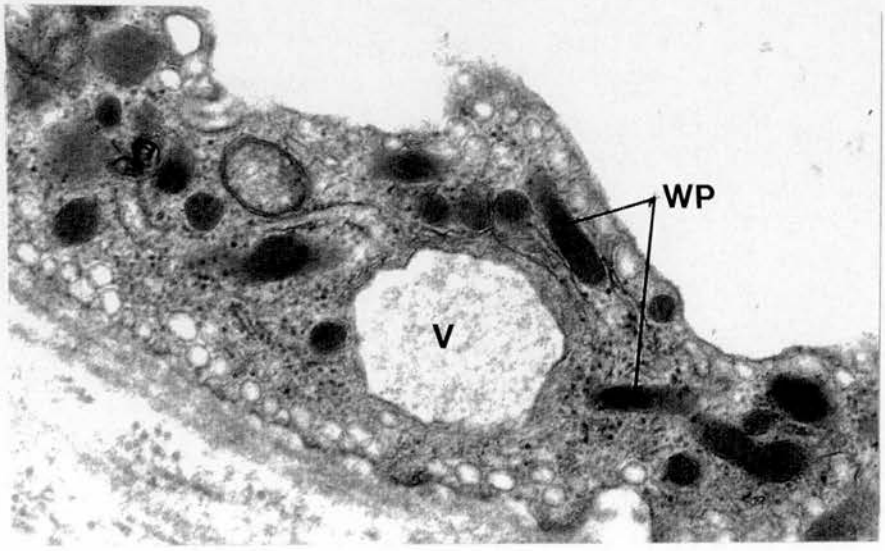


Figure 34b *Enlargement of area containing vacuole (V). Weibel-Palade (WP) bodies are also shown.*



Discussion

This study has demonstrated that there are ultrastructural differences between endothelial cells in patients undergoing operation for ruptured aortic aneurysm and patients undergoing elective operation for aortic aneurysm. These changes were found in two different types of tissue. This suggests that widespread endothelial cell activation in small vessels is an early event following aortic aneurysm rupture.

The changes were less marked in skeletal muscle. The reason for this may be that there are intrinsic differences between endothelial cells in these two tissues or it may be that the stimulus which is causing these changes varies between the tissues.[272] For example in response to worsening hypovolaemic shock the blood supply to adipose tissue may be reduced earlier than in skeletal muscle thereby rendering adipose tissue hypoxic for a longer period of time.

The most marked structural difference was the presence of budding which was found in at least one capillary from all patients with rupture but in none of the capillaries from the patients in the control group. A less marked finding was the reduction in intracellular vesicles in endothelial cells from patients with rupture.

The ultrastructural changes in endothelial cells that we found are similar to those described in heart capillaries of dogs subjected to a period of hypoxia produced by ligation of a coronary artery.[273-275] These studies describe loss of cytoplasmic vesicles at an early stage, 10-30 minutes, and the production on "blebs" on the luminal surface of the cell after more prolonged ischaemia 60-180 minutes. It must be noted that the "blebs" described are of greater size than the budding which is described here.

The endothelial cell budding has the appearance of shedding of membrane bound vesicles.[276] Shedding is the release of cell surface constituents either in soluble form

or in association with plasma membrane, as opposed to secretion which is the release of soluble proteins from secretory granules in the in the cytoplasm to the exterior of the cell.

In cell culture studies of human umbilical vein endothelial cells a number of agonists, such as complement protein C5b-9, have been shown to stimulate the shedding of membrane bound vesicles of a similar size to those observed in the present studies.[277] These membrane bound vesicles have been shown to be strongly procoagulant, promoting the production of the prothrombinase complex. It is should be noted that tissue factor, being an integral membrane glycoprotein, is expressed by shedding of membrane bound vesicles and it may be that this is the cause of increased prothrombinase activity.[192,278]

Intracellular vacuoles were found more often in endothelial cells from patients with ruptured aneurysm. Such ultrastructural appearances have been described in animal studies of endothelial cell injury but their origin is unknown.[193,194] In those animal studies endothelial injury was induced by helium-neon laser and the appearance of vacuoles within the endothelial cell was found to be an early sign of cell injury. Aggregation of platelets on the cell surface was found to be associated with the development of vacuoles in the endothelial cell, indicating that this is an ultrastructural change which is associated with procoagulant functional changes. However no such studies have been carried out in humans.

The cause of the endothelial cell changes found in these patients has not been indicated by these studies. Several stimuli are known to cause endothelial cell activation: hypoxia, cytokines, endotoxin, activated leucocytes, thrombin and histamine. As noted above in septic shock the trigger for endothelial cell activation is TNF-α, but it seems unlikely that patients with ruptured aneurysm have endotoxaemia at the time of admission. It seems more likely that in these patients

the stimulus is direct endothelial cell hypoxia. All the patients studied were hypotensive on admission. Hypovolaemic shock results in widespread tissue hypoperfusion and hypoxia which should be most marked in tissues such as subcutaneous fat. Such hypoxia has been shown to cause endothelial cell activation in cell culture and animal models in as little as 30 minutes.[279] In addition the ultrastructural changes in endothelial cells are similar to those expected to occur with hypoxia,[280] but it must be noted that endothelial cells have a limited variety of structural changes in response to stimuli.

The functional significance of these structural changes is a matter for speculation. As stated above it has been shown that hypoxia induces structural changes in endothelial cells in canine models and in one study it was shown that such cells demonstrate abnormal function in terms of vasodilatory reserve.[281] However no human or animal studies have been carried out relating changes in endothelial cell structure to changes in endothelial cell function in terms of modification in interactions with the haemostatic system.

It has been shown in cell culture models that hypoxia can induce endothelial cells to produce procoagulant substances such as tissue factor.[282-284] This has not yet been shown to be of significance in clinical studies but it does demonstrate that a mechanism exists whereby endothelial cells, activated by hypoxia, could stimulate thrombin production and ultimately trigger DIC.

In addition endothelial cells play an important role in leucocyte activation, adhesion and migration both directly via expression of adhesion molecules and indirectly via cytokine expression. Thus endothelial cell activation secondary to hypoxia might cause leucocyte adhesion and activation in hypoxic tissues. Subsequent reperfusion would wash out these activated white cells and cytokines contributing to multiorgan failure in the early post-operative period.[285]

In summary the structural changes that have been demonstrated in endothelial cells from patients with rupture are known to be associated with alteration in function and are compatible with the structural changes caused by hypoxia. The endothelial cell budding looks identical to the process of shedding of membrane bound vesicles. It is by this process that tissue factor is expressed and it is known from cell culture studies that tissue factor is expressed by endothelial cells in response to hypoxia. It may be speculated that the ultrastructural changes described above represent tissue factor expression. If it is so then this may well be the mechanism which triggers DIC in these patients. It must be emphasised that this is speculative and further, more sophisticated studies looking at function in addition to structure, are required to test this hypothesis.

In conclusion these studies have demonstrated that:-

1. Electron microscopy of tissue biopsy at time of operation is a feasible method for the examination of endothelial cells.

2. Ultrastructural changes are present in endothelial cells of small vessels in patients with aortic aneurysm rupture.

3. These changes are present in adipose tissue and skeletal muscle

Future studies

While the above studies have indicated that ultrastructural changes do occur in endothelial cells at an early stage following rupture they do not provide information concerning the function and activity of these cells. Immunogold electron microscopy has been shown to be a valuable technique for investigating endothelial cell function.[286] However these techniques have previously only been described in cell culture models and animal models which allow vascular perfusion with fixative prior to biopsy. Light microscopy with immuno staining wuould be easier to perform but in the samll vessels being studied there would be insufficient resolution to determine whether the area being stained was in the lumen, on the surface of the endothelial cell, within the cytoplasm of the cell or in the subendothelial layer. This is important as some of the factors of interest, such as tissue factor and von Willebrand factor, would be a normal finding in the subendothelium or within the cell cytoplasm but would be abnormal if expressed on the cell surface. Immunoelectron microcroscopy should provide this degree of resolution.

An example of immunoperoxidase staining of a capillary within adipose tissue obtained from a patient with a ruptured aortic aneurysm is shown in Figure 36. In this example the primary antibody used was anti-vWF. Staining can be seen within the vessel lumen and on the endothelial cell surface. Immunoperoxidase staining is easier to perform but produces inferior results to those which are expected from immunogold staining. With immunoperoxidase it is less clear-cut where staining has occurred as it merely shows up as a darker area and it is more difficult to carry out quantatative analysis. This example of immunoperoxidase staining demostrates that it is possible to immunologically stain fixed adipose tissue and subsequently to examine these tissue by electron microscopy without loss of ultrastructural detail.

Figure 35 *Immunogold technique. The staining can be carried out on after fixation, before embedding or after embedding if an embedding agent such as LR White is used rather than araldite. The gold microsphere appears as a dark dot on electron microscopy.*

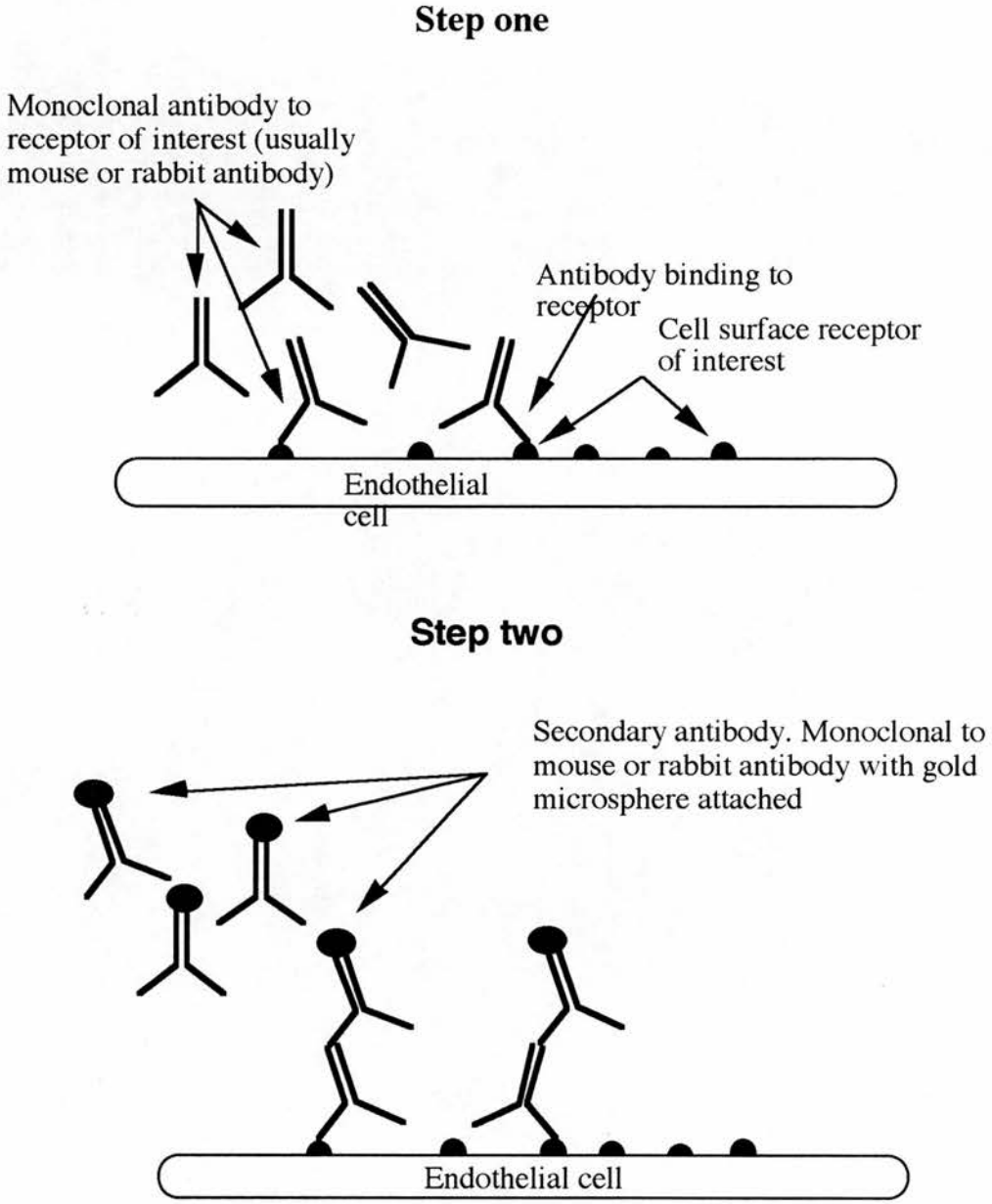
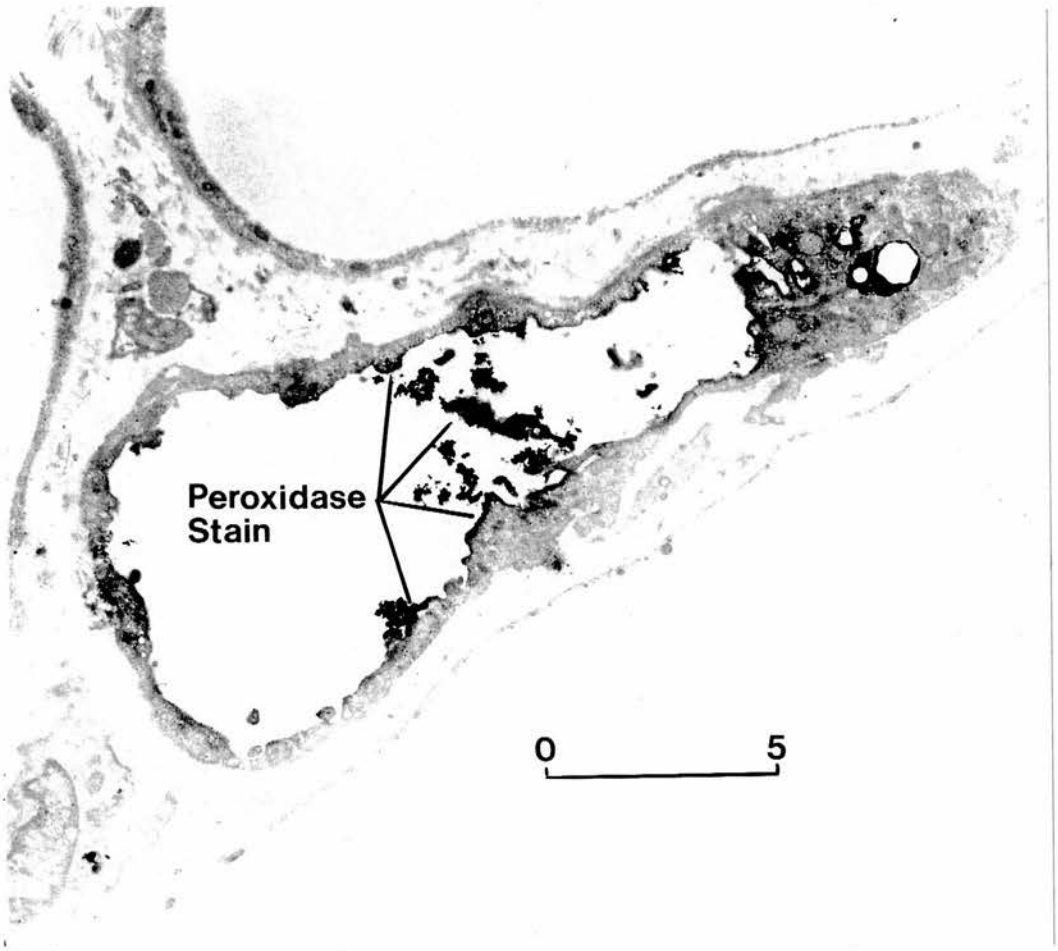


Figure 36 *Endothelial cell from adipose tissue stained by immunoperoxidase with primary antibody against vWF*



Chapter 8

Fibrin Sealant as a Topical Haemostatic Agent in Vascular Surgery: A Randomised Clinical Trial

Current knowledge of fibrin based adhesives and sealants

Fibrin based glues have several theoretical advantages over other tissue adhesives: tissue compatibility, biodegradability and the ability to adhere to wet surfaces.[287] Fibrin sealant may be expected to be biocompatible as it is a naturally occurring human protein which is being employed at a site where it can normally be expected to be present. It may even promote healing since the first stage in normal wound healing is the production of a fibrin network into which fibroblasts and capillaries migrate. These expectations have been bourne out in various experiments.

Fibrin sealant is readily biodegradable via the physiological mechanism of fibrinolysis. Fibrin sealant injected subcutaneously in rats was partially lysed at 3 days and no traces of it could be found at 7 days.[288] When examined at 3-6 months following implantation there was no evidence of residual fibrin sealant in knitted dacron grafts which had been sealed with fibrin sealant, in ovine and canine models.[289] In comparison grafts sealed with cross-linked collagen all had residual sealant at 3-6 months. Likewise animal models of PTFE graft anastomoses and arteriotomies sealed with fibrin glue have shown that there is complete resorption after 2-4 weeks.[290,291]

Fibrin sealant does not excite an inflammatory response and does not cause late fibrosis. Pretreatment of Dacron arterial grafts with fibrin sealant does not reduce fibrous ingrowth and neovascularisation.[292] Canine femoral arteries, in which a suture line had been sealed with fibrin glue, revealed no fibrosis or foreign body reaction on examination after 3 weeks.[291] Similarly in a canine model of PTFE graft anastomoses sealed with fibrin glue, no inflammatory reaction was found when these were examined at times ranging from 1 day to 3 months.[290] Fibroblasts could be seen migrating into the fibrin glue patch within 2-3 weeks of surgery. In this study the use of oxidised cellulose gauze was often associated with the presence of

multinucleated giant cells. The high degree of biocompatibility indicated by these studies is supported in a number of other publications.[288, 293-295]

There is evidence from animal studies that not only does fibrin sealant not inhibit wound healing but that it may in fact improve wound healing. In a study of skin wounds in rats which were closed either by adhesive tape alone or by adhesive tape and fibrin sealant it was found that when examined after 8 days the wounds sealed with fibrin sealant were significantly stronger. Since at this stage all traces of the fibrin sealant had disappeared, the improvement in wound strength must be due to superior wound healing.[296]

Further evidence of biocompatibility is provided by animal experiments which have shown that the development of intra-abdominal adhesions is actually reduced by the use of fibrin sealant.[297-299]

Methods of Production

The fibrin sealant kit used in these studies was provided by the Scottish National Blood Transfusion Service. Human fibrinogen was manufactured at the SNBTS Protein Fractionation Centre from pooled donor plasma cryoprecipitate. Human thrombin preparation was supplied by the Centre Regional de Transfusion Sanguine, Lille (CRTS) France.

Many other methods of producing fibrin sealant have been described. These involve the extraction of fibrinogen from various sources such as single units of fresh frozen plasma,[300, 301] autologous blood[302-304] and pericardial blood.[305] These techniques evolved as a result of a ban of commercially prepared fibrinogen in the United States by the Food and Drug administration in 1978 which was introduced because of concerns about the risk of transmission of hepatitis in fibrinogen produced from pooled donor plasma.[306] Unfortunately these methods are time-consuming and cumbersome and the concentration of fibrinogen yielded is unpredictable and often sub-optimal.[296, 307]

In addition, these techniques do not solve all the perceived problems with donor plasma derived fibrin sealant as a source of thrombin is still required. If human thrombin is used then exposure to homologous blood products is not avoided and the use of bovine thrombin is associated with its own complications and hazards. Bovine thrombin is a foreign protein and has been shown to cause immunisation.[308] Such thrombin inhibitors cross react with human thrombin which may inhibit normal coagulation and thus cause a bleeding problem.[309-311]

It has been shown that the fibrinogen and thrombin content of the components is important in the properties of the final clot. Increasing the concentration of fibrinogen and thrombin increase the tensile strength of the clot.[296, 307] However

increasing the concentration beyond a certain level has detrimental effects. In vivo studies in adult male Wistar rats demonstrated wound strength at 8 days was reduced when glue was used that had very high concentrations of fibrinogen and thrombin.[296] The explanation for this effect is that fibrin clot produced using more concentrated thrombin and fibrinogen inhibits macrophage and neutrophil migration and may have similar effects on fibroblasts.[312, 313] It appears that the optimum level of fibrinogen is 29 - 39g/l and thrombin is 200 - 500 iu/ml. The fibrin sealant supplied by SNBTS has concentrations of thrombin and fibrinogen which fall within these ranges.[314]

Viral Inactivation

Concern has been expressed that viral transmission could occur as a result of fibrin sealant use. Theoretically virus remaining in the preparation could be adsorbed and retained by the patient. There are reports in the literature which implicate cryoprecipitated fibrinogen, administered intravenously, as a mechanism for the transmission of HIV-1 and Hepatitis C.[306, 315] Therefore the method of production has been designed and the plasma derived components are treated to minimise the risk of viral transmission. To date, studies have failed to show evidence of viral transmission in over one million patients treated throughout Europe with fibrin sealant prepared by current methods.[316]

All blood donations are screened for Hepatitis B surface antigen and antibody to Hepatitis C, Human Immunodeficiency Virus 1 and 2. The plasma is treated by terminal heat treatment at 80°C for 72 hours, sometimes called extreme or severe heat treatment. This treatment has an excellent safety record with no reported cases of viral transmission from Factor VIII or IX manufactured by this method.[317-321] Virus inactivation studies have been carried out on human fibrinogen manufactured by this method.[314] Studies using plasma inoculated by vaccinia, HIV1, and Semliki Forest virus (SLFV), which is a Togavirus belonging to the same family as Hepatitis C virus. These studies showed viral inactivation levels of >1.4 log10 reduction in vaccinia, >6.0 log10 reduction in HIV, and >5.2 log10 reduction in SLFV. This level of viral inactivation is similar to that achieved in the manufacture of factor VIII Z8 which has an excellent safety record.

Virus inactivation of the CRTS Human Thrombin is carried out by 6 hour incubation in a solvent/detergent mixture as developed by the New York Blood Centre (NYBC).[322] Virus inactivation studies of human thrombin manufactured by this method showed viral inactivation levels of >5.6 log10 with VSV and >4.8 log10

with SindBis virus. This level of viral inactivation is equivalent to that achieved in the production of NYBC antihemophilic factor which has an excellent safety record.[323, 324]

Clinical Experience of Fibrin Sealant

The first description of the use of modern fibrin sealant was in 1972 as a tissue adhesive for nerve repairs.[91] Since then it has been used in a wide variety of clinical settings: otolaryngology,[325] plastic surgery,[326-328] neurosurgery,[316,329,330] thoracic surgery,[294,295,331-334] hepatobiliary surgery,[335-337] pancreatic surgery,[338,339] surgery for abdominal trauma,[340-342] colorectal surgery,[343] paediatric surgery,[344] gynaecology,[345, 346] urology,[347] ophthalmology[348] and cardiac surgery.[293,349-354] One area in which it appears to be particularly valuable is in patients with defective haemostatic mechanisms, either congenital or secondary to anticoagulant therapy, who are undergoing otherwise minor surgery such as tooth extraction and circumcision.[355-359]

Cardiovascular Surgery

The possibilities for using fibrin glue in cardiovascular surgery were recognised by Spangler early on during its development.[92] Borst (1982) further developed and promoted the use of fibrin sealant in this field describing its use for sealing knitted grafts, puncture holes and suture lines in vascular anastomoses.[349, 350] These studies were not controlled trials but the authors concluded that fibrin sealant was a useful agent even in the presence of coagulopathy and/or heparinisation and they reported that it was most useful in sites difficult or impossible to approach, locations dangerous to suture and in arresting diffuse haemorrhage.

A similar study describing the use of fibrin sealant in paediatric cardiac surgery recommended its use for resealing Dacron conduits, complex reconstructions of pulmonary arteries, complex and multiple suture lines, Gore-tex patches in high pressure systems, bleeding near major coronary artery branches, bleeding from raw

areas of the heart at operation and all bleeding points that are difficult to control by conventional means.[354]

A randomised controlled trial of fibrin sealant in patients undergoing repeat cardiac surgery showed that, compared with conventional haemostatic techniques, fibrin sealant had a superior success rate in achieving haemostasis at the sternal edge within 5 minutes, and patients treated with fibrin sealant had lower operative blood loss and a decreased incidence of emergency resternotomy.[351]

Fibrin glue was reported, in a non-controlled study, as being useful in operations for acute aortic dissection, both for sealing the suture line of the anastomosis and for sticking dissected layers of aorta together to produce a stronger wall which held sutures more securely.[360] This is further supported by a controlled trial of patients with acute aortic dissection in which fibrin sealant was found to reduce peri-operative blood loss and overall mortality.[353] Some centres now use fibrin sealant routinely during operation for aortic dissection.[361]

Peripheral Vascular Surgery

There have been few studies of fibrin sealant in peripheral vascular surgery. In a randomised trial, using a canine model of peripheral vascular surgery with PTFE grafts, fibrin sealant was shown to be more effective than oxidised regenerated cellulose in controlling anastomotic bleeding.[290] Those cases treated with fibrin sealant achieved haemostasis more quickly, had lower operative blood loss and a lower incidence of groin haematomas. A similar controlled study using a canine model demonstrated that in cases where the vascular anastomoses was reinforced with fibrin sealant there was significantly less blood loss and that a higher pressure was required to burst the anastomosis.[291] The only clinical report of fibrin sealant

use in peripheral vascular surgery is a report of two cases in which fibrin sealant was used to reinforce the aortic stump during operation for aorto-duodenal fistula.[362] There no other studies of fibrin sealant in peripheral vascular surgery in humans but reports of its efficacy in cardiovascular surgery suggest that it should be effective in this setting.

Methods and materials

Fibrin sealant kit

The fibrin sealant kit used in these studies was provided by the Scottish National Blood Transfusion Service. Human fibrinogen was manufactured at the SNBTS Protein Fractionation Centre from pooled donor plasma cryoprecipitate. Human thrombin preparation was supplied by the Centre Regional de Transfusion Sanguine, Lille (CRTS) France.

The kit comprised 4 x 1ml syringes, a dual syringe applicator device (Figures 35 & 36) and four vials:-

1. Lyophilised human fibrinogen, 200mg, with factor XIII, 50 iu and fibrinectin

2. 7ml solution of 20 mmol/l Tris containing aprotinin, 21,000 kallikrein inactivator units. For reconstitution of fibrinogen

3. Lyophilised human thrombin, 1,000 NIH units

4. 10ml solution of 40 mmol/l calcium chloride. For reconstitution of thrombin.

The fibrin sealant was prepared at the start of operation. The fibrinogen was reconstituted using 5ml of the Tris/aprotinin solution and the thrombin was reconstituted using 5ml of calcium chloride solution. The syringes and dual syringe applicator device were supplied in sterile packaging. This was opened in an aseptic technique onto the sterile surgical field. The scrub nurse then drew up 1 ml of each solution into separate 1ml syringes. The syringes were placed in the dual applicator and a mixing devices was attached to the ends of the syringes. A blunt ended 18

gauge needle was attached to the mixing device (Figure 37 & 38). The complete system was kept in the sterile field at room temperature until required.

Figure 37 *Dual syringe applicator*

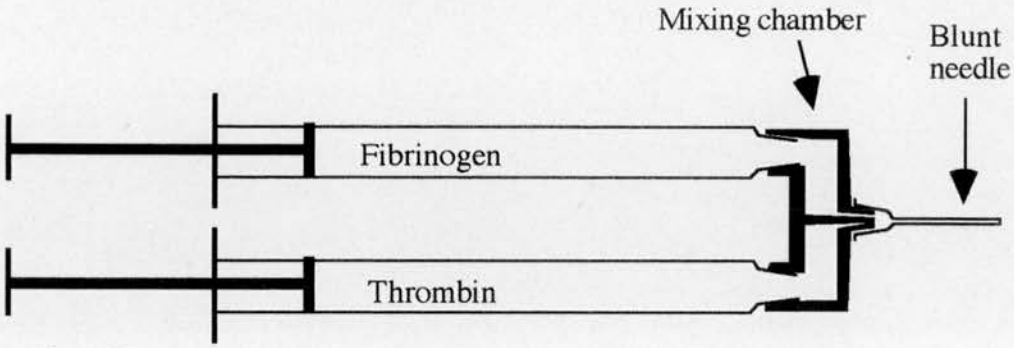
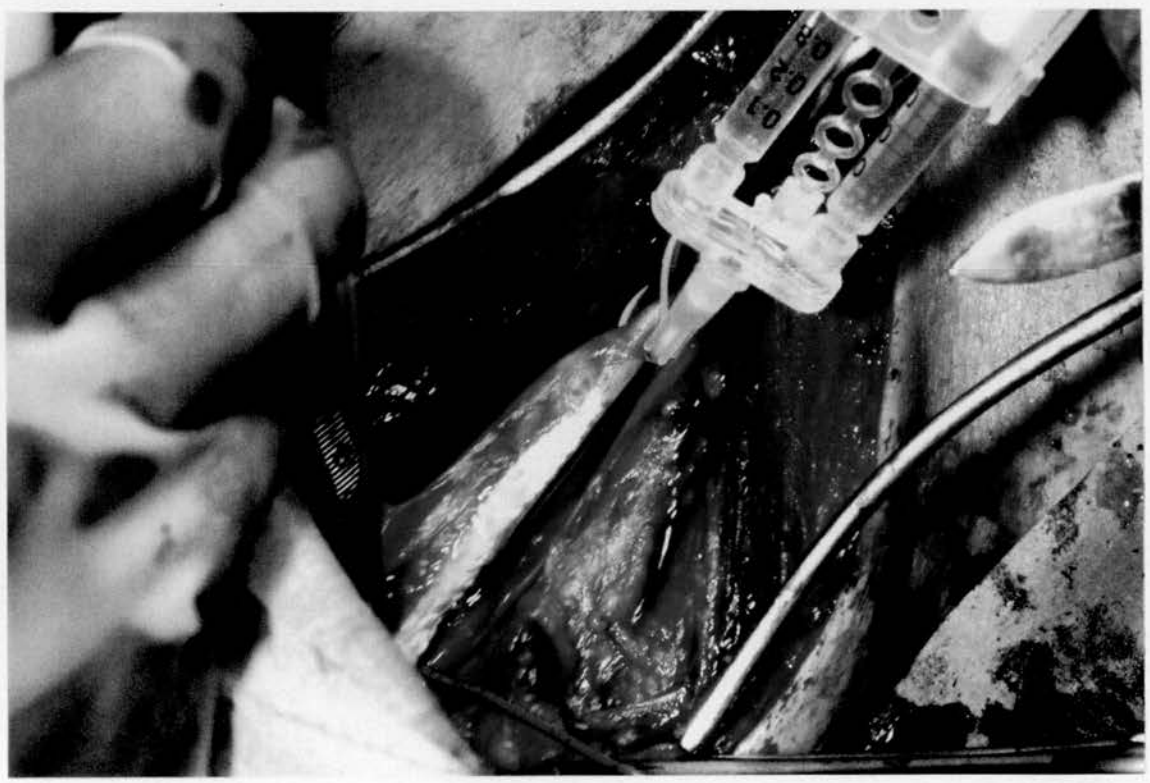


Figure 38 *Applying fibrin sealant to the suture line of a PTFE patch in internal carotid artery using dual syringe applicator*



Study design

The study was a prospective, randomised, non-blinded trial. An initial pilot study involving 8 patients was carried out in order to familiarise surgeons and theatre staff with the use of fibrin sealant prior to the commencement of the trial.

Patients were recruited from the vascular surgery unit of the Royal Infirmary of Edinburgh. Three groups of patients were recruited:-

(1) Patients undergoing carotid endarterectomy with arteriotomy closure using a polytetrafluoroethylene (PTFE) patch.

(2) Patients undergoing elective arterial bypass graft using a PTFE graft.

(3) Patients undergoing elective abdominal aortic aneurysm repair.

All patients gave informed written consent and the trial was approved by the local ethical committee. Past medical history and drug use within the previous 14 days was recorded. To allow assessment of the viral safety of the sealant patients who were known to be seropositive for anti-HIV, who had a history of hepatitis or whose liver function tests were outside the normal range were excluded. In addition, patients with a history of severe reactions to blood products or concurrent disease which might compromise their ability to be retained within the study were also excluded. A venous blood sample was taken for haemoglobin, haematocrit, platelet and white cell count, prothrombin time, activated partial thromboplastin time and liver function tests. A serum sample was stored at -40^{o} C for later viral studies if required.

Following entry into the trial the patients were randomised in a computer generated sequence to treatment or control. In the treatment group, following completion of the vascular anastomosis, fibrin sealant was applied to the suture line using a dual

syringe technique.[93] The clamps were released two minutes after application was complete. In the control group nothing was applied to the suture line. The time taken to achieve haemostasis from release of the clamps was recorded. Surgical treatment was otherwise identical in the two groups.

In both groups any further manoeuvres, such as application of pressure, haemostatic gauze or reversal of heparin, required to aid haemostasis were recorded. The length of operation, blood loss and blood product usage were recorded.

Patients were followed-up for 26 weeks. Blood was taken at 24 hours and 5 days for haemoglobin, haematocrit, platelet count and white cell count, and at 6 weeks and 12 weeks for liver function tests and at 26 weeks for virology testing.

Carotid endarterectomy

Patients and operative details

The mean age of patients was 64.5 years, range 48 - 75 years and there was no difference in mean age between the patients in the treatment and control groups. Fifteen patients were taking regular aspirin and both the patients not taking aspirin were in the control group. No patients had any abnormality on pre-operative coagulation screen.

Operation was carried out under general anaesthetic in all cases. Heparin 5,000 iu was given by intravenous bolus before the carotid artery was clamped. The heparin was not reversed. Arteriotomy closure was performed using a 0.5 mm PTFE patch (WL Gore) and 7.0 goretex suture.

After seventeen patients had been randomised (8 treatment and 9 control), both the surgeon and anaesthetist thought that there was a clinically significant reduction in bleeding in patients treated with fibrin sealant and requested that an interim analysis be performed with a view to stopping the trial. A statistical analysis was performed on the available data which confirmed the clinical impression and the randomisation of patients undergoing carotid endarterectomy was stopped at this point on ethical grounds.

Results

The time taken to achieve haemostasis was significantly shorter in the treatment group than the control group - $p < 0.005$ by Mann-Whitney test (Figure 39). There was no significant difference in total operative time. Operative blood loss was lower in the treatment group (median 420ml range 300-500ml) than in the control group (median 550ml range 350-1200ml) but this difference was not statistically significant (Figure 40).

In all patients (9/9) in the control group and in one patient (1/8) in the treatment group oxidised cellulose gauze was used to aid haemostasis. No patients required any blood products.

Complications

One patient in the control group suffered a perioperative cerebrovascular accident on the ipsilateral side to operation. He made a good recovery from this and was left with reduced co-ordination in the right hand but no loss of power. There was no evidence of arterial embolism or thrombosis in any patient in the treatment group. There were no wound complications in any patient in either group.

Figure 39 *Time to achieve haemostasis in carotid endarterectomy*

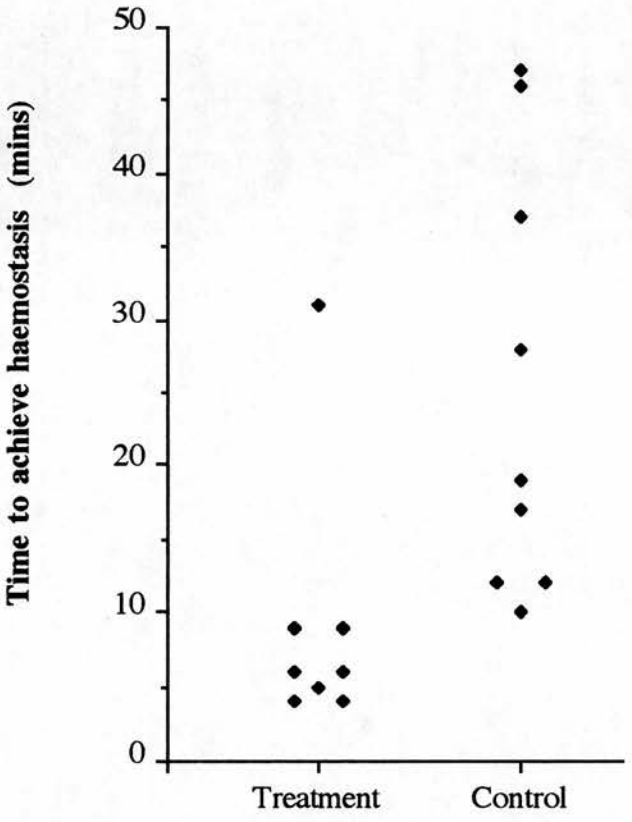
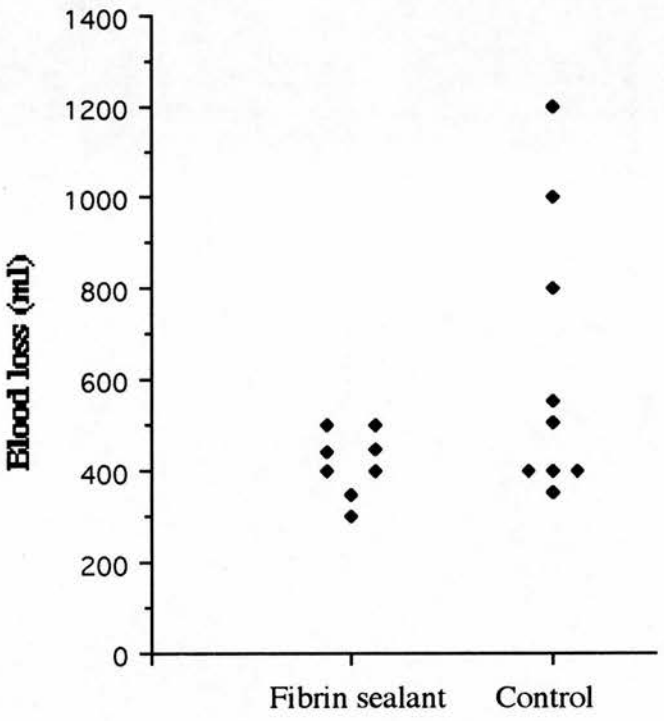


Figure 40 *Operative blood loss in carotid endarterectomy*



Patients and operative details

Twenty patients undergoing bypass grafting using PTFE bypass graft were randomised. The mean age of patients was 67.6 years, range 56 - 85 years and there was no difference in mean age between the patients in the treatment and control groups. Five patients in the control group and five in the treatment group were taking aspirin prior to operation. No patients had any abnormality on pre-operative coagulation screen. In all cases the anastomosis at the inflow end of the graft was observed. These were all performed as end-to-side anastomoses.

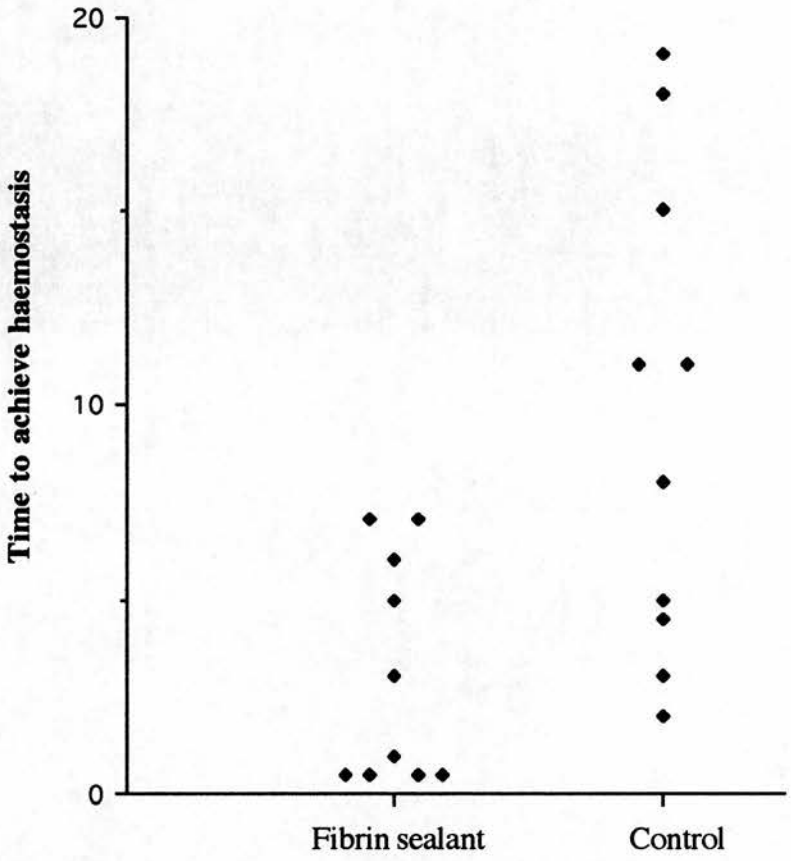
Heparin 5,000 iu was given by intravenous bolus before the artery was cross-clamped. The heparin was not reversed. 8mm and 10mm externally reinforced PTFE grafts (WL Gore) were used and the anastomoses were performed using 5.0 goretex suture.

Results

The times to achieve haemostasis in the control and treatment groups are shown in Figure 41. The median time taken to achieve haemostasis in the fibrin sealant group was 2 minutes compared with 9.5 minutes in the control group. This difference was statistically significant - $p = 0.012$ by Mann-Whitney. Haemostasis was achieved immediately on release of the clamps in 4/10 patients in the treatment group compared with 0/10 in the control group - $p = 0.14$ by Fisher exact test. There was no difference in overall operating time, blood loss or blood product requirement.

There were no embolic events in any patient but one patient in the control group suffered an early graft occlusion.

Figure 41 *Time taken to achieve haemostasis in PTFE bypass grafting*



Patients and operative details

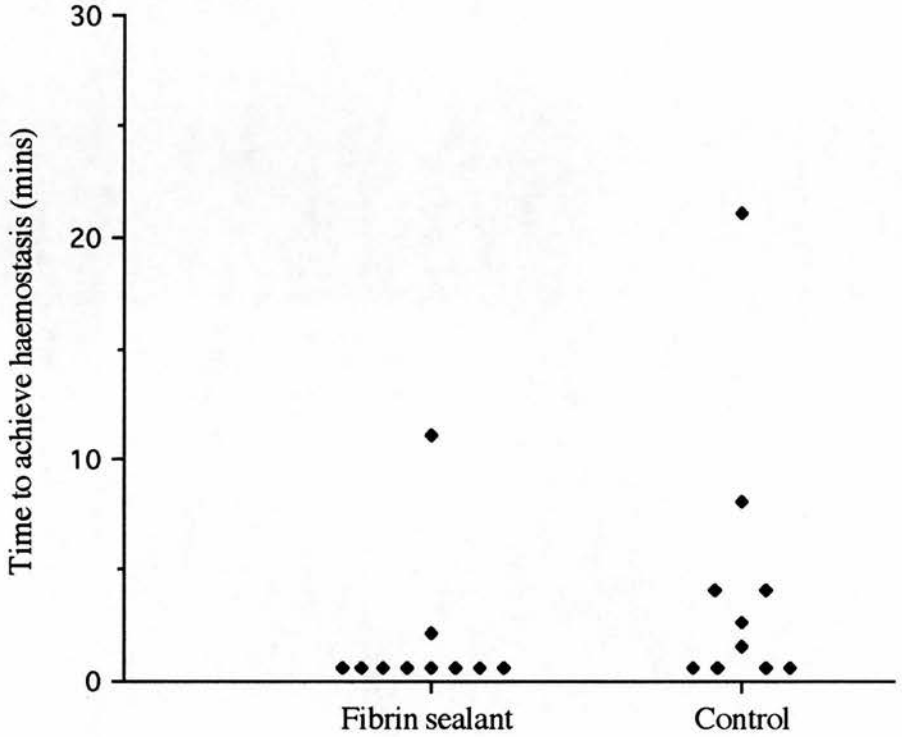
Twenty patients were randomised. The mean age of patients was 67.6 years, range 56 - 85 years and there was no difference in mean age between the patients in the treatment and control groups. Three patients in the control group and three in the treatment group were taking aspirin prior to operation. No patients had any abnormality on pre-operative coagulation screen.

Operation was carried out under general anaesthetic with thoracic epidural. Heparin 5,000 iu was given by intravenous bolus before the aorta was cross-clamped. The heparin was not reversed. Woven dacron aortic grafts (Vascutek) were used, and the anastomosis was performed with 5.0 Prolene sutures (Ethicon).

Results

The times to achieve haemostasis in the control and treatment groups are shown in Figure 42. The time taken to achieve haemostasis in the fibrin sealant group was shorter but this difference did not reach statistical significance - $p = 0.08$ by Mann-Whitney. Haemostasis was achieved immediately on release of the clamps in 8/10 patients in the treatment group compared with 4/10 in the control group - $p = 0.08$ by Fisher exact test. There was no difference in overall operating time, blood loss or blood product requirement.

Figure 42 *Time taken to achieve haemostasis in aortic aneurysm repair*



Complications

There were three major complications:-

The first patient was in the treatment group. He developed a coagulopathy post operatively and required laparotomy in the early post-operative period for control of haemorrhage. At laparotomy there was bleeding from multiple sites including both the treated proximal anastomosis and the untreated distal anastomosis. He made a good recovery from this and was almost ready for discharge when he suffered a severe cerebrovascular accident 10 days post-operatively. He made no recovery and died 24 hours later.

The second case was a patient in the treatment group. He underwent an uncomplicated aortic aneurysm repair but was found to be paraplegic on awaking from the anaesthetic. This is a well recognised but uncommon and unpredictable complication of aortic surgery thought to be due to an abnormal pattern of arterial supply to the spinal cord which is disrupted during operation.[238, 363-367]

The third patient was in the control group. This patient underwent uncomplicated aneurysm repair and was making good recovery when he suffered a fatal myocardial infarction 48 hours after operation.

No other patient suffered from any thrombo-embolic complication or wound infection.

Results of virology tests

Follow-up was completed on 43 patients, 24 in the treatment group and 21 in the control group. At time of entry to the trial 39/43 patients had antibodies to Hepatitis A, 2/43 to Hepatitis B core antigen, 2/43 to Hepatitis B surface antigen, 18/43 to parvovirus B19 and none had antibodies to Hepatitis C virus. No patient had Hepatitis B surface antigen present at any time. Only one seroconversion occurred; a patient in the treatment group developed antibodies to parvovirus B19 at 26 weeks. This patient received no other blood products between time of entry to the study and 26 weeks post-operatively.

Discussion

Fibrin sealant is not a new compound. It has been available for almost 20 years and there are over 1,000 publications on the Medline database concerning its clinical use.[368] However nearly all of these publications are anecdotal or small non-controlled studies. There are only three previous randomised controlled trials: one concerning its use in operation for aortic dissection,[353] another regarding it use in reducing bleeding from the sternal edge in patients undergoing sternotomy during re-do cardiac surgery[351] and the third concerning its use in hepatic resection.[336]

The present studies are significant for a number of reasons: They are the first studies of its clinical use in peripheral vascular surgery, they are randomised controlled trials, unlike nearly all other publications on this subject, and the patients were followed up for six months to determine viral safety.

These trials indicate that fibrin sealant is effective as a topical haemostatic agent in vascular surgery. This was shown in three different types of vascular anastomoses: PTFE patch closure, end-to-side anastomosis with PTFE bypass graft and end-to-end anastomosis from aorta to woven dacron graft. The benefit was greatest in the group of patients undergoing carotid endarterectomy with PTFE patch, not because fibrin sealant was more effective in this situation but rather because these patients have more prolonged bleeding than those undergoing aneurysm repair or bypass grafting and thus the benefit was greater.

The only statistically significant difference was in the shortening of the time taken to achieve haemostasis. The overall operating times were not significantly shorter. This is partly due to the fact that the bleeding time is relatively short in terms of overall operating time and therefore a significant reduction in time to achieve haemostasis does not result in a significant difference in overall operating time. In

addition while waiting for bleeding to stop at one site the surgeon can be proceeding with the operation at another site. For example during bypass grafting the surgeon may be performing the lower anastomosis while waiting for the upper anastomosis to stop bleeding.

The blood loss in the patients undergoing carotid endarterectomy was lower in the treatment group than in the control group but this was not statistically significant. It may be that if the trial had not been stopped this difference might have reached statistical significance. In the other two arms of the trial there was no difference in the overall blood loss which was as expected. The amount of blood lost from anastomotic bleeding in aortic aneurysm repair and bypass grafting is small compared to the overall blood loss.

An important concern about using fibrin sealant at vascular anastomoses is that it might promote intravascular thrombosis leading to early graft occlusion or embolism. No evidence of this has been found in this study. In fact the only intraoperative thromboembolic event and the only early graft occlusion were in patients in the control group.

Another common concern is that fibrin sealant may cause transmission of viruses.[368] In vitro studies have shown that the method of production of fibrin sealant is effective in killing viruses present in the collected plasma. Other blood products produced using these methods have been found not to transmit viruses.[317-321, 323, 324] The follow-up studies in these patients did not show any evidence of viral transmission. Only one seroconversion occurred and that was to parvovirus B19. This virus is usually transmitted by means other than by transfusion of blood products. It is a common pathogen as shown by the fact that half the patients in this study had been infected at some time. Therefore this single seroconversion does not provide definite evidence of viral transmission.

The significant question which arises is whether the routine use of fibrin sealant should be recommended for peripheral vascular surgery. There was a clear benefit in terms of reducing anastomotic bleeding in carotid endarterectomy with PTFE patch. Prolonged bleeding is common during this procedure and the routine use of fibrin sealant is justified. However prolonged anastomotic bleeding is relatively uncommon in aortic aneurysm repair and PTFE bypass grafting and, although fibrin sealant is effective in reducing anastomotic bleeding in these procedures, its routine use cannot be justified.

In conclusion these studies indicate that:-

1. Fibrin sealant shortens the time required to achieve haemostasis at vascular anastomoses.

2. Fibrin sealant reduced operative blood loss during carotid endarterectomy but this was not statistically significant.

3. The use of fibrin sealant at vascular anastomoses is not complicated by intravascular thrombosis

4. The use of intraoperative topical fibrin sealant is not complicated by viral transmission.

Future studies

Future developments with fibrin sealant extend in two directions. The first is to use it in different clinical settings. The second is to develop new formulations and new methods of application in order to improve its efficacy and ease of use in established clinical settings.

Clinical situations in which fibrin sealant might be expected to be useful are those where there is commonly prolonged bleeding which is not easily controlled by conventional means. For example during aortic aneurysm surgery for rupture there is often prolonged bleeding from vascular anastomoses especially when a coagulopathy is present. Fibrin sealant might be expected to be effective in this situation as it has been shown to be effective in the presence of a coagulopathy, either congenital or secondary to warfarin therapy,[356, 358] and we have shown above that it reduces the time taken to achieve haemostasis at end to end aorto-dacron graft anastomoses. Unfortunately it would be difficult to conduct prospective randomised trials in these patients because of the emergency nature of the surgery and the difficulty in obtaining informed consent. Studies of fibrin sealant in this situations would probably have to be non-controlled.

The current formulation and method of application of fibrin sealant has several disadvantages. It takes 10-15 minutes to dissolve the fibrinogen and thus the sealant needs to be prepared in advance. This is not a problem in clinical trials as it is known before the start of operation whether fibrin sealant is to be used. However in normal clinical practice topical haemostatic agents are not commonly used on a routine basis as prolonged haemorrhage is uncommon. Topical haemostatic agents are more commonly used only after a bleeding problem arises and are therefore required immediately. A formulation in which fibrin sealant could be stored ready for immediate use would be a major improvement. Unfortunately in its current

formulation the fibrinogen component gels at room temperature, needing to be heated to above 37°C to return to solution, and it therefore could not easily be stored ready-made.

If a ready to use formulation were available then the indications for fibrin sealant in vascular surgery could be extended. For example, as stated above, prolonged anastomotic bleeding during bypass grafting is an uncommon occurrence and thus the routine use of fibrin sealant for all cases is not justified. When it does occur the currently available fibrin sealant is of little use as it takes at least 15 minutes to prepare, by which time the bleeding would have almost stopped. A fibrin sealant available in the operating theatre, ready for use, would be very useful for these situations.

A second disadvantage with the current formulation of fibrin sealant is that on release of the clamps there is a tendency for the fibrin sealant to be "blown off" the patch by the pressure of blood within the lumen. Combining the sealant with something to hold it in place might be useful. The use of collagen fleece in combination with fibrin sealant has been reported in cardiac surgery.[354] Since completing the carotid endarterectomy arm of the trial we have used fibrin sealant in combination with collagen fleece for carotid endarterectomy on four occasions. This limited experience has suggested that fibrin sealant in this formulation is more effective but a clinical trial would be required to prove this.

Another advantage of combination with collagen fleece is that by holding this fibrin sealant in place it is more likely to be effective in situations where there is active bleeding. The use of fibrin sealant at vascular anastomoses, in comparison with its use other sites, is easier because bleeding at the anastomosis can be stopped by clamping the relevant vessels while the sealant is setting. Where there is active bleeding the components of the sealant may be washed away before a strong fibrin

network is formed.

The development of a fibrinogen impregnated collagen fleece with a long shelf-life might solve both problems. The main problems to be overcome are the development of a formulation of fibrinogen which stays in solution at room temperature with a useful shelf-life and a means by which to sterilise fibrinogen in solution without degrading it. Any new formulation would require a randomised clinical trial to determine how well it performed in relation to current fibrin sealant and, as shown by the above studies, patients undergoing peripheral vascular surgery would be suitable for this purpose.

A further possible application for fibrin sealant is in the treatment of prosthetic graft infections. In current practice graft infection requires the removal of the graft which is commonly followed by amputation. Antibiotic treatment is unable to fully eradicate infection in the presence of a foreign body and replacement of the graft invariably results in recurrent infection. In animal studies a fibrin glue - antibiotic suspension has been shown to be effective in the prevention of graft infection in a contaminated field.[369] This model does not exactly mirror the clinical situation as there was no initial infected graft to be removed, micro-organisms were added at the time of graft insertion and thus the graft was not inserted into a site with established infection. Furthermore the animals were sacrificed at day 17 post-operatively at which time there was evidence of continued infection on tissue culture although there was no gross infection. It may be that if this experiment was continued the animals would eventually develop graft infection. Despite these reservations fibrin glue - antibiotic suspension might be effective in the situation of graft infection. This will require further animal experimentation before trials in humans could be considered.

Chapter 9

Summary

Disturbance of the haemostatic system may be of paramount importance in the pathogenesis of many of the complications of aortic aneurysm surgery. The first and most obvious of these is the excessive bleeding often encountered during operations for ruptured aneurysm which is due to disseminated intravascular coagulation. As shown above post-operative haemorrhage is strongly associated with this coagulopathy and is found not only in patients undergoing operation for rupture but also in patients undergoing elective operations.

Excessive and inappropriate thrombosis may be as important as the bleeding tendency. In patients with ruptured aneurysms there is a significant morbidity and mortality associated with intraoperative lower limb arterial thrombosis. It seems likely that as well as the clinically apparent thrombosis in the lower limbs there is also occult thrombosis in various vital organs, such as kidney, lung and heart, which may lead to multiorgan failure and thus fatally complicate an otherwise technically successful operation.[370] There was an unexpectedly high incidence of thrombotic events such as myocardial infarction and stroke in the patients requiring re-operation for control of haemorrhage. It may be the case that these patients develop a pro-thrombotic tendency in the early post-operative period which is responsible for the high incidence of thrombotic events. Other authors have also reported a high incidence of thrombotic events in the early period following repair of a ruptured aortic aneurysm.[163,371,372]

Re-examining the hypothesis presented above in light of the findings presented in this thesis it is apparent that some refinements can be made. Contrary to earlier reports, there was little evidence of systemic activation of the soluble coagulation and fibrinolytic systems in patients with asymptomatic infrarenal aortic aneurysms. Levels of fibrinogen were at the upper end of the normal range although this might be expected in a group of patients with a high prevalence of atherosclerosis. In no

patient was there significant prolongation of either the PTR or APTT. It does not appear that there are significant pre-existing defects in the soluble coagulation system that might account for the high incidence of coagulopathy in patients undergoing elective aneurysm repair or suffering aneurysm rupture.

However, the presence of an aortic aneurysm appears to have a marked effect on platelets. Platelet counts in patients with aneurysms were low, although usually within the normal range. Levels of soluble glycocalicin were elevated suggesting either increased turnover of platelets or a chronic low level of activation or both. The fact that patients with carotid artery disease had high platelet counts and normal levels of glycocalicin suggests that the effect on platelets is related to the presence of an aneurysm rather than atherosclerosis.

The elevated levels of glycocalicin may represent activation of circulating platelets. It has been demonstrated in vitro that activation of platelets can lead to the generation of a protease which cleaves glycoprotein 1b (GP1b) close to the site of its insertion onto the platelet membrane. The soluble degradation product thus released is glycocalicin.[373] GP1b is central to the process of platelet adhesion at the site of vessel wall injury and thus vital for clot formation and haemostasis. It is therefore easy to envisage that platelets deficient in GP1b would be dysfunctional. Such loss of GP1b occurs during platelet storage in blood banks and has been proposed as the reason for the decreased functional ability of stored platelets.[373] Thus in these patients there may be a combination of dysfunctional platelets and a mild degree of thrombocytopenia which could significantly compromise the coagulation system. Haemostatic function in the resting state might be near normal but the threshold at which clinical bleeding problems occur would be much lower and there would be little reserve in the face of a major challenge to the haemostatic system such as aneurysm rupture or aortic operation.

There were elevated levels of markers of thrombin and platelet activation at time of admission in patients with ruptured aortic aneurysm. This was found in nearly all patients even those who were not hypotensive on admission and who did not develop a clinical bleeding problem. The more sophisticated markers of activation of platelets and the coagulation system did not predict outcome any better than the simple platelet count. It may be that the platelet count is a sensitive measure of the overall insult to the coagulation system and the patient in general.

There was a marked rise in markers of thrombin, plasmin and platelet activation, and a significant drop in platelet numbers by the end of operation. The relative contributions of overall duration of operation, duration of cross-clamping and reperfusion cannot be determined from these studies. It would be useful to examine the changes which occur in the haemostatic system during operation for rupture in isolation without the confusing effects of a variable degree of hypoxic shock prior to operation.

Unfortunately the studies of changes in the haemostatic system which occur during elective aortic repair, although interesting in their own right, were found to be a poor model for the events which occur during operation for rupture. For example, there was a marked rise in FPA and BTG during operation for ruptured aneurysm which was not found in patients undergoing elective operations. This difference may be attributable to the effect of systemic heparin. It was not anticipated during the design of these studies that heparin would have such a markeds effect.

However the time-dependent activation of platelets, thrombin and plasmin which occurred during the phase of initial dissection in elective operations was striking. It seems likely that this would have continued if heparin was not given and indeed this was seen in the patient with the contained rupture. The data from the patient with contained rupture indicated that there was steadily increasing activation of platelets

and thrombin throughout operation. In the elective patients there was evidence of platelet activation following reperfusion and this seemed to be related to the duration of cross-clamping. These findings support the hypothesis that the trauma of surgery, the hypoperfusion associated with cross-clamping and the effect of reperfusion of ischaemic tissue cause activation of the coagulation system which may contribute to the process leading to DIC.

As stated above the findings in patients undergoing elective aortic repair are interesting in their own right. If disturbance of the haemostatic system is important in much of the morbidity and mortality associated with aortic surgery then greater understanding of these events might quite quickly lead to new therapeutic strategies to improve the prognosis of patients undergoing aortic surgery. There are already a number of potent drugs available by which the haemostatic system can be manipulated but their use has developed empirically. For example, systemic heparin is given during elective aortic surgery in order to prevent arterial thrombosis during cross-clamping and it was thought that it increased bleeding problems. However a randomised study has shown that it has no effect on the rate of thrombotic episodes or operative blood loss but it did show that heparin reduced the rate of perioperative myocardial infarction.[258] The most significant conclusion from this study is that intraoperative manipulation of the haemostatic system can prevent complication in the early post-operative period and improve outcome. Greater understanding of the pathogenic mechanism of post-operative MI and its relationship to the haemostatic system might lead to the development of more effective means of manipulating the haemostatic system.

The means by which severe shock causes activation of the haemostatic system are unknown. Current knowledge obtained from cell culture models of the effects of hypoxia on endothelial cells indicates that there is a pathway by which hypoxic activation of endothelial cells could lead to DIC. However, there have been no

previous clinical studies of endothelial cells. The electron microscopic studies of endothelial cell are significant in that for the first time it has been shown that there are changes in endothelial cells of shocked patients. These ultrastructural changes are similar to those that have been shown to occur in animal models in response to shock. These responses are known to be associated with increased procoagulant activity.

These data supports the hypothesis that events in tissues rendered hypoxic secondary to hypovolaemic shock are responsible for triggering DIC but it is still a long way from providing proof and does provide details of the pathogenic pathway which leads to DIC. It is important to determine the functional changes in endothelial cell which might be done by immunoelectron microscopy but this will still fall short of proving the link. Definitive proof will require an animal model in which various steps of the hypothesis can be blocked to determine their significance and these findings would have to be confirmed by observational studies in humans.

Finally what significance do these findings have for the management of the individual patient ? The management of hypovolaemic shock is a difficult issue. The simple reversal of hypovolaemia at an early stage using intravenous colloid, crystalloid, red cell concentrate and blood components does not seem to be the answer. Many authors believe that the maintenance of a relatively hypovolaemic state until surgical control of haemorrhage is achieved improves outcome, not only in ruptured aneurysm but also in trauma.[374-376] A randomised study of patients admitted with bleeding peptic ulcers indicated that aggressive early resuscitation with blood transfusion was associated with worse outcome.[377] Some of these authors have proposed that hypovolaemic shock may in some way enhance haemostasis, either by direct haemodynamic effects or by effects on the haemostatic systems. These mechanisms are poorly understood. It is clear that at some stage hypovolaemic shock no longer enhances haemostasis but sets in train the events that

lead to disseminated intravascular coagulation with disastrous results. The early identification of these patients and the process which leads to DIC may be vital to improving their outcome. It seems likely that the same processes which in the early stages of hypovolaemic shock improve haemostasis may lead to DIC when the state of shock is sustained beyond a certain point. For example there were ultrastructural changes in endothelial cells and elevated levels of markers of platelet and thrombin activity at the time of admission in patients who underwent successful repair of ruptured aneurysm and who did not develop a clinical coagulopathy. There were similar changes in patients who developed a coagulopathy and died.

Manipulation of the haemostatic system by pharmacological means using drugs such as heparin, aprotinin, anti-thrombin III or hirudin has theoretical drawbacks. It may disturb physiological processes which are advantageous in some patients and may not even be efficacious in those patients who develop or are destined to develop a coagulopathy. A recent case report highlights the dangers of meddling with the haemostatic system in these patients (see appendix 1).[380]

One therapeutic modality which has few theoretical drawbacks and several attractive features is early platelet transfusion. It may be that patients with aneurysms have dysfunctional platelets prior to rupture and there is certainly evidence of platelet activation at an early stage following rupture. Furthermore thrombocytopenia appears to be one of the strongest predictors of poor outcome. A recent paper reporting improved results in the repair of thoracoabdominal aortic aneurysms apportioned much of the improvement to a policy of early transfusion of platelets preventing the onset of clinical coagulopathy.[378] A randomised trial of immediate platelet transfusion in patients admitted with ruptured aortic aneurysm could be justified.

Fibrin sealant may well prove to be useful as a haemostatic agent in patients

undergoing aortic surgery who develop a coagulopathy. It would be difficult to conduct randomised trials in this setting due to problems in getting informed consent. The randomised studies of its use in elective surgery indicate that it is an effective haemostatic agent at suture lines in dacron aortic grafts. It has been shown to be safe in that it does not produce thromboembolic events and that does not transmit viral infection. In elective surgery suture line bleeding is not commonly a problem and it is thus of limited use but in surgery for rupture suture line bleeding is a significant problem especially when a coagulopathy is present. Fibrin sealant has been shown in other studies to be effective in the presence of a coagulopathy.[356, 358] The major problem with the current fibrin sealant is the time taken in preparation, in particular the reconstitution of the fibrinogen component. A ready to use fibrin sealant available in theatre would be very useful although the impact on overall mortality is likely to be quite small.

References

1. Meade RH. An introduction to the history of general surgery. London: W.B. Saunders, 1968:105-21.
2. Brock RC. The life and work of Astley Cooper. Edinburgh: E&S Livingstone, 1952
3. McGuire H. Aneurysm of the external iliac of the left side, both common iliacs and lower end of aorta; ligation of the aorta - death. Am J Med Sci 1868;56:415.
4. Watson JH. Ligature of the abdominal aorta. Br Med J 1869;i:270.
5. Monteiro CB. Abdominal aorta tied (correspondence). Lancet 1842;i:334.
6. South. Very large aneurysm of the external and common iliac arteries, occupying a considerable portion of the right half of the abdomen; formidable operation of ligation of the abdominal aorta just above its bifurcation; survival of the patient 43 hours. Lancet 1856;ii:47.
7. Murray J. Ligature of the abdominal aorta. London Med Gaz 1834;14:68.
8. James JH. Case of aneurysm of the external iliac for which the femoral artery and subsequently the aorta were tied. Med Chir Tr 1830;16:1.
9. Editorial. Lancet 1828;2:607.
10. Milton H. Ligature of the abdominal aorta for ruptured aneurysm of that vessel - death. Lancet 1891;1:85.
11. Matas R. Ligation of the abdominal aorta: report of the ultimate result - one year five months and nine days after the ligation of the abdominal aorta at the bifurcation. Ann Surg 1925;81:457-64.
12. Matas R. Aneurysm of the abdominal aorta at its bifurcation into the common iliac arteries. Ann Surg 1940;112:909-22.
13. Elkin DC. Aneurysm of the abdominal aorta. Ann Surg 1940;112:895-906.

14. Brooks B, Blalock A, Johnson GS. Ligation of the terminal aorta; an experimental study. Arch Surg 1928;17:794.

15. Brooks B. Ligation of the aorta; a clinical and experimental study. JAMA 1926;87:722.

16. Velpeau AA. Memoire sur la figure de l'acupuncture des artes dans le traitment des aneurismes. Gaz Med (Paris) 1831;2:1.

17. Moore CH, Muschison C. On a method of procuring the consolidation of fibrin in certain incurable aneurysms. With the report of a case in which an aneurysm of the ascending aorta was treated by the insertion of wire. Med Chir Trans 1864;47:129.

18. Power D. The palliative treatment of aneurysm by "wiring" with Colt's apparatus. Br J Surg 1921;9:27-36.

19. Peacock JH, Brown GJA. Wiring in the treatment of aortic aneurysms. Br J Surg 1968;55:344-55.

20. Halsted WS. Clinical and experimental contributions to surgery of the thorax. In: Surgical papers. Baltimore: John Hopkins Press, 1924: 321. vol 1).

21. Biggar IA. The surgical treatment of aneurysm of the abdominal aorta. Review of the literature and report of two cases, one apparently successful. Ann Surg 1940;112:879-94.

22. Hufnagel CA. Preserved homologous arterial transplants. Bull Am Coll Surg 1947;32:321.

23. Dubost C, Allery M, Oeconemous N. Resection of aneurysm of abdominal aorta; re-establishment of continuity by a preserved human arterial graft, with result after 5 months. Arch Surg 1952;64:405.

24. Voorhees AB. The development of arterial prostheses. A personal view. Arch Surg 1985;120:289.

25. DeBakey ME, Cooley DA, Crawford ES, Morris GC. Clinical application of a new flexible knitted dacron arterial substitute. Arch Surg 1957;74:944.

26. Creech O. Endoaneurysmorraphy and treatment of aortic aneurysm. Ann Surg 1966;164:935.
27. Ratnoff OD. The evolution of knowledge about haemostasis. In: Disorders of haemostasis and thrombosis. 1986:
28. Hunter J. A treatise on the blood, inflammation, and gun-shot wounds. Philadelphia: Webster, 1817
29. Buchanan A. On the coagulation of the blood and other fibriniferous liquids. Lon Med Gaz 1845;1:617.
30. Mellanby J. The coagulation of blood. J Physiol 1909;38:28-112.
31. de Blainville HMD. Injection de matiere cerebrale dans les veins. Gazette Medicale de Paris 1834;2:524.
32. Schmidt. Zur blutlehre. Leipzig: Vogel, 1892
33. Donne A. De l'origine des globules du sang, de leur formation et leur fin. C R Acad Sci 1842;14:366-8.
34. Denys J. Etudes sur la coagulation du sang. Cellule 1886;3:445-6.
35. Osler W. An account of certain organisms occurring in the liquor sanguis. Proc Royal Soc 1874;22:391-8.
36. Hayem G. Sur le mechanism de l'arret des hemorrhages. C R Acad Sci 1882;95:18-21.
37. Morawitz P. The chemistry of blood coagulation. Ergebnise der Physiologie biologischen Chemi und experimentalle Pharmacologie 1905;4:307-422.
38. Woolridge LD. In: Horsley V, Starling E, ed. On the chemistry of the blood and other scientific papers. London: Trubner, 1893:
39. Mills CA. The action of tissue extracts in the coagulation of blood. J Biol Chem 1921;46:167-92.
40. Schneider CL. Fibrin embolism (disseminated intravascular coagulation) with defibrination as one of the end results during placenta abruptio. Surg Obstet Gynecol 1951;92:27-34.

41. Penick GD, Roberts HR, Webster WP, Brinkhouse KM. Hemorrhagic states secondary to intravascular clotting. Arch Pathol 1958;66:708-14.
42. DeLee JB. A case of fatal haemorrhagic diathesis with premature detachment of the placenta. Am J Obst Gynec 1901;44:785.
43. Williams JW. Premature separation of the normally implanted placenta. Surg Obst Gynecol 1915;21:541-54.
44. Dieckman WJ. Blood chemistry and renal function in abruptio placentae. Am J Obst Gyne 1936;31:734-44.
45. Obata I. J Immunol 1919;4:111.
46. Schneider CL. Am J Physiol 1947;149:123.
47. Fulton LD, Page EW. Proc Soc Exper Biol 1948;68:594.
48. Page EW, Fulton LD, Glendening MB. The cause of the blood coagulation defect following abruptio placentae. Am J Obs Gynae 1951;61:1116-22.
49. MacFarlane RG. Fibrinolysis following operation. Lancet 1937;i:10-2.
50. Dastre A. Fibrinolyse dans le sang. Arch de physiol norm et path 1893;5:661-3.
51. MacFarlane RG, Biggs R. Observations on fibrinolysis: Spontaneous activity associated with surgical operations, trauma, &c. Lancet 1946;2:862-4.
52. Christensen LR, McLeod CM. Proteolytic enzyme of serum: Characterisation, activation and reaction with inhibitors. J Gen Physiol 1945;28:559.
53. Friesen SR, Nelson RM. The occurrence of massive generalized wound bleeding during operation with reference to the possible role of blood transfusions in its etiology. Am Surgeon 1951;17:609-22.
54. Coon WC, Hodgson PE. Fibrinolysis in surgery patients. Possible relationship to a hemorrhagic diathesis. Surg Gynecol Obstet 1952;95:717-24.
55. Tagnon HJ, Levenson SM, Davidson CS, Taylor FHL. The occurrence of fibrinolysis in shock, with observations on the prothrombin time and the plasma fibrinogen during hemorrhagic shock. Am J Med Sci 1946;211:88-96.
56. Biggs R, MacFarlane RG, Pilling J. Observations on fibrinolysis; experimental

activity produced by exercise or adrenaline. Lancet 1947;i:402-5.

57. Zucker MB, Siegel M, Cliffton EE, Belleville JW, Howland WS, Grossi CE. Generalized excessive oozing in patients undergoing major surgery and receiving multiple blood transfusions. J Lab Clin Med 1962;50:849-61.

58. Bieger R, Vreeken J, Stibbe J, Loegliger EA. Arterial aneurysm as a cause of consumptive coagulopathy. N Eng J Med 1971;285:152-4.

59. Fine NL, Applebaum J, Elguezabal A, Castleman L. Multiple coagulation defects in association with dissecting aneurysm. Arch Int Med 1967;119:522-6.

60. Gibney EJ, Bouchier-Hayes D. Coagulopathy and abdominal aortic aneurysm. Eur J Vasc Surg 1990;4:557-62.

61. Krevans JR, Jackson DP. Hemorrhagic disorder following massive whole blood transfusions. JAMA 1955;159:171-7.

62. Phillips LL. Alterations in the blood clotting system in disseminated intravascular coagulation. Am J Card 1967;20:174-84.

63. Todd JS, Phillips LL. Fibrinolytic hemorrhage in surgical patients. Surg Obstet Gynecol 1962;114:333-40.

64. Schnetzer GW, Penner JA. Chronic intravascular coagulation syndrome associated with atherosclerotic aortic aneurysm. Southern Med J 1973;66:264-8.

65. Mulcare RJ, Royster TS, Weiss HJ, Phillips LL. Disseminated intravascular coagulation as a complication of abdominal aortic aneurysm repair. Ann Surg 1974;180:343-9.

66. ten Cate JW, Timmers H, Becker AE. Coagulopathy in ruptured or dissecting aortic aneurysm. Am J Surg 1975;59:171-6.

67. Kazmier FJ, Didischeim P, Fairbanks VJ. Intravascular coagulation and arterial disease. Thromb Diath Haemorrh 1969;suppl 36:295-303.

68. Siebert WT, Natelson EA. Chronic consumptive coagulopathy accompanying abdominal aortic aneurysm. Arch Surg 1976;111:539-41.

69. Diskin CJ, Weitberg AB. Minidose heparin therapy. Treatment of chronic intravascular coagulation syndrome. Arch Intern Med 1980;140:263-6.

70. Fousier LS, Morrow NE, Davis RB. Platelet dysfunction associated with abdominal aortic aneurysm. Am J Clin Path 1980;74:701-5.

71. Mukaiyama H, Shionoya S, Ikezawa T, Kamiya T, Hamaguchi M, Saito H. Abdominal aortic aneurysm complicated with chronic disseminated intravascular coagulation: a case of surgical treatment. J Vasc Surg 1987;6:600-4.

72. Thompson RW, Adams DH, Cohen JR, Mannick JA, Whittemore AD. Disseminated intravascular coagulation caused by abdominal aortic aneurysm. J Vasc Surg 1986;4:184-6.

73. MacNeily AE, Graham AM. Coagulopathy induced by aorto-iliac aneurysms. Can J Surg 1988;31:27-30.

74. Miyata T, Tada Y, Takagi A, Oshima A, Shirakawa M, Idezuki Y. Disseminated intravascular coagulation caused by abdominal aortic aneurysm. J Cardiovasc Surg 1988;29:494-7.

75. Goto H, Kimoto A, Kawaguchi H. Surgical treatment of abdominal aortic aneurysm complicated with chronic disseminated intravascular coagulation. J Cardiovasc Surg 1985;26:280-2.

76. Green PJ, Lecointe-Jones A, Majer R, Weir P, Day L. Idiopathic chronic disseminated intravascular coagulation. Blood Coag Fibrinol 1990;1:113-4.

77. Fisher D, Yawn D, Crawford E. Preoperative disseminated intravascular coagulation associated with aortic aneurysm. Arch Surg 1983;118:1252-5.

78. Friedman SG. A history of vascular surgery.New York: Futura, 1989:1-15.

79. Dale A. The beginnings of vascular surgery. Surgery 1974;76:849-66.

80. Zimmerman LM, Veith I. Great ideas in the history of surgery. Baltimore: Williams and Wilkie, 1961:179-92.

81. Horsley V. Note on haemostasis by application of living tissue. Br Med J

1914;ii:8.

82. Cushing H. The control of bleedimg in operation for brain tumors. Ann Surg 1911;:1-19.

83. Grey E. Fibrin as a haemostatic in cerebral surgery. Surg Gynecol Obstet 1915;21:452-4.

84. Cushing H, Bovie WT. Electrosurgery as an aid to the removal of intracranial tumours. Surg Gynec Obstet 1928;47:751-84.

85. Pearce JA. Electrosurgery. Cambridge: University press, 1986:1-15.

86. Caulk JR. Infiltration anesthesia of the internal vesical orifice for the removal of minor obstructions: presentation of a cautery punch. J Urol 1920;4:399-408.

87. Bumpus HC. Punch operation for prostatic obstruction. Surg Clin N Amer 1927;7:1473-8.

88. Bergel S. Uber wirkungen des fibrins. Dtsch Med Wochenschr 1909;35:633.

89. Tedrick R, Warner E. Fibrin fixation of skin transplants. Surgery 1944;15:90.

90. Cronkite E, Lozner E, Deaver J. Use of thrombin and fibrinogen in skin grafting. JAMA 1944;124:967.

91. Matras H, Dinges H, Lassman H, Mammoli B. Zur nahtlosen interfaszikularen nerventransplantation im tierexperiment. (Suture-free interfascicular nerve transplantation in animal experiments). Wien Med Wochenshr 1972;122:517-23.

92. Spangler H. Gewebeklebung und lokale blutstillung mit fibrinogen, thrombin und blutgerinnungsfaktor XIII. Wien Klin Wochenschr 1976;88:suppl 49:1-18.

93. Matras H. Fibrin seal: the state of the art. J Oral Maxillofac Surg 1985;43:605-11.

94. Liddington MI, Heather BP. The relationship between aortic diameter and body habitus. Eur J Vasc Surg 1992;6:89-92.

95. Lindholm L, Ejlertsson G, Forsberg R, Norgren L. Low prevalance of abdominal aortic aneurysm in hypertensive patients. Act Med Scan

1985;218:305-10.

96. Collins J, Araujo H, Sutton GLJ, Lindsell D. Oxford screening programme for abdominal aortic aneurysm in men aged 65 to 74 years. Lancet 1988;ii:613-5.

97. Editorial. Suggested standards for reporting on arterial aneurysms. J Vasc Surg 1991;13:444-50.

98. Ellis M, Powell JT, Greenhalgh RM. The limitations of ultrasound for the surveillance of abdominal aortic aneurysms. Br J Surg 1991;78:614-6.

99. Collin J, Walton J. Is abdominal aortic aneurysm a familial disease? Br Med J 1989;299:493.

100. Bengtsson H, Norrgård O, Angquist KA, et al. Ultrasonographic screening of the abdominal aorta among siblings of patients with abdominal aortic aneurysms. Br J Surg 1989;76:589-91.

101. Allardice JT, Allwright GJ, Wafula JMC, Wyatt AP. High prevalence of abdominal aortic aneurysm in men with peripheral vascular disease: screening by ultrasonography. Br J Surg 1988;75:240-42.

102. Galland RB, Simmons MJ, Torrie EPH. Prevalence of abdominal aortic aneurysm in patients with peripheral vascular disease. Br J Surg 1991;78.

103. Smith FC, Grimshaw GM, Paterson IS, Shearman CP, Hamer JD. Ultrasonographic screening for abdominal aortic aneurysm in an urban community. Br J Surg 1993;80:1406-9.

104. Akkersdijk GJM, Puylaert JBCM, de Vries AC. Abdominal aortic aneurysm as an incidental finding in abdominal ultrasonography. Br J Surg 1991;78:1261-3.

105. Armour RH. Survivors of ruptured abdominal aneurysms: the iceberg's tip. Br Med J 1977;ii:1055-7.

106. Bickerstaff LK, Pairolero PC, Hollier LH, et al. Thoracic aortic aneurysms: A population based study. Surgery 1982;92:1103-8.

107. Ingoldby CJH, Wujanto R, Mitchell JE. Impact of vascular surgery on community mortality from ruptured aortic aneurysms. Br J Surg 1986;73:551-

3.

108. Mealy K, Salman A. The true incidence of ruptured abdominal aortic aneurysms. Eur J Vasc Surg 1988;2:405-8.

109. Thomas PRS, Stewart RD. Abdominal aortic aneurysm. Br J Surg 1988;75:733-6.

110. Rantakokko V, Havia T, Inberg MV, Vanttinen E. Abdominal aortic aneurysms: a clinical and autopsy study of 408 patients. Acta Chir Scand 1983;149:151-5.

111. Johansson G, Swedenborg J. Ruptured abdominal aortic aneurysm: a study of incidence and mortality. Br J Surg 1986;73:101-3.

112. Drott C, Arfvidsson B, Ortenvall P, Lundhom K. Age standardized incidence of ruptured aortic aneurysms in a defined Swedish population between 1952-88. Br J Surg 1992;79:175-9.

113. Dent A, Kent SJS, Young TW. Ruptured aortic anerysm: What is the true mortality. Br J Surg 1986;73:551-3.

114. Budd JS, Finch DR. Management of abdominal aortic aneurysm. Br Med J 1988;297:484.

115. Bengtsson H, Bergqvist D. Ruptured abdominal aortic aneurysm: a population based study. J Vasc Surg 1993;18:74-80.

116. Pleumeekers HJCM, Hoes AW, van der Does E, Van Urk H, Grobbee DE. Epidemiology of abdominal aortic aneurysms. Eur J Vasc Surg 1994;8:119-28.

117. Office of Population. Mortality statistics. England and Wales.London: HMSO, 1984

118. Naylor AR, Webb J, Fowkes FGR, Ruckley CV. Trends in abdominal aortic aneurysm surgery in Scotland (1971-1984). Eur J Vasc Surg 1988;2:217-21.

119. Lilienfield DE, Gunderson PD, Sprafka JM. Epidemiology of aortic aneurysms: I. Mortality trends in the United States, 1951 to 1981. Arteriosclerosis 1987;7:637-43.

120. Castleden WM. Abdominal aortic aneurysms in Western Australia: descriptive epidemiology and patterns of rupture. Br J Surg 1986;73:551-3.

121. Fowkes FGR, Macintyre CCA, Ruckley CV. Increasing incidence of aortic aneurysms in England and Wales. Br Med J 1989;298:33-5.

122. Jenkins AML, Ruckley CV, Nolan B. Ruptured aortic aneurysm. Br J Surg 1986;73:395-8.

123. Samy AK, Whyte B, MacBain G. Abdominal aortic aneurysm in Scotland. Br J Surg 1994;81:1104-6.

124. Estes E. Abdominal aortic aneurysms: A study of 102 cases. Circulation 1950;29.

125. Delin A, Ohlsen H, Swedenborg J. Growth rate of abdominal aortic aneurysms as measured by computed tomography. Br J Surg 1985;72:530-532.

126. Bernstein EF, Dilley RB, Goldberger LE, Gosink BB, Leopold GR. Growth rate of small aneurysms. Surgery 1976;80:765-773.

127. Nevitt MP, Ballard DJ, Hallett JW. Prognosis of abdominal aortic aneurysms. N Engl J Med 1989;321:1009-14.

128. Darling RC. Ruptured arteriosclerotic abdominal aortic aneurysms. A pathologic and clinical study. Am J Surg 1970;119:397-40.

129. Darling RC, Massina CR, Brewster DC, Ottinger LW. Autopsy study of unoperated abdominal aortic aneurysms. The cause of early resection. Circulation 1977;56((suppl 2)):161-4.

130. Sterpetti AV, Cavallaro A, Cavallari N, Allegrucci P, Tamburelli A. Factors influencing the rupture of abdominal aortic aneurysms. Surg Gynecol Obstet 1991;173:175-8.

131. Inzoli F, Boschetti F, Zappa M, Longo T, Fumero R. Biomechanical factors in abdominal aortic aneurysm rupture. Eur J Vasc Surg 1993;7:667-74.

132. Sterpetti AV, Schultz RD, Feldhaus RJ, Cheng SE, Peetz DJJ. Factors influencing enlargement rate of small abdominal aortic aneurysms. J Surg Res

1987;43:211-9.

133. Guirguis EM, Barber GG. The natural history of abdominal aortic aneurysms. Am J Surg 1991;162:481-3.

134. Johansen G, Swedenborg J. Ruptured abdominal aortic aneurysms: A study of incidence. Br J Surg 1986;73:101-3.

135. Stonebridge PA, Callam MJ, Bradbury AW, Murie JA, Jenkins AMcL, Ruckley CV. Comparison of long-term survival following successful repair of ruptured and nonruptured abdominal aortic aneurysm. Br J Surg (in press) 1993;.

136. Szilagyi DE, Smith RF, DeRusso FJ, et al. Contribution of abdominal aortic aneurysmectomy to prolongation of life. Ann Surg 1966;164:678-9.

137. Johnston WJ. Ruptured abdominal aortic aneurysm: Six year follow-up results of a multicenter prospective study. J Vasc Surg 1994;19:888-900.

138. De Bakey ME, Crawford ES, Cooley DA, Morris GC, Royster TS, Abbott WP. Aneurysm of abdominal aorta. Analysis of results of graft replacement one to eleven years after operation. Ann Surg 1964;160:622-38.

139. Mannick JA, Brooks JW, Bosher LH, Hume DM. Ruptured aneurysm of the abdominal aorta. New Engl J Med 1964;271:915-20.

140. Kouchoukos NT, Levy JF, Butcher HR. Mortality from ruptured abdominal aortic aneurysm. Am J Surg 1967;113:232-5.

141. Hicks GL, Eastland MW, DeWeese JA, May AG, Rob CG. Survival improvement following aortic aneurysm resection. Ann Surg 1975;181:863-9.

142. Marsh CH. Experiences with abdominal aortic aneurysms in a district general hospital. Annals of the Royal College of Surgeons of England 1980;62:294-296.

143. Diehl JT, Cali RF, Hertzer NR, Beven EG. Complications of abdominal aortic reconstruction. Ann Surg 1983;197:49-56.

144. Fielding JWL, Black J, Ashton F, Slaney G. Ruptured aortic aneurysms:

postoperative complications and their aetiology. Br J Surg 1984;71:487-91.

145. Donaldson MC, Rosenberg JM, Bucknam CA. Factors affecting survival after ruptured aortic aneurysm. J Vasc Surg 1985;2:564-70.

146. Shackleton CR, Schechter MT, Bianco R, Hildebrand HD. Preoperative predictors of mortality risk in ruptured abdominal aortic aneurysm. J Vasc Surg 1987;6:583-9.

147. Amundsen S, Skjaervan R, Trippestad A, Soreide O. Abdominal aortic aneurysms - a study of factors influencing postoperative mortality. Eur J Vasc Surg 1989;3:405-9.

148. Murphy JL, Barber GG, McPhail NV, Scobie TK. Factors affecting survival after rupture of abdominal aortic aneurysms: effect of size on management and outcome. Canadian J Surg 1990;33:201-5.

149. Ouriel K, Geary K, Green RM, Fiore W, Geary JE, DeWeese JA. Factors determining survival after ruptured aortic aneurysm: the hospital, the surgeon, and the patient. J Vasc Surg 1991;11:493-6.

150. Harris LM, Faggioli GL, Fiedler R, Curl GR, Ricotta JJ. Ruptured abdominal aortic aneurysms: factors affecting mortality rate. J Vasc Surg 1991;14:819-20.

151. Johansen K, Kohler TR, Nocholls SC, Zierler RE, Clowes AW, Kazmers A. Ruptured abdominal aortic aneurysm: the Harbourview experience. J Vasc Surg 1991;13:240-5.

152. Cohen JR, Birnbaum E, Kassan M, Wise L. Experience in managing 70 patients with ruptured abdominal aortic aneurysms. New York State Journal of Medicine 1991;91:97-100.

153. Gloviczki P, Pairolero PC, Mucha P, et al. Ruptured abdominal aortic aneurysms: repair should not be denied. J Vasc Surg 1992;15:851-9.

154. McCready RA, Siderys H, Pittman JN, et al. Ruptured abdominal aortic aneurysms in a private hospital: a decade's experience (1980-1989). Ann Vasc Surg 1993;7:225-8.

155. D'Angelo F, Vaghi M, Mattassi R, Bisetti P, Tacconi A. Changing trends in the outcome of urgent aneurysms surgery. A retrospective study on 170 patients treated in the years 1966-1990. J Cardiovasc Surg 1993;34:237-9.

156. Katz DJ, Stanley JC, Zelenock GB. Operative mortality rates for intact and ruptured aortic aneurysms in Michigan: an eleven-year statewide experience. J Vasc Surg 1994;19:804-15.

157. Callam MJ, Haiart D, Murie JA, Ruckley CV, Jenkins AML. Ruptured aortic aneurysm: A proposed classification. Br J Surg 1991;78:1126-9.

158. Soreide O, Grimsgaard C, Myhre HO, Solheim K, Trippestad A. Time and cause of death for 301 patients operated on for abdominal aortic aneurysms. Age and Ageing 1982;11:256-60.

159. Bauer EP, Redaelli C, von Segesser LK, Turina MI. Ruptured abdominal aortic aneurysms: predictors for early complications and death. Surgery 1993;114:31-5.

160. AbuRahma AF, Woodruff BA, Luncente FC, Stuart SP, Boland JP. Factors affecting survival of patients with ruptured abdominal aortic aneurysm in a West Virginia community. Surg Gynecol Obstet 1991;172:377-82.

161. Tromp Meesters RC, van der Gaaf Y, Vos A, Eikelboom BC. Ruptured aortic aneurysm: early post-operative prediction of mortaity using an organ system failure score. Br J Surg 1994;81:512-6.

162. Davies MJ, Murphy WG, Murie JA, et al. Preoperative coagulopathy in ruptured abdominal aortic aneurysms predicts poor outcome. Br J Surg 1993;80:974-6.

163. Bradbury AW, Bachoo P, Milne AA, Duncan JL. Platelet count and the outcome of operation for ruptured abdominal aortic aneurysm. J Vasc Surg 1995;21:484-91.

164. Spero JA, Lewis JH, Hasiba U. Disseminated intravascular coagulation: findings in 346 patients. Thromb Haemost 1980;43:28.

165. Schaffner W, McLeod AC, Koenig MG. Thrombocytopenic Rocky Mountain spotted fever. Arch Int Med 1965;116:857.

166. Gralnick HR, Bagley J, Abrell E. Heparin treatment for the hemorrhagic diathesis of acute promyelocytic leukemia. Am J Med 1972;52:167.

167. Editorial. Disseminated intravascular coagulation and head injury. Lancet 1982;:531.

168. Kearney TJ, Bent L, Grode M, Lee S, Hiatt JR, Shabot MM. Coagulopathy and catecholamines in severe head injury. J Trauma 1992;32:608-11.

169. Hutton RA, Warrell DA. Action of snake venom components on the haemostatic system. Blood Reviews 1993;7:176-89.

170. Broze GJ. The tissue factor pathway of coagulation: factor VII, tissue factor, and tissue factor pathway inhibitor. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, ed. Haemostasis and thrombosis. 3rd ed. Edinburgh: Churchill Livingstone, 1994: 349-78. vol 1).

171. Jackson C. Factor X. Prog Thromb Haem 1984;7:55-110.

172. Jesty J, Spencer AK, Nemerson Y. The mechanism of the activation of factor X. J Biol Chem 1974;249:5614-22.

173. Rao LV, Rappaport SI. Activation of factor VII bound to tissue factor: a key early step in the tissue factor pathway of blood coagulation. Proc Natl Acad Sci USA 1988;85:6687-91.

174. Radcliffe P, Nemerson Y. Mechanism of activation of bovine factor VII. Products of cleavage by factor X. J Biol Chem 1976;251:4797-802.

175. Nakagaki T, Foster DC, Berkner K, Kisiel W. Initiation of the extrinsic pathway of blood coagulation: evidence for the tissue factor dependent autoactivation of human coagulation factor VII. Biochemistry 1991;30:10819-24.

176. Broze GJ. The lipoprotein-associated coagulation inhibitor that inhibits the factor VII-tissue factor complex also inhibits factor Xa: insight into its possible

mechanism of action. Blood 1988;71:335-43.

177. Rosing J, Tans G, Govers Riemslag JW. The role of phospholipids and factor Va in the prothrombinase complex. J Biol Chem 1980;255:274-283.

178. van Diejen G, Tans G, Rosing J, Hemker HC. The role of phospholipid and FVIIIa in the activation of bovine factor X. J Biol Chem 1981;256:3433-43.

179. Roth GJ. Developing relationships: Arterial platelet adhesion, glycoprotein Ib, and leucine rich glycoproteins. Blood 1991;77:5-19.

180. Roth GJ. Platelets and blood vessels: the adhesion event. Immunology Today 1992;13:100-5.

181. Shroit AJ, Zwaal RFA. Transbilayer movement of phospholipids in red cell and platelet membranes. Biochimica et Biophysica Acta 1991;1071:313-329.

182. Takagi T, Doolittle RF. Amino acid sequence studies on factor XIII and the peptide released during its activation by thrombin. Biochemistry 1974;13:750-6.

183. Chung SI. Kinetic studies with transglutaminases. J Biol Chem 1972;247:2798-2807.

184. Lorand L, Losowsky MS, Miloszewski KJM. Human factor XIII. Fibrin-stabilizing factor. Prog Thromb Haem 1980;5:245-90.

185. Pizzo SV. Serpin receptor 1: a hepatic receptor that mediates the clearance of antithrombin III-proteinase complexes. Am J Med 1989;87:3B.

186. Bauer KA, Rosenberg RD. Role of antithrombin III as a regulator of in vivo coagulation. Sem Haematol 1991;28:10-18.

187. Levin EG, Marzec U, Anderson J, Harker LA. Thrombin stimulates tissue plasminogen activator release from cultured human endothelial cells. J Clin Invest 1984;74:1988-95.

188. Collen D. On the regulation and control of fibrinolysis. Thromb Haemost 1980;43:77-89.

189. Bajaj MS, Kuppswamy MN, Saito H, Spitzer SG, Bajaj SP. Cultured normal

human hepatocytes do not synthesize lipoprotein associated coagulation inhibitor: evidence that endothelium is the principal site of its synthesis. Proc Natl Acad Sci USA 1990;87:8869-73.

190. Weibel ER, Palade GE. New cytoplasmic components in arterial endothelia. J Cell Biol 1964;23:101-12.
191. Wagner DD. The Weibel-Palade body: the storage granule for von Willebrand factor and P-selectin. Thromb Haem 1993;70:105-10.
192. Bona R, Lee E, Rickles F. Tissue factor apoprotein: intracellular transport and expression in shed membrane vesicles. Thromb Res 1987;48:487-500.
193. Povlishock JT, Rosenblaum WI. Injury of brain microvessels with a helium-neon laser and evans blue can elicit local platelet aggregation without endothelial denudation. Arch Pathol Lab Med 1987;111:415-21.
194. Povlishock JT, Rosenblaum WI, Sholley MM, Wei AP. An ultrastructural analysis of endothelial change paralleling platelet aggregation in a light/dye model of microvascular insult. Am J Pathol 1983;110:148-60.
195. Brinkman H-JM, Mertens K, Holthuis J, Zwart-Huinick LA, Grijm K, Van Mourik JA. The activation of human blood coagulation factor X on the surface of endothelial cells: a comparison with various vascular cells, platelets and monocytes. Br J Haem 1994;87:332-42.
196. Levi M, ten Cate H, van der Poll T, van Deventer SJ. Pathogenesis of disseminated intravascular coagulation in sepsis. JAMA 1993;270:975-9.
197. van Deventer SJH, Buller HR, ten Cate JW, Aarden LA, Hack CE, Sturk A. Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic and complement pathways. Blood 1990;76:2520-6.
198. Michie HR, Manogue KR, Spriggs DR, et al. Detection of circulating tumour necrosis factor after endotoxin administration. N Engl J Med 1988;318:1481-6.
199. van der Poll T, Buller HR, ten Cate H, et al. Activation of coagulation after administration of tumour necrosis factor to normal subjects. N Engl J Med

1990;322:1622-7.

200. Semeraro N, Colucci M. Changes in the coagulation-fibrinolysis balance of endothelial cells and mononuclear phagocytes: role in disseminated intravascular coagulation associated with infectious diseases. Int J Clin Lab Res 1992;21:214-20.
201. Conkling PR, Greenberg CS, Weinberg JB. Tumour necrosis factor induces tissue factor like activity in human leukaemia cell line U937 and peripheral blood monocytes. Blood 1988;72:128-33.
202. Rabiner SF, Friedman LH. The role of intravascular haemolysis and the reticulo-endothelial system in the production of a hypercoagulable state. Br J Haem 1968;14:105.
203. Conway EM, Rosenberg RD. Tumour necrosis factor suppresses transcription of the thrombomodulin gene in endothelial cells. Mol Cell Biol 1988;8:5588-92.
204. Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumour necrosis factor. J Exp Med 1986;163:740-5.
205. Taylor FB, Chang A, Esmon CT, D'Angelo A, Vigano-D'Angelo S, Blick KE. Protein C prevents the coagulopathic and lethal effects of *escherichia coli* infusion in the baboon. J Clin Invest 1987;79:918-25.
206. Takahashi H, Tatewaki W, Wada K, Niwano H, Shibita A. Fibrinolysis and fibrinogenolysis in disseminated intravascular coagulation. Thromb Haem 1990;63:340-4.
207. Davidson JF, McNicol GP, Frank GL. Plasminogen activator producing tumour. Br Med J 1969;1:88.
208. Bick RL. Disseminated intravascular coagulation. Hematology - Oncology clinics of North America 1992;6:1259-85.
209. Feinstein DI. Diagnosis and management of disseminated intravascular coagulation: the role of heparin therapy. Blood 1982;60:284-7.

210. Micallef-Eynaud PD, Ludlam CA. Aortic aneurysms and consumptive coagulopathy. Blood Coagulation & Fibrinolysis 1991;2(3):477-81.

211. Nyui S, Inoue S, Sato T, Nakase A. Repair of an aortoiliac aneurysm associated with chronic disseminated intravascular coagulopathy - a case report. Japanese J Surg 1991;21:110-3.

212. Blauhut B, Kramar H, Vinazzer H, Bergman H. Substitution of antithrombin III in shock and DIC: a randomized study. Thromb Res 1985;39:81-89.

213. Rubin RN, Colman RW. Disseminated intravascular coagulation. approach to treatment. Drugs 1992;44:963-71.

214. Schramm W. In: Marx R, Thies, ed. Klinische und ambulante anwendung klassicher antikoagulantien. Stuttgart: Schattauer, 1974: 139.

215. Bick RL. Clinical relevance of antithrombin III. Sem Throm Haem 1982;8(4):276-287.

216. Fourrier F, Chopin C, Huart JJ, Runge I, Caron C, Goudeman J. Double-blind, placebo-controlled trial of antithrombin III concentrates in septic shock with disseminated intravascular coagulation. Chest 1993;104:882-8.

217. Vinazzer H. Clinical use of antithrombin III. Vox Sang 1987;53:193-198.

218. Lane DA, Olds RJ, Thein SL. Antithrombin III and its deficiency. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, ed. Haemostasis and thrombosis. Edinburgh: Churchill Livingstone, 1994:

219. Okajima K, Imamura H, Koga S, Inoue M, Takatsuki K, Aoki N. Treatment of patients with disseminated intravascular coagulation by protein C. Am J Haematol 1990;33:277-8.

220. Maruyama I. Therapeutic strategy of newly developing medicines for disseminated intravascular coagulation--activated protein C and thrombomodulin. Japanese Journal of Clinical Medicin 1993;51:99-106.

221. Tsuzuki T, Toyama K, Nakayasu K, Iida S, Ueda M, Toizumi A. Disseminated intravascular coagulation after hepatic resection. Surgery 1990;107:172-6.

222. Gando S, Tedo I, Kubota M. Posttrauma coagulation and fibrinolysis. Critical Care Medicine 1992;20:594-600.

223. Taenaka N, Shimaa Y, Hirata T, et al. Gabexate mesilate (FOY) therapy of disseminated intravascular coagulation due to sepsis. Crit Care Med 1983;11:735-8.

224. Umeki S, Dachi M, Watanabe M, Yaji S, Soejima R. Gabexate as a therapy for disseminated intravascular coagulation. Arch Int Med 1988;148:1409-12.

225. Day KC, Hoffman LC, Palmier MO, et al. Recombinant lipoprotein associated coagulation inhibitor inhibits tissue thromboplastin induced intravascular coagulation in the rabbit. Blood 1990;76:1538-45.

226. Markwardt F. Past, present and future of hirudin. Haemostasis 1991;21 Suppl 1:11-26.

227. Mulcare R, Royster T, Phillips L. Intravascular coagulation in surgical procedures on the abdominal aorta. Surg Gynecol Obstet 1976;143:730-4.

228. Straub PW. Chronic intravascular coagulation: localized or generalized? With evidence for thrombus turnover. Thromb Haemost 1973;56(suppl):1-10.

229. Booth NA, Buckler PW, Dawson AA, Ah See AK, Bennett B. Haemorrhage associated with large abdominal aortic aneurysms. Clin Lab Haematol 1984;64:123-31.

230. Tromholt N, Jorgensen SJ, Hesse B, Hansen MS. *In vivo* demonstration of focal fibrinolytic activity in abdominal aortic aneurysms. Eur J Vasc Surg 1993;7:675-9.

231. Mashiah A, Thomson JM, Poller L, Charlesworth D. Changes in the coagulation of blood during resection of the abdominal aorta. Surg Gynecol Obstet 1979;149:214-6.

232. von Sommoggy S, Fraunhofer S, Wahba A, Blumel G, Maurer PC. Coagulation in aortofemoral bifurcation bypass grafting. Eur J Vasc Surg 1991;5:247-53.

233. Roumen RM, van der Vliet JA, Wevers RA, Goris RJ. Intestinal permeability is

increased after major vascular surgery. J Vasc Surg 1993;17:734-7.

234. Baigrie RJ, Lamont PM, Morris PJ. Portal endotoxin and cytokine responses to abdominal aortic surgery. Br J Surg 1992; 79:1235.

235. Paterson IS, Smith FC, Tsang GM, Hamer JD, Shearman CP. Reperfusion plasma contains a neutrophil activator. Ann Vasc Surg 1993;7:68-75.

236. Goldstone J. Aneurysms of the aorta and iliac arteries. In: Moore WS, ed. Vascular surgery. A comprehensive review. 3rd ed. London: WB Saunders, 1991: 315-6.

237. Mutirangura P, Stonebridge PA, Clason AE, et al. A ten year review of non-ruptured aortic aneurysms. Br J Surg 1989;76:1251-4.

238. Crawford ES. Symposium. Prevention of complications of abdominal aortic reconstruction. Introduction. Surgery 1983;93:91-6.

239. Slootmans FCW, van der Vliet JA, Reinarts HHM, van Roye SFS, Buskens FGM. Relaparotomies after ruptured abdominal aortic aneurysm repair. Eur J Vasc Surg 1994;8:342-5.

240. Valeri CR, Feingold H, Cassidy G, Rango G, Khuri S, Altschule M. Hypothermia induced reversible platelet dysfunction. Ann Surg 1987;205:175-81.

241. Kahn HA, Faust GR, Richard R, Tedesco R, Cohen JR. Hypothermia and bleeding during abdominal aortic aneurysm repair. Ann Vasc Surg 1994;8:6-9.

242. Gaffney PJ. FDP. Lancet 1972;ii:1422.

243. Gaffney PJ, Brasher M. Subunit structure of the plasmin induced degradation products of crosslinked fibrin. Biochimica et Biophysica Acta 1973;295:308-13.

244. Rylatt DB, Blake AS, Cottis LE. An immunoassay for human D dimer using monoclonal antibodies. Thromb Res 1983;31:767-78.

245. Beer JH, Buchi L, Steiner B. Glycocalicin: A new assay - the normal plasma levels and its potential usefulness in selected diseases. Blood 1994;83:691-702.

246. Bessos H, Murphy WG. A new competitive binding enzyme linked immunosorbent assay for glycocalicin in plasma and platelet concentrate supernatants. Thromb Res 1990;59:497.

247. Gershlick AH. Are there markers of the blood-vessel wall interactions and of thrombus formation that can be used clinically. Circulation 1990;81(suppl I):28-34.

248. Nossel HL, Younger LR, Wilner GD, Procupez T, Canfield RE, Butler VP. Radioimmunoassay of human fibrinopeptide A. Proc Natl Acad Sci 1971;68:2350.

249. Nossel HL, Yudelman I, Canfield RE, et al. Measurement of Fibrinopeptide A in human blood. J Clin Invest 1974;54:43-53.

250. Walz DA. Platelet released proteins as molecular markers for the activation process. Semin Thromb Haemost 1984;10:270-9.

251. Kaplan KL, Owen J. Plasma levels of β–thromboglobulin and platelet factor 4 as indices of platlet activation in vivo. Blood 1981;57:199-202.

252. Zahavi JL, Kakkar VV. β-Thromboglobulin - a specific marker of of in vivo platelet release reaction. Thrombo Haemostasis 1980;44:23-9.

253. Ludlam CA. Evidence for the platelet specificity of B-thromboglobulin and studis on its concentration in healthy individuals. Br J Haem 1979;41:271-8.

254. Dawes J, Smith RC, Pepper DS. The release, distribution and clearance of human B-thromboglobulin and platelet factor 4. Thromb Res 1978;12:851-81.

255. Ernst E. Fibrinogen: its emerging role as a cardiovascular risk factor. Angioology 1994;45:87-93.

256. Zahavi J, Price AJ, Westwick J, et al. Enhanced in-vivo platelet release reactions, increased thromboxane synthesis and decreased prostacyclin release after tourniquet ischaemia. Lancet 1980;2:663-7.

257. Utsunomiya T, Krausz MM, Dunham B, et al. Maintnenance of cardiodynamis with aspirin during abdominal aortic anerysmectomy (AAA). Ann Surg

1981;194:602-8.

258. Thompson J, Clyne CAC. Intraoperative heparinization reduces the risk of myocardial infarction during elective aortic surgery. Br J Surg 1993;81:614-5.

259. Dougherty TF, Dolowitz DA. Physiologic actions of heparin not related to blood clotting. Am J Card 1981;14:18-24.

260. Hobson RW, Wright JG, Fox BS, Kerr JC. Heparinization reduces endothelial permeability and hydrogen ion accumulation in a canine skeletal muscle ischaemia reperfusion model. J Vasc Surg 1988;7:585-91.

261. Dauber IM, Van Benthuysen KM, McMurtry IF, et al. Functional coronary microvascular injury evdent as increased permeability due to brief ischemia and reperfusion. Circ Res 1990;66:986-98.

262. Tsao PS, Aoki N, Lefer DJ, Johnson G, Lefer AM. Time course of endothelial dysfunction and myocardial injury during myocardial ischenia and reperfusion in the cat. Circulation 1990;82:1402-12.

263. Quillen JE, Sellke FW, Brooks LA, Harrison DG. Ischemia-reperfusion impairs endothelium-dependent relaxation of coronary microvessels but does not affect large arteries. Circulation 1990;82:586-94.

264. Radomski MW, Palmer RMJ, Moncada S. Comparative pharmacology of endothelium derived relaxing factor, nitric oxide and prostacyclin in platelets. Br J Pharmacol 1987;92:181-7.

265. Azuma H, Ishikawa M, Sekizaki S. Endothelial dependent inhibition of platelet aggregation. Br J Pharmocol 1986;88:441-5.

266. Sneddon JH, Vane JR. Endothelium-derived relaxing factor reduces platelet adhesion to bovine endothelial cells. Proc Natl Acad Sci USA 1988;85:2800-4.

267. Lindblad B, Bergqvist D, Wakefield TW, Stanley JC. Time-related anticoagulation after regional and systemic administration of heparin in patients undergoing aortiliac surgery. Eur J Vasc Surg 1994;8:574-7.

268. Getaz EP, Louw JH. The coagulopathy asociated with aortic aneurysm repair.

Postgraduate Med J 1977;53:668-71.

269. Takahashi H, Tatewaki W, Wada K, Hanao M, Shibita A. Thrombin vs plasmin generation in disseminated intravascular coagulation associated with various underlying disorders. Am J Haem 1990;33:90-95.

270. Amundsen S, Skjærven R, Trippestad A, Søreide O. Abdominal aortic aneurysms: Is there an association between surgical volume, surgical experience, hospital type and operative mortality. Acta Chir Scand 1990;156:323-8.

271. Taylor FB, Chang A, Morrissey JH, et al. Lethal E. coli septic shock is prevented by blocking tissue factor with monoclonal antibody. Circulatory Shock 1991;33:127-34.

272. Kuzu I, Bicknell R, Harris AL, Jones M, Gatter KC, Mason DY. Heterogeneity of vascular endothelial cells with relevanc to diagnosis of vascular tumours. J Clin Pathol 1992;45:143-8.

273. Armiger LC, Gavin JB. Changes in the microvasculature of ischemic and infarcted myocardium. Lab Invest 1975;33:51-6.

274. Kloner RA, Ganote CE, Jennings RB. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. J Clin Invest 1974;54:1496-508.

275. Kloner RA, Ellis SG, Lange R, Braunwald E. Studies of experimental coronary artery reperfusion. Effects on infarct size, myocardial function, biochemistry, ultrastructure and microvascular damage. Circulation 1983;68(suppl I):8-15.

276. Black PH. Shedding from the cell surface of normal and cancer cells. Adv Cancer Res 1980;32:75-199.

277. Hamilton KK, Hattori R, Esmon CT, Sims PJ. Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for the assembly of the prothrombinase enzyme complex. J Biol Chem 1990;265:3809-14.

278. Robinson RA, Worfolk L, Tracy PB. Endotoxin enhances the expression of

monocyte prothrombinase activity. Blood 1992;79:406-16.

279. Michiels C, Arnould T, Houbion A. Human endothelial cells submitted to hypoxia reoxygenation: implication of free radicals, xanthine oxidase and energy deficiency. J Cell Biol 1992;153:53-61.

280. Hammersen F, Hammersen E. The structural reaction pattern of endothelial cells to injurious stimuli. Agents and Actions 1983;13:442-50.

281. Forman MB, Puett DW, Bingham SE, et al. Preservation of endothelial cell structure and function by intracoronary prefluorochemical in a canine preparation of reperfusion. Lab Invest 1987;76:469-79.

282. Gertler JP, Welbe DA, Ocasio VH, Abbott WM. Hypoxia induces procoagulant activity in cultured human venous endothelium. J Vasc Surg 1991;13:428-33.

283. Shreeniwas R, Ogawa S, Cozzolino F. Macrovascular and microvascular endothelium during long-term hypoxia: alteration in cell growth, monolayer permeability, and cell surface coagulant function of cultured bovine endothelium. J cell Physiol 1991;146:8-17.

284. Ogawa S, Geriach H, Esposito C, Pasaglan-Macaulay A, Brett J, Stern D. Hypoxia modulates the barrier and coagulant function of cultured bovine endothelium. J Clin Invest 1990;85:1090-8.

285. Bradbury AW, Murie JA, Ruckley CV. The role of the leukocyte in the pathogenesis of vascular disease. Br J Surg 1993;80:1503-12.

286. Milici AJ, Porter GA. Lectin and immunolabeling of microvascular endothelia. J Electron Microscopy 1991;19:305-15.

287. Gibble JW, Ness PM. Fibrin Glue: The perfect operative sealant. Transfusion 1990;30(8):741-747.

288. Harris DM, Siedentop KH, Ham KR, Sanchez B. Autologous fibrin tissue adhesive biodegradation and systemic effects. Laryngoscope 1987;97:1141-4.

289. Jonas R, Schoen F, Ziemer G, Britton L, Castaneda A. Biological sealants and knitted dacron conduits: Comparison of collagen and fibrin glue pretreatments

in circulatory models. Ann Thorac Surg 1987;44:283-90.

290. Kram H, Nugent P, Reuben B, Shoemaker W. Fibrin glue sealing of polytetrofluoroethylene vascular graft anastamoses: comparison with oxidosed cellulose. J Vasc Surg 1988;8:563-8.

291. Jakob H, Campbell C, Qui Z, Pick R, Repogle R. Use of fibrin sealant for reinforcing arterial anastomoses. J Vasc Surg 1984;1:171-179.

292. Jonas R, Schoen F, Levy R, Castaneda A. Biological sealants and knitted dacron: porosity and histological comparisons of vascular graft materials with and without collagen and fibrin glue pretreatments. Ann Thorac Surg 1986;41:657-663.

293. Sponitz WD, Dalton MS, Baker JW, Nolan SP. Reduction of perioperative hemorrhage by anterior mediastinal spray application of fibrin glue during cardiac operations. Ann Thorac Surg 1987;44:529-31.

294. McCarthy PM, Trastek VF, Bell DG, et al. The effectiveness of fibrin glue for reducing experimental pulmonary air leak. Ann Thorac Surg 1988;45:203-5.

295. Goldman CD, Blocker SH, Ternberg JL, Crouch EC. Management of experimental pneumothorax in weanling rabbits with the use of fibrin glue sclerosant. Arch Surg 1986;121:565-8.

296. Byrne D, Hardy J, Wood R, McIntosh R, Cuschieri A. Effect of fibrin glues on the mechanical properties of healing wounds. Br J Surg 1991;78:841-3.

297. de Virgilio C, Dubrow T, Sheppard BB, et al. Fibrin glue inhibits intra-abdominal adhesion formation. Arch Surg 1990;125:1378-81.

298. Chmielewski GW, Saxe JM, Dulchavskey SA, Diebel LN, Bailey JK. Fibrin gel limits intra-abdominal adhesion formation. Am Surg 1992;58:592-3.

299. Sheppard BB, De Virgilio C, Bleiweis M, Milliken JC, Robertson JM. Inhibition of intra-abdominal adhesion: fibrin glue in a long term model. Am Surg 1993;59:786-90.

300. Dresdale A, Rose EA, Jeevanandam V, Reemtsma K, Bowman FO, Malm JR.

Preparation of fibrin glue from single-donor fresh-frozen plasma. Surgery 1985;97:750-4.

301. DePalma L, Criss VR, Luban NL. The preparation of fibrinogen concentrate for use as fibrin glue by our different methods. Transfusion 1993;33:717-20.
302. Siedentop KH, Harris DM, Sanchez B. Autologous fibrin tissue adhesive. Laryngoscope 1985;95:1074-6.
303. Kjaergard HK, Weis-Fogh US, Sorensen H, Thiis J, Rygg I. Autologous fibrin glue - a simple method of preparation by means of ethanol. Surg Gynecol Obstet 1992;175:72-3.
304. Weis-Fogh US. Fibrinogen prepared from small blood samples for autologous use in a tissue adhesive. Eur Surg Res 1988;20:381-9.
305. Kjaergard HK, Weis-Fogh US. Preparation of fibrin glue from pericardial bood. Ann Thorac Surg 1993;55:543-4.
306. Bove JR. Fibrinogen - Is it worth the risk? Transfusion 1978;19:129-36.
307. Siedentop KH, Harris DM, Sanchez B. Autologous fibrin tissue adhesive: factors influencing bonding power. Laryngoscope 1988;98:731-3.
308. Banninger H, Hardegger T, Tobler A, et al. Fibrin glue in surgery: frequent development of inhibitors of bovine thrombin and human factor V. Br J Haem 1993;85:528-32.
309. Zehnder JL, Leung LLK. Development of antibodies to thrombin and factor V with recurrent bleeding in a patient exposed to topical bovine thrombin. Blood 1990;76:2011-6.
310. Stricker RB, Lane PK, Leffert JD, Rodgers GM, Shuman MA, Corash L. Development of antithrombin antibodies following surgery in patients with prosthetic cardiac valves. Blood 1988;72:1375-80.
311. Rapaport SI, Zivelin A, Minow RA, Hunter CS, Donnelly K. Clinical significance of antibodies to bovine and human thrombin and factor V after surgical use of bovine thrombin. Am J Clin Path 1992;97:84-91.

312. Rotsein OD, Pruett TL, Simmons RL. Fibrin in peritonitis: V. fibrin inhibits phagocytic killing of Escherichia coli by human polymorphonulear leucocytes. Ann Surg 1986;203:413-9.

313. Ciano PS, Colvin RB, Dvorak AM, McDonagh J, Dvorak HF. Macrophage migration in fibrin gel networks. Lab Invest 1986;54:62-70.

314. Scottish national Blood Transfusion Service. Fibrin sealant kit. Investigators' brochure. SNBTS, 1993:

315. Wilson SM, Pell P, Donegan EA. HIV-1 transmission following the use of cryoprecipitated fibrinogen as a gel/adhesive. Transfusion 1991;31:515.

316. Cain JE, Dryer RF, Barton BR. Evaluation of dural closure techniques. Suture methods, fibrin adhesive sealant and cyanoacrylate polymer. Spine 1988;13:720-725.

317. Williams MD, Cohen BJ, Bedall AC, Pasi KJ, Hill FGH, Mortimer PP. Transmission of parvovirus B19 by clotting factor concentrates. Vox Sang 1990;58:177-81.

318. Skidmore SJ, Pasi KJ, Mawson SJ, Williams MD, Hill FGH. Serological evidence that dry heating of clotting factor concentrate prevents transmission of non-A, non-B hepatitis. J Med Virol 1990;30:50-2.

319. Pasi KJ, Hill FGH. Safety trial of heated British factor VIII concentrate (8Y). Arch Dis Childhood 1989;64:88-93.

320. Ludlam CA, Chapman D, Cohen B, Litton PA. Antibodies to Hepatitis C virus in haemophilia. Lancet 1989;2:560-1.

321. Evans JA, Pasi KJ, Williams MD, Hill FGH. Consitently normal CD4+, CD8+ levels in haemophiliac boys only treated with a virally safe factor VIII concentrate (BPL 8Y). Br J Haem 1991;79:457-461.

322. Prince AM, Horowitz B, Brotman B. Sterilisation of hepatitis and HTLV-III viruses by exposure to tri(n-butyl)phosphate and sodium cholate. Lancet 1986;1:706.

323. Noel L, Guerois C, Maisonneuve P, Verroust F, Laurian Y. Antibodies to hepatits C in haemophilia. Lancet 1989;1:560.

324. Horowitz MS, Rooks C, Horowitz B, W HM. Virus safety of solvent detergent treated antihaemophilic factor concentrate. Lancet 1988;2:186-8.

325. Staindl O. Tissue adhesion with highly concentrated human fibrinogen in otolaryngology. Ann Otol 1979;88:413-8.

326. Marchac D, Renier D. Fibrin glue in craniofacial surgery. J Craniofacial Surg 1990;1:32-4.

327. Vobel A, O'Grady K, Toriumi DM. Surgical tissue adhesives in facial plastic and reconstructive surgery. Facial Plastic Surg 1993;9:49-57.

328. Achauer BM, Miller SR, Lee TE. The hemostatic effect of fibrin glue on graft donor sites. J Burn Care Rehab 1994;15:24-8.

329. Pomeranz S, Constantini S, Umansky F. The use of fibrin sealant in cerebrospinal fluid leakage. Neurochirurgia 1991;34:166-9.

330. Lee KC, Park SK, Lee KS. Neurosurgical application of fibrin adhesive. Yonsei Med J 1991;32:53-7.

331. Mouritzen C, Dromer M, Keinecke HO. The effect of fibrin glueing to seal bronchial and alveolar leakages after pulmonary resections and decortications. Eur J Cardio-Thorac Surg 1993;7:75-80.

332. Thetter O. Fibrin adhesive and its application in thoracic surgery. Thorac Cardiovasc Surg 1981;29:290.

333. Jessen C, Sharma P. Use of fibrin glue in thoracic surgery. Ann Thorac Surg 1985;39:521-4.

334. Kjaergard HK, Weis-Fogh US. Autologous fibrin glue for sealing vascular prostheses of high porosity. Cardiovascular Surgery 1994;2:45-7.

335. Chisholm RA, Jones SN, Lees WR. Fibrin sealant as a plug for the post liver biopsy needle track. Clin Radiol 1989;40:627-8.

336. Kohno H, Nagasue N, Chang YC, Taniura H, Yamanoi A, Nakamura T.

Comparison of topical haemostatic agents in elective hepatic resection: a clinical prospective randomized trial. World J Surg 1992;16:966-9.

337. de la Garza JL, Rumsey E. Fibrin glue and hemostasis in liver trauma. J Trauma 1990;30:512-3.
338. Marczell AP, Stierer M. Partial pancreaticoduodenectomy (Whipple procedure) for pancreatic malignancy: occlusion of a non-anastomosed pancreatic stump with fibrin sealant. HPB Surg 1992;5:251-9.
339. Kram HB, Clark SR, Ocampo HP, Yamaguchi MA, Shoemaker WC. Fibrin glue sealing of pancreatic injuries, resections and anastomoses. Am J Surg 1991;161:479-81.
340. Kram HB, del Junco T, Clark SR, Ocqampo HP, Shoemaker WC. Techniques of splenic preservation using fibrin glue. J Trauma 1990;30:97-101.
341. Ochsner MG, Maniscalco-Theberge ME, Champion HR. Fibrin glue as a haemostatic agent in hepatic and splenic trauma. J Trauma 1990;30:884-7.
342. Salvino CK, Esposito TJ, Smith DK, et al. Laparoscopic injection of fibrin glue to arrest intraparenchymal abdominal hemorrhage: an experimental study. J Trauma 1993;35:762-6.
343. Hjortup A, Moesgaard F, Kjaergard J. Fibrin adhesive on the treatment of perineal fistulas. Dis Colon Rectum 1991;34:752-4.
344. Kinahan TJ, Johnson HW. Tisseel in hypospadias repair. Can J Surg 1992;35:75-7.
345. Feichtinger W, Barad D, Feinman M, Barg P. The use of fibrin sealant for embryo transfer. Fertility & Sterility 1990;54:733-4.
346. Adamyan LV, Myinbayev OA, Kulakov VI. Use of fibrin glue in obstetrics and gynaecology: a review of the literature. Internat J Fertility 1991;36:81-8.
347. Levinson AK, Swanson DA, Johnson DE, Greskovich FJ, Stephenson RA, Lichtiger B. Fibrin glue for partial nephrectomy. Urology 1991;38:314-6.
348. Mester U, Zuche M, Rauber M. Astigmatism after phacoemulsification with

posterior chamber lens implantation: small incision technique with fibrin adhesive for wound closure. J Cataract Refrac Surg 1993;19:616-9.

349. Borst HG, Haverich A, Walterbusch G, Maatz W. Fibrin adhesive: An important hemostatic adjunct in cardiovascular operations. J Thorac Cardiovasc Surg 1982;84:548-553.
350. Borst HG, Haverich A. Fibrin seal in cardiovascular surgery. Proceedings if a workshop. Thorac Cardiovasc Surg 1982;30:195-224.
351. Rousou J, Gonzalez-Lavin L, Cosgrove D, et al. Randomized clinical trial of fibrin sealant in patients undergoing resternotomy or reoperation after cardiac operations. J Thorac Cardiovasc Surg 1989;97:194-203.
352. Rousou JA, Engelman RM, Breyer RH. Fibrin glue: an effective hemostatic agent for non-suturable intraoperative bleeding. Ann Thorac Surg 1984;38:409-10.
353. Seguin JR, Frapier J-M, Colson P, Chaptal P. Fibrin sealant improves surgical results of type A acute aortic dissections. Ann Thorac Surg 1991;52:745-749.
354. Stark J, de Leval M. Experience with Fibrin Seal (Tisseel) in operations for congenital heart defects. Ann Thorac Surg 1984;38:411-3.
355. Raborn GW, Hohn FL, Grace MG, Arora BK. Tisseel, a two component fibrin tissue sealant system: report of a trial involving anticoagulated dental patients. Canadian Dental Assoc J 1990;56:779-81.
356. Rakocz M, Mazar A, Varon D, Spierer S, Blinder D, Martinowitz U. Dental extractions in patients with bleeding disorders. The use of fibrin glue. Oral surgery, oral medicine, oral pathology 1993;75:280-2.
357. Martinowitz U, Mazar AL, Taicher S, et al. Dental extraction for patients on oral anticoagulant therapy. Oral surg, oral med, oral path 1990;70:274-7.
358. Martinowitz U, Varon D, Jonas P, et al. Circumcision in haemophilia: the use of fibrin glue for local haemostasis. J Urol 1992;148:855-7.
359. Zusman SP, Lustig JP, Baston I. Postextraction hemostasis in patients on

anticoagulant therapy: the use of a fibrin sealant. Quintessence Int 1992;23:713-6.

360. Guilmet D, Bachet J, Goudot B, et al. Use of biological glue in acute aortic dissection. Preliminary clinical results with a new surgical technique. J Thorac Cardio Surg 1979;77.

361. Dottori V, Spagnolo S, Passerone G, et al. Ten years of surgery of aortic dissections and aneurysms. Minerva Cardioangiologica 1992;40:431-6.

362. Glimaker H, Bjorck CG, Hallstensson S, Ohlsen L, Westman B. Avoiding blow-out of the aortic stump by reinforcement with fibrin glue. A report of two cases. Eur J Vascr Surg 1993;7:346-8.

363. Szilagyi DE. A second look at the etiology of spinal cord damage in surgery of the abdominal aorta. J Vasc Surg 1993;17:1111-3.

364. Salam AA, Sholkamy SM, Chaikof EL. Spinal cord ischemia after abdominal aortic procedures: is previous colectomy a risk factor? J Vasc Surg 1993;17:1108-10.

365. Friedman SG, Moccio CG. Spinal cord ischemia following elective aortoiliac reconstruction. Ann Vasc Surg 1988;2:295-7.

366. Picone AL, Green RM, Ricotta JR, May AG, Deweese JA. Spinal cord ischaemia following operation on the abdominal aorta. J Vasc Surg 1986;3:94-103.

367. Szilagyi DE, Hageman JH, Smith RF, Elliott JP. Spinal cord damage in surgery of the abdominal aorta. Surgery 1978;83:38-56.

368. Cederholm-Williams SA. Fibrin glue. Br Med J 1994;308:1570.

369. Ney AL, Kelly PH, Tsukayama DT, Bubrick MP. Fibrin glue-antibiotic suspension in the prevention of prosthetic graft infection. J Trauma 1990;30:1000-6.

370. Milne AA, Murphy WG, Drummmond GB, Paterson DA, Ruckley CV. Disseminated intravascular coagulation following aortic aneurysm repair using

aprotinin and intra-operative autotransfusion. *Lancet* 1994;344:470-1.

371. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. New Engl J Med 1995;332:635-41.
372. Bradbury AW, Milne A, Bachoo P, Duncan JD. Regarding "The incidence of deep vein thrombosis in patients undergoing aortic aneurysm resection". J Vasc Surg 1994;20:318-9.
373. Fox JEB. Shedding of adhesion receptors from the surface of activated platelets. Blood Coag Fibrinol 1994;5:291-304.
374. Westaby S. Resuscitation in thoracic trauma. Br J Surg 1994;81:929-31.
375. Crawford ES. Ruptured abdominal aortic aneurysm: an editorial. J Vasc Surg 1991;13:348-50.
376. Assalia A, Schein M. Resuscitation for haemorrhagic shock. Br J Surg 1993;80:213.
377. Blair SD, Janvrin SB, McCollum CN, Greenhalgh RM. Effect of early blood transfusion on gastrointestinal haemorrhage. Br J Surg 1986;73:783-5.
378. Gilling-Smith GL, Worswick L, Knight PF, Wolfe JHN, Mansfield AO. Surgical repiar of thoracoabdominal aortic aneurysm: 10 years' experience. Br J Surg 1995;82:624-9.
379. Heyns A du P. Thrombopoiesis and platelet kinetics. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, ed. Haemostasis and thrombosis. Edinburgh: Churchill Livingstone, 1994:
380 Milne AA, Murphy WG, Drummond GB, Paterson DA, Ruckley CV. Disseminated intravascular coagulation following aortic aneurysm repair using aprotinin and intra-operative autotransfusion. Lancet 1994; 344: 470-1.

Appendix 1

Publications Arising from this Thesis.

Current PRACTICE IN SURGERY

Patient management

Complex surgical coagulopathies

A. A. Milne, M. J. Davies, W. G. Murphy

Introduction

Patients bleed at surgery. In most patients a combination of good surgical technique and physiological haemostatic mechanisms results in cessation of bleeding or in acceptable levels of haemostatic control. In some patients bleeding does not stop. These patients have one of two things: inadequate surgical haemostasis, or failure of physiological mechanisms. The distinction between these two should be emphasised. Surgical bleeding occurs when there is a large defect in a vessel wall that cannot be sealed by the haemostatic process but than can be corrected surgically. Vessel constriction may result in the apparent cessation of bleeding for a while but bleeding will resume when vasospasm ceases. The treatment of this bleeding is surgical: the placement of a ligature, under-running or electrocautery. In microvascular bleeding, due to failure of physiological haemostatic mechanisms, there is bleeding from a multitude of very small vessels. A previously dry operating field may start to ooze blood over a large area. The treatment of this sort of bleeding is to correct the underlying coagulopathy; re-operation may be disastrous. Identification of the cause of the coagulopathy and determining the best treatment can be difficult. Patients may have a combination of problems that include pre-existing compromised coagulation due to liver disease or aspirin therapy, dilutional coagulopathy due to massive blood loss, or disseminated intravascular coagulation due to head injury, sepsis or hypovolaemic shock. During a bleeding episode new factors may come into play and the relative contribution of established factors may alter. These complex surgical coagulopathies require a rational and pragmatic approach to diagnosis and management. Traditionally, abnormal results of routine laboratory tests such as low platelet count or prolonged clotting times have been seen as necessary to confirm the presence of a bleeding disorder. In surgical patients this can be inappropriate. A normal clotting screen does not contradict a clinical diagnosis of abnormal bleeding, and abnormal screening tests do not necessarily indicate the primary lesion in the haemostatic system.

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The formation of the haemostatic plug

Coagulation begins with the release of tissue factor from damaged cells and with the simultaneous exposure of subendothelial collagen to the plasma. Three interactive steps ensue: 1) Activation of platelets; 2) Generation of the enzyme thrombin; 3) Generation of the enzyme plasmin.

Activation of platelets

Once the endothelial lining of a vessel is disturbed platelets enter the breach. Dynamic flow characteristics of the moving stream of blood are important in this initial concentration of platelets into the endothelial gap. In particular the presence of an adequate concentration of red blood cells is necessary to promote localisation of platelets at the periphery of the blood flow.[1] At the injury site several biochemical mechanisms may operate to bind and activate the platelets (Fig. 1). Important among these mechanisms is the binding of platelets to von Willebrand factor (vWf). VWf is a soluble plasma protein that binds

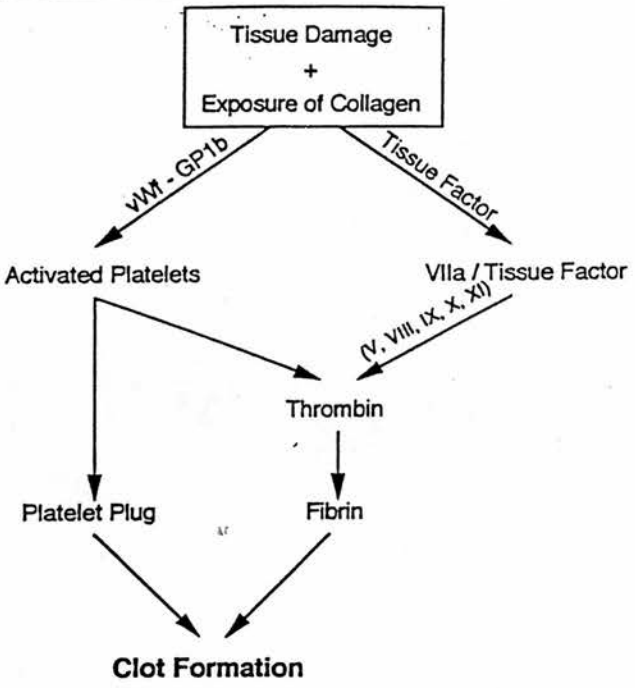

Fig. 1

in its native form to exposed collagen in the wound. This results in a conformational change in the vWf molecule, so that it now exposes previously hidden binding sites for platelets. Platelets entering the wound bind to the vWf molecule through an important receptor on the platelet membrane, glycoprotein (GP) Ib. This activates the platelets.[2] Activated platelets bind to collagen, and to each other, and promote the formation of a stable clot through activation of the clotting cascades. The initial localisation and activation of platelets can fail and a coagulopathy ensue if: 1) the collagen is abnormal (scurvy, Ehlers-Danlos syndrome); 2) the von Willebrand factor is absent or abnormal (von Willebrand's disease); 3) the platelets are reduced in number or function (thrombocytopenia from any cause, renal disease, aspirin therapy, other acquired or congenital platelet function defects including Dextran therapy and congenital absence of the GPIb receptor); 4) the haematocrit is critically reduced (below 20%).[1]

Laboratory testing of activation of platelets. There is no foolproof test of this extremely important step in the coagulation process, and routine clotting screens can miss serious defects. The bleeding time will usually, but not invariably be prolonged, and a platelet count will detect thrombocytopenia if it is present. The prothrombin time and activated partial thromboplastin time (APTT) are not affected by platelet function and may be entirely normal. However, in von Willebrand's disease there is an associated deficiency of factor VIII, which causes a prolonged APTT. Extended testing of platelet function may be necessary to identify the cause of a coagulopathy.

Generation of the enzyme thrombin

On the surface of the immobilised and activated platelets at the site of injury a series of self-amplifying steps takes place that results in the local generation of the serine protease thrombin. In addition to activated platelets and tissue factor from damaged cells, this cascade requires a series of circulating proenzymes (factors VII, X, XI, IX) to be activated sequentially and amplified, along with their necessary plasma cofactors (VIII & V). Deficiencies of these factors occur: they are well characterised but very uncommon, and unlikely to present *de novo* to the surgeon. Thrombin is pivotal in the clotting process: it amplifies its own formation from precursors, acts as a potent platelet activator, and generates insoluble fibrin from circulating fibrinogen.

Fibrin and fibrinogen. Fibrinogen is present in massive amounts in plasma. After albumin and the immunoglobulins, it is the most plentiful plasma protein. It has two major functions: in its soluble form it is the ligand through which platelets bind together; after proteolysis by thrombin it forms a dense and insoluble fibrin mesh on the platelet plug.

Laboratory testing of generation of thrombin. The pathways involved in the generation of the enzyme from its plasma precursors are tested in vitro by a combination of 3 or 4 tests: a prothrombin time which to be normal requires physiological amounts of factors VII, X, V and prothrombin; an activated partial thromboplastin time which requires physiological amounts of prothrombin, factors V, VIII, IX, X and XI, and the contact activating factors, and either a thrombin time or a plasma fibrinogen level to assess the amount of fibrinogen available. It has been customary to conceptualise the clotting process in terms of these tests: if the tests are normal then haemostasis must be intact, if the tests are abnormal then haemostasis must be abnormal. However, these tests are an artificial simulation of the clotting process: they use artificial substitutes for several factors that are essential for in vivo haemostasis such as activated platelets, von Willebrand factor, and collagen; they require an intact contact activation system to work in vitro, which humans do not. These tests should appropriately be used as a necessary aid to the formation of a diagnosis along with the rest of the clinical picture.

Generation of the enzyme plasmin

Plasmin is formed in the clot by the action of an enzyme (tissue plasminogen activator, released from damaged tissue) on a precursor in the plasma — plasminogen. Plasminogen is preferentially concentrated from the plasma into the forming clot, where after activation by tissue plasminogen activator it can cause maximum breakdown of formed fibrin while at the same time it is protected from the potent inhibitor in the plasma, alpha 2-antiplasmin. The plasmin mechanism serves to limit clot formation to the site of injury, where clotting activation is strong enough to overcome plasmin-induced clot breakdown.

Laboratory testing for plasmin generation. It is not usual to need to test for an intact fibrinolytic system, except in the investigation of a thrombotic tendency. Evidence

of plasmin activation is a useful diagnostic test for disseminated intravascular coagulation (DIC). Breakdown products of fibrin caused by excessive plasmin activation in DIC can be detected in the plasma. One of 2 tests, D-dimers or fibrin degradation products can be measured. D-dimers are more specific for DIC.

Diagnosis and management of coagulopathies

From a clinical viewpoint, coagulopathies fall into two main categories: those diagnosed surgery, and those presenting at or after surgery.

Preoperative diagnosis of coagulopathy

Diagnosis. In common with other clinical conditions, coagulopathy should be diagnosed by a sequence of history, physical examination and laboratory analysis. If conscious the patient should be asked specific questions.

Does the patient bleed or bruise abnormally?
Is there a family history of abnormal bleeding?
Has the patient taken aspirin within the previous week?
Has the patient had warfarin therapy?

Specific question should inquire about bleeding at previous surgery, or after dental extraction.

Physical signs of liver disease or of skin bleeding other than senile purpura will predict for abnormal haemostasis. The presence of hypotensive shock, sepsis, or obstetric complications suggests that an acquired coagulopathy may already be established.

Laboratory analysis for bleeding problems includes an assessment of liver and renal function, as well as a clotting screen, consisting of a platelet count, an activated partial thromboplastin time (APTT) and a prothrombin time (PT). If an abnormal finding is made on the clinical or laboratory assessment a haematological consultation should be obtained. (In the absence of predictive indicators, a bleeding time to assess the risk of excessive bleeding at surgery due to abnormal platelet function or von Willebrand's disease has such poor positive and negative predictive values as to be of little use as a screening test.[3])

Appropriate management will depend on the cause of the coagulopathy and on the time available for therapeutic intervention. Management in specific clinical conditions is discussed in later sections.

Table 1—Clinical conditions that can contribute to microvascular bleeding

Hypotension	
Sepsis	
Head injury	
Dilutional	
Direct surgical cause:	cardiopulmonary bypass, liver transplant
Pre-existing disorder:	liver disease
	renal disease
	drug therapy (heparin, warfarin, fibrinolytic therapy, aspirin)
	vitamin K deficiency
	immune thrombocytopenia
	congenital defect
	myelodysplastic syndrome with platelet dysfunction
	myeloma
	scurvy

Diagnosis and management of coagulopathy presenting during surgery

A pragmatic and stepwise approach is outlined.

Step 1. When unexpected diffuse microvascular bleeding develops during surgery contributing clinical causes should be identified from the available history (Table 1). Several factors may be present at the same time.

Step 2. A clotting screen is sent to the laboratory immediately. Laboratory testing is performed to guide therapy by helping to identify the pathological mechanisms operating in an established coagulopathy. It is not intended to confirm the presence of one.

Step 3. If there is not time to wait for the clotting screen result haemostatic therapy is given on the basis of the presumed clinical causes identified by history and examination.

Step 4. If the clotting screen result agrees with the clinical diagnosis appropriate therapy is given as discussed in the next section and the effects checked clinically and by repeat laboratory testing.

If the clotting screen result is not in agreement with the clinical diagnosis, both the presumed clinical cause and any identified laboratory abnormality should usually be corrected. For example in a patient bleeding following cardio-pulmonary bypass who has a normal platelet count and a prolonged prothrombin time, it can be assumed on clinical grounds that a platelet function defect is present as well as the clotting factor abnormality detected by the prolonged prothrombin. Both should be treated.

Clinical conditions associated with coagulation defects

Congenital defects of coagulation

Congenital defects in coagulation are rare. The most common conditions, haemophilia A, Christmas disease (haemophilia B) and von Willebrand's disease have incidences of 1–5 per 100 000. Rarer conditions include deficiencies of other clotting factors and platelet function defects. Surgery in patients with known congenital clotting disorders must be carried out with the close cooperation of a haematologist who has a special interest in this area. Adequate amounts of appropriate replacement therapy must be available before elective surgery is performed.

Haemophilia A (deficiency of factor VIII) and haemophilia B (Christmas disease; deficiency of factor IX) are clinically similar. Both are inherited as X-linked recessive disorders. Patients present with a mild, moderate or severe bleeding tendency, depending on their factor levels. Patients with severe disease tend to bleed into muscles and joints with minimal trauma. They may pre-

sent with other serious haemorrhage such as acute upper GI bleeds or intracranial haemorrhage. Patients with mild disease may have only a very slight bleeding tendency, but manifest as serious bleeding at surgery.

Von Willebrand's disease, deficiency of von Willebrand factor, is an autosomal dominant condition with an incidence of 2.5 per 100 000. Mucocutaneous bleeding and profuse haemorrhage following dental extraction or minor surgery are common. Unexpected bleeding following surgical challenge may be the presenting feature, even in adulthood.

Liver disease

Liver failure is commonly associated with coagulation problems. Patients with liver failure have several reasons for developing clotting abnormalities. There is reduced absorption of vitamin K, reduced synthesis of many clotting factors (including II, VII, IX and X, and fibrinogen), thrombocytopenia caused by hypersplenism, and low grade DIC due to impaired synthesis of inactivators in the liver and failure to clear activated clotting factors. Abnormal coagulation studies can be shown in 85% of patient with liver disease although only 15% have clinical bleeding problems.[4] Abnormal clotting tests and thrombocytopenia are commonly found in patients undergoing liver biopsy. However, these do not correlate well with bleeding problems.[5] It is advisable to raise the platelet count above 100×10^9 per litre by platelet transfusion and to correct the prothrombin time to less than 1.7, if possible, with vitamin K and fresh plasma before liver biopsy is performed.[6]

Renal failure

Chronic uraemia is associated a mild bleeding tendency mainly ascribed to impaired platelet function,[7,8] although a low haematocrit[9,10] and abnormal endothelial cell function[11] also contribute. In these patients, the presence of haemostatic impairment can be identified and quantified by the bleeding time. Correction of a prolonged bleeding time can be achieved by raising the haematocrit to about 30%, and by dialysis. Additional effective measures are desmopressin infusion, cryoprecipitate infusion, or platelet transfusion. The use of blood products should be avoided unless lack of time in an actively bleeding patient compromises the use of pharmacological alternatives.

Acute renal failure can complicate a complex coagulopathy but is unlikely to contribute significantly to the bleeding tendency. Extended ultra-filtration can cause a consumptive thrombocytopenia, and systemic anticoagulation will worsen the impairment of haemostasis.

In addition, chronic renal failure and nephrotic syndrome are associated with a thrombotic tendency, caused by low levels of the antithrombotic proteins, antithrombin III and protein C and by impaired release of tissue plasminogen activator.[8]

Drug therapy

Many drugs interfere with normal coagulation and can cause bleeding problems both in emergency and elective settings.

Heparin. Heparin directly impairs thrombin generation and activity by active site enzyme inhibition. It is given in a low dose regime for prevention of deep venous thrombosis (DVT) or in a higher, therapeutic dose, for treatment of acute thromboembolic disease or to prevent clotting of extracorporeal systems. Low dose heparin is associated with a small increase (2%) in the incidence of minor bleeding and wound haematomas but no increase in the incidence of serious bleeding.[12] In patients treated with therapeutic heparin the reported incidence of clinically significant bleeding varies from 1–15%.[13] Heparin has an in vivo half life of a few hours. Its effect on haemostasis can be rapidly reversed by infusion of protamine sulphate. It is not reversed by plasma therapy.

Heparin can cause several serious complications in patients other than a bleeding tendency from its pharmacological activity. Up to 7% of patients treated with prophylactic heparin will develop an immune thrombocytopenia.[14] This usually takes approximately 10 days of treatment to develop on first exposure to the drug, though on subsequent exposure the onset may be much quicker. Though usually mild, severity of the thrombocytopenia can be profound, with the platelet count less than 10×10^9 per litre. Other complications related to heparin associated thrombocytopenia are thrombosis, either venous or arterial, and acute skin necrosis at the site of subcutaneous injections.[15] These effects can be induced by very small amounts of heparin, even by heparin flushes of indwelling lines. Management of a patient with heparin-associated thrombocytopenia depends on the clinical situation, the certainty of the diagnosis, and the risks involved in ignoring it. In general a thrombocytopenic patient on heparin therapy should have the heparin stopped. This includes line flushes and subcutaneous low dose therapy. Anticoagulation can be continued according to the clinical situation with warfarin, aspirin, prostacyclin, ancrod, or a specific non-cross-reactive low molecular weight heparin, of which Orgaran™ is the only one available at the time of writing.[15] Laboratory confirmation of the diagnosis is possible in a few centres, although more generally applicable tests have recently been developed.[16] Bleeding thrombocytopenic patients should be treated by reversal of heparin with protamine, and platelet transfusion combined with intravenous immunoglobulin. Non-heparin anticoagulation can be re-established about 24 h after bleeding has stopped. Patients with extending venous thrombosis or with arterial thrombosis in association with heparin associated thrombocytopenia should be treated by fibrinolytic therapy or surgical clot removal, followed by alternative systemic anticoagulation.

Aspirin. Aspirin invariably and irreversibly causes impairment of platelet function. After a single oral dose impairment of platelet function may persist for up to a week. This may contribute to clinical haemostatic impairment in surgical patients. This effect is more

likely to be clinically significant when other factors are contributing to a bleeding tendency. For example, recent prospective trials in patients undergoing coronary artery bypass grafting have failed to show a difference in bleeding in patients taking a single preoperative aspirin dose compared to controls.[17] However, aspirin in combination with low dose heparin has been reported to cause an increase in serious bleeding in surgical patients.[18] Clinical bleeding in patients who have ingested aspirin within the previous week may need to be treated with platelet transfusions. Other factors will probably be contributing to the haemostatic failure and will need to be treated also.

Warfarin. Warfarin exerts its anticoagulant effect by interfering with the terminal steps in the synthesis of the vitamin K dependent clotting factors II, VII, IX and X, all of which are terminally gamma-carboxylated serine proteases. The failure to achieve terminal gamma carboxylation prevents these proteins forming enzyme-substrate complexes on the surface of activated platelets.

Warfarin is clearly associated with an increased incidence of bleeding.[19–21] There is a good correlation between degree of anticoagulation and incidence of bleeding. In combination with low dose heparin, warfarin causes a marked increase in serious bleeding complications and this combination should be avoided.[22] Patients on warfarin therapy are at increased risk of both bleeding and thromboembolic episodes in the perioperative period. In general warfarin therapy can be stopped prior to operation. Anticoagulation can be maintained with heparin during the immediate perioperative period and warfarin can be restarted as soon as the patient can swallow.

Patients who develop serious bleeding on warfarin at any level of anticoagulation should have the warfarin effect reversed by combination therapy using clotting factor concentrate of II, IX and X, and either factor VII concentrate (if available) or fresh frozen plasma (FFP).[6] In combination with factor concentrate, about 300 ml of FFP will provide enough factor VII.[23] The half life of factor VII in vivo is less than 2 h, and repeated testing and infusion may be necessary. The patient should also receive vitamin K 10 mg by injection.

Thrombolytic therapy. Patients may require surgery shortly after receiving thrombolytic therapy for one of several reasons including emergency bypass grafting, persistent bleeding after arterial catheterisation, and intracranial haemorrhage. Thrombolytic therapy results in depletion of fibrinogen by direct enzymatic digestion by plasmin. Levels of factors V and VIII in the plasma are also reduced by plasmin digestion. In addition, plasmin causes a platelet functional defect. Often the patients will also have been started on heparin. Patients undergoing cardiac surgery have a considerable increase in bleeding after failed thrombolytic therapy.[24] Management of these patients involves stopping the infusion of the thrombolytic agent, and of heparin if used. If the patient is not actively bleeding, results of a clotting screen can be awaited. If there is active bleeding, or if the fibrinogen level is less than 1 g per litre, cryoprecipitate is given: 10 units, given as a single pool, will raise the plasma level by approximately 0.7 g per litre in an average adult. Cryoprecipitate also contains factors V and VIII, and this dose will probably be adequate to correct the deficiencies. Heparin should be reversed with protamine sulphate. If these measures fail platelets should be transfused. If the bleeding is immediately life-threatening antifibrinolytic agents, tranexamic acid or epsilon aminocaproic acid, should be given.[25] The fibrinogen levels should be checked repeatedly after treatment, since fibrinolysis may persist after infusion of cryoprecipitate.

Massive blood loss

Massive blood loss rarely occurs in isolation. Usually there is a degree of shock and there is often associated major trauma. Degree and length of shock and the number of units of red cell concentrate (RCC) transfused are strong indicators of overall outcome in these patients.[26,27] Despite this there have been several well designed studies that have managed to isolate and identify the problems occurring with blood loss alone. Significant coagulation defects tend to occur after about 20 units of RCC have been transfused.[28,29] It should be emphasised that patients can receive very large transfusions and still exhibit normal clotting. Of those who develop abnormality on laboratory testing most do not have clinical bleeding problems. In one study only 29% of patients transfused 20–30 units of RCC developed abnormal clotting.[28] The difficulty in predicting the patients who will develop clotting problems and at what stage this will occur, means that rigid protocols of platelet and FFP administration in conjunction with massive transfusions are inappropriate. Initial resuscitation is usually carried out with crystalloid with or without colloid, followed by RCC when available. After massive transfusion with RCC, which lack platelets and other clotting factors, a dilutional coagulopathy develops: a platelet count of less than 100×10^9 per litre is most clearly associated with bleeding problems. Reduction of clotting factors, in particular V and VIII, associated with a mild prolongation of clotting test times, is common but does not correlate to the risk of developing microvascular bleeding. Marked prolongation of the clotting times, however, is usually associated with a drop to less than 20% of normal plasma levels of factors V and VIII with hypofibrinogenaemia and suggests the onset of a consumptive coagulopathy. A patient who develops microvascular bleeding during the course of resuscitation after blood loss should be treated with platelet and fresh frozen plasma transfusion. If the clotting screen reveals fibrinogen levels of less than 1 g per litre, cryoprecipitate (a pool of 10 units in an adult) should also be given.

Disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC) occurs when coagulation is triggered by some abnormal stimulus

to take place in the general circulation, and is not confined to a region of damaged tissue. Many disease processes can cause DIC (Table 1). The factors that trigger DIC may differ.[30] Brain is a rich source of tissue thromboplastin: the high incidence of DIC in patients with head injury, especially when brain tissue itself is injured is most likely due to tissue thromboplastin release, causing direct intravascular activation of tissue factor and subsequent thrombin activation. In septic shock DIC can be precipitated by endotoxin, and perhaps by direct activation by the bacterial cell wall. Direct endothelial hypoxia may precipitate DIC in hypotensive shock.

The clinical picture in DIC can be diverse. Some patients have a primarily thrombotic disease process with multiple infarcts and organ failure; others have a mainly haemorrhagic disorder. DIC can be acute and overwhelming with complete defibrination, profound thrombocytopenia and uncontrollable haemorrhage; it can also present as a sub-clinical disorder detected only on coagulation studies. Coagulation studies typically show thrombocytopenia, prolongation of clotting times, hypofibrinogenaemia, and the presence of circulating fibrin degradation products. It has not proved possible to complete good laboratory or clinical studies of DIC until recently. The use of primate models of endotoxic shock have elucidated the mechanisms of DIC in this setting. Coagulation is activated by endotoxin through tumour necrosis factor (TNF), probably via interleukin 6, causing expression of tissue factor in monocytes and endothelial cells.[31] Exogenous tissue factor pathway inhibitor, a naturally occurring anticoagulant in human plasma, prevented the onset of DIC and improved survival in these studies. Other possible therapeutic agents that may emerge in the future for treatment of DIC include antithrombin III (ATIII) and activated Protein C, both of which are naturally occurring thrombin inhibitors. Clinical trials to date of ATIII have been inconclusive[32] or disappointing.[33,34] It is likely that in established DIC no single agent will be sufficient, but that a combination of inhibitors of cytokines and antithrombins will be necessary. At present standard management of DIC involves removal of the cause if possible, and replacement of the consumed clotting factors, especially fibrinogen, factors VIII and V, and platelets, with transfusions of cryoprecipitate, fresh frozen plasma and platelet concentrates. There is no evidence to support the use of heparin in DIC.

Cardiopulmonary bypass

Following cardiopulmonary bypass (CPB) 10–20% of patients may develop a degree of haemostatic failure, more commonly after repeat procedures. CPB has wide ranging effects on coagulation: platelets, the complement system, leucocytes and coagulation factors are all affected.[35–41] Thrombocytopenia and platelet dysfunction are probably the most significant abnormalities resulting from CPB. Platelets are activated during the procedure. These activated platelets form circulating aggregates, secrete granule contents and may inappropriately activate coagulation. Platelets may be directly activated by contact with the CPB circuit or the effect may be mediated by thrombin, plasmin, or leucocytes. CPB activates leucocytes and production and granulocyte elastase and oxygen free radicals can be shown.[39] Leucocyte activation is probably mediated by the complement system: increased levels of C3a and C5a are found early after the commencement of CPB.[40] Elastase from activated leucocytes is a direct platelet agonist.[42] Plasmin levels rise during CPB: tPA release from ischaemic tissue may contribute to this phenomenon. Plasmin is also a platelet-activating enzyme.

Other effects of CPB include a reduction of plasma levels of clotting factors II, V, VII, VIII, IX and X, elevation of factor XII and activation of fibrinolysis. The significance of these effects is uncertain. It has become apparent that aprotinin, a broad spectrum inhibitor of serine proteases, including kallikrein, plasmin and elastase reduces or completely prevents the coagulopathy induced by cardiopulmonary bypass.[43] The mechanism for this effect is unclear but prevention of activation platelet is probably involved.[44]

Patients undergoing cardiopulmonary bypass may have additional causes of coagulopathy such as aspirin ingestion, incomplete warfarin reversal, sepsis associated with endocarditis, or recent fibrinolytic therapy. Reoperated patients, those with recent aspirin ingestion, and patients with septic endocarditis are likely to benefit from aprotinin infusion.[45] Patients presenting with excessive microvascular bleeding after heparin reversal at the end of bypass can be treated with platelet transfusion, and by correcting of any residual prolongation of the clotting times with fresh frozen plasma. However, the platelet function defect induced by cardiopulmonary bypass is usually self limiting, and reverses naturally within 2 h. The patient should be spared the hazards of exposure to blood products for minor levels of microvascular bleeding.

Liver transplant

Patients undergoing liver transplant have a complex coagulopathy that arises from the pre-existing coagulopathy of end-stage liver failure complicated by the effects of the operation itself, particularly during the anhepatic phase. There is increased fibrinolysis caused by increased levels of plasmin from a combination of increased release of tPA from injured endothelium, reduced clearance of tPA and a lack of hepatic synthesis of alpha-1 antiplasmin.[46,47] These abnormalities resolve at varying rates following reperfusion of the transplanted liver. Aprotinin has been reported to reduce bleeding in liver transplantation, though this is disputed.[48–50] The most significant action is probably inhibition of free plasmin.

The fast-moving changes in coagulation during liver transplantation require monitoring that is more immediate than the 20–30 min required for a conventional coagulation screen. Some centres report useful results from thromboelastastography. This is a device which can be used in the operating theatre to give an assessment of overall function of the haemostatic system, including

platelet function from a single sample.[49,51] However the technique does not give immediate results, and no objective studies are available showing benefit from its use. Much of the immediate treatment of excessive blood loss during transplant remains the empirical use of platelet, plasma, and cryoprecipitate infusions.

Ruptured aortic aneurysm

Emergency surgery for ruptured abdominal aortic aneurysm still carries a mortality rate of about 50%.[52–55] Several factors are associated with poor prognosis: low systolic blood pressure at presentation, advanced age, excessive operative blood loss and coagulopathy. Coagulopathy has been shown to carry a particularly poor prognosis.[56,57,58] Patients with a poor prognosis associated with coagulopathy can be identified by means of routine haematological investigation at time of admission. In a recent study patients with a platelet count > 100 had a mortality of 7.4%. Patients who had a platelet count of < 100 had a mortality of 82%,[57] presumably due to established tissue hypoxic damage and associated consumptive coagulopathy before resuscitation and surgery. Future studies directed towards pharmacological intervention in this poor prognosis group may improve the outcome in patients with ruptured aneurysms who survive to reach hospital.

References

1. Escolar G, Garrido M, Mazzara R, et al. Experimental basis for the use of red cell transfusion in the management of anaemic-thrombocytopenic patients. Transfusion 1988; 28: 406–411.
2. Kroll M H, Harris T S, Moake J L, et al. Von Willebrand factor binding to platelet GPIb initiates signals for platelet activation. J Clin Invest 1991; 88: 1568–1573.
3. Lind S E. Prolonged bleeding time. Am J Med 1984; 77: 305–312.
4. Roberts H R, Cedebaum A I. The liver and blood coagulation: Physiology and pathology. Gastroenterology 1972; 63: 297–319.
5. Ewe K. Bleeding after liver biopsy does not correlate with indices of peripheral coagulation. Digestive Dis Sci 1981; 26: 388–393.
6. British Committee for Standards in Haematology. Guidelines for the use of fresh frozen plasma. Transfusion Medicine 1992; 2: 57–63.
7. George J N, Shattill S J. The clinical importance of abnormalities of platelet function. New Engl J Med 1991; 324: 27–39.
8. Remuzzi G. Bleeding in renal failure. Lancet 1988; i: 1205–1208.
9. Fernandez F, Goudable C, Sie P, et al. Low haematocrit and prolonged bleeding time in uraemic patients: effects of red cell transfusions. Brit J Haematol 1985; 59: 139–148.
10. Livio M, Marchesi D, Remuzzi G, et al. Uraemic bleeding: role of anaemia and beneficial effects of red cell transfusions. Lancet 1982; ii: 1013–1015.
11. Remuzzi G, Cavenaghi A E, Mecca G, et al. Prostacyclin-like activity and bleeding in renal failure. Lancet 1977; ii: 1195–1197.
12. Collins R, Scrimgeour A, Yusef S, Peto R. Reduction in fatal pulmonary embolism and venous thrombosis by peri-operative administration subcutaneous heparin. N Engl J Med 1988; 318(18): 1162–1171.
13. Clagett G P, Reisch J S. Prevention of thromboembolism in general surgical patients. Ann Surg 1988; 208(2): 227–239.
14. Kelton J G, Sheridan D, Santos A, et al. Heparin-induced thrombocytopenia: laboratory studies. Blood 1988; 72: 925–930.
15. Warketin T E. International Society for Haemostasis and Thrombosis, Subcommittee on Platelet Immunology. 1993: in press.
16. Greinacher A, Mockl M, Mueller-Eckhardt C. Prospective comparison of two laboratory assays for diagnosing heparin-associated thrombocytopenia (HAT): platelet aggregation assay (PA) and heparin induced platelet aggregation assay (HIPA). Thrombosis and Haemostatis 1993; 69: 1331.
17. Taggart D P, Siddiqui A, Wheatly D J. Low-dose preoperative aspirin therapy, postoperative blood loss and transfusion requirements. Ann Thorac Surg 1990; 50: 424–428.
18. Yett H S, Skillman J J, Salzman E W. The hazards of aspirin plus heparin. N Engl J Med 1978; 298: 1092.
19. Hull R, Hirsh J, Jay R, Carter C, al e. Different intensities of oral anticoagulant therapy in the treatment of proximal vein thromboses. N Engl J Med 1982; 307: 1676–1681.
20. Saour J N, Sieck J O, Mamo L A R, Gallus AS. Trial of different intensities of anticoagulation in patients with prosthetic heart values. N Engl J Med 1990; 322: 428–432.
21. Turpie A G G, Gunstensen J, Hirsh J, Nelson H, Gent M. Randomised comparised of two intensities of oral anticoagulant therapy after tissue heart valve replacement. Lancet 1988; i: 1242–1245.
22. Torosian M, Michelson E L, Morganroth J et al. Aspirin and coumadin related bleeding after coronary bypass graft surgery. An Intern Med 1978; 89: 325–328.
23. Mannucci P M, Franchi F, Dioguardi N. Correction of abnormal coagulation in chronic liver disease by combined use of fresh frozen plasma and prothrombin complex concentrates. Lancet 1976; ii: 542–545.
24. Ferguson T B, Muhlbaier L H, Salai D L et al. Coronary bypass grafting after failed elective and failed emergent surgical interventions. J Thorac Cardiovasc Surg 1988; 95: 761–771.
25. Sane D S, Califf R M, Topol E J et al. Bleeding during thrombolytic therapy for acute myocardial infarction: mechanisms and management. Ann Intern Med 1989; 111: 1010–1022.
26. Wilson R F, Mammen E, Walt A. Eight years of experience with massive blood transfusions. J Trauma 1971; 1: 275–285.
27. Miller R D, Robbins T O, Tong M J, Barton S L. Coagulation defects associated with massive blood transfusions. Ann Surg 1971; 174: 794–801.
28. Ciavarella D, Reed R L, Counts R B et al. Clotting factor levels and the risk of diffuse microvascular bleeding in the massively transfused patient. Brit J Haematol 1987; 67: 365–368.
29. Counts R B, Haisch C, Simon T L, Maxwell N G, Heimach D M, Carrico C J. Haemostasis in massively transfused trauma patients. Ann Surg 1979; 190: 91–99.
30. Takahashi H, Tatewaki W, Wada K et al. Thrombin vs plasmin generation in disseminated intravascular coagulation associated with various underlying disorders. Am J Haem 1990; 33: 90–95.
31. Taylor F B. International Society for Thrombosis and Haemostasis: Subcommittee on Disseminated Intravascular Coagulation. Elsevier, Amsterdam. 1993 (in press)
32. Fourrier F, Chopin C, Goudemand G et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation — compared patterns of antithrombin III, protein C and protein S deficiencies. Chest 1992; 101: 816–823.
33. Blauhut B, Necek H, Vinazzer H et al. Substitution therapy with an antithrombin III concentrate in shock and DIC. Thrombosis Research 1982; 27: 271–278.
34. Vinazzer H. Clinical use of antithrombin III concentrates. Vox Sanguinis 1987; 53: 193–198.
35. Gralnick H R, Fisher R D. The haemostatic response to open heart operations. J Thorac Cardiovasc Surg 1971; 61: 909–915.
36. Addonizio V R, Smith J B, Guiod L R et al. The relationship between thromboxane synthesis and platelet protein release during simulated extracorporeal circulation. Blood 1979; 54: 371–376.
37. George J N, Picket E B, Saucerman S, et al. Platelet surface glycoproteins: studies on resting and activated platelets and platelet microparticles in normal subjects and observations in patients during adult respiratory distress syndrome and cardiac surgery. J Clin Invest 1986; 78: 40–48.
38. Colman RW. Platelet and neutrophil activation in cardiopulmonary bypass. Ann Thorac Surg 1990; 49: 32 –34.
39. Hind C R K, Griffin J F, Pack S et al. Effect of cardiopulmonary bypass on circulating concentrations of leucocyte elastase and free radical activity. Cardiovascular Research 1998; 22: 37–41.
40. Wachtfogel Y Y T, Harpel P C, Edmunds L H, Colman R W. Formation of C1s-C1-inhibitor, kallikrein-C1-inhibitor complexes during cardiopulmonary bypass. Blood 1989; 73: 468–471.
41. Wenger R K, Lukasiewicz H, Mikuta B S, Niewiarowski S, Edmunds L H. Loss of platelet fibrinogen receptors during clinical cardiopulmonary bypass. J Thorac Cardiovasc Surg 1989; 97: 235–239.
42. Kornecki E, Ehrlich Y H, De Mars D D et al. Exposure of

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Postoperative Haemorrhage Following Aortic Aneurysm Repair

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Between 1988 and 1993, 17 (3%) out of a total 654 patients underwent reoperation for control of haemorrhage following repair of abdominal aortic aneurysm in a vascular surgery unit. The first operation was performed for rupture in 12 cases and electively in five. The incidence of reoperation for postoperative bleeding was 1.7% following elective operation and 3.3% following emergency operation. Case-controls, matched for sex and primary operation, were identified. The mortality rate in those requiring reoperation was 58% compared with 23% in the control group ($p = 0.037$). Seven patients suffered progressive deterioration and died in the early postoperative period. Of the remaining ten patients, four suffered unexpected serious complications; two a fatal cerebro-vascular accident (CVA), one a fatal myocardial infarction (MI) and the fourth a non-fatal CVA. The patients requiring reoperation had greater blood loss ($p < 0.05$), greater transfusion requirements and lower core temperatures ($p < 0.05$) at the end of their first operation than the control group. All except one of the patients who bled had evidence of coagulopathy and had lower platelet counts than the control group both before and after the first operation. At reoperation there were multiple minor bleeding points in 11 patients, no active bleeding points in two patients and a discrete bleeding point in four patients. In conclusion, re-operation for control of postoperative haemorrhage is an uncommon complication which is strongly associated with coagulopathy, may predispose to "rebound" postoperative thrombotic episodes, and carries a poor prognosis.

Introduction

Abdominal aortic aneurysm is a common condition and the number of operations being performed is steadily rising.[1-4] This increase is due to a combination of increasing awareness of the condition, greater ease of diagnosis due to the wide availability of ultrasound, a greater willingness to perform operations in older and less fit patients [5] and a true increase in the incidence of the condition.[6] The incidence of haemorrhage both during and after operation, which formerly accounted for the majority of deaths after aneurysm repair, was much reduced by the introduction of the "inlay" technique which avoids the need for extensive dissection.[7] Despite this and other technological improvements, haemorrhage remains one of the more common complications of aneurysm repair.[3,8-10] The purpose of this study was to determine the incidence, nature and outcome of postoperative haemorrhage as a preliminary to examining ways of avoiding this complication.

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Methods and Materials

The case records of all patients undergoing laparotomy for control of haemorrhage following aortic aneurysm repair, during the period 31 July 1988–1 August 1993 in the Vascular Surgery Unit of the Royal Infirmary of Edinburgh, were identified by means of the Vascular Audit system. Seventeen patients were identified, 10 male and 7 female, with a mean age of 73 ± 5 years. In five the first operation had been elective and in 12 the operation had been performed for rupture. During this period 291 elective operations and 363 emergency operations were carried out for abdominal aortic aneurysm in the Vascular Unit giving an incidence of operation for postoperative bleeding of 1.7% after elective operations and 3.3% after emergency operations. Case-controls, matched for sex and indication for operation, were selected from operation records during the same period by the following method. For each study patient the next patient of the same sex undergoing operation for the same indication was selected. The mean age of the control group was 71 ± 8 years.

Operations were carried out either by a consultant vascular surgeon or by a senior registrar with

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Table 1. Haematological and operative findings in patients who required re-operation after rupture and control patients undergoing operation for rupture

	Study Group		Controls		
	Median	Range	Median	Range	
Preoperative platelet count ($\times 10^9/l$)	103	81–385	253	244–336	NS
Platelet count after first operation ($\times 10^9/l$)	92	40–140	112	64–175	NS
Fibrinogen after first operation (g/l)	1.12	0.6–2.9	1.30	0.6–3.8	NS
Prothrombin ratio after first operation	1.6	1.1–3.3	1.4	1.0–2.2	NS
Temperature at the end of operation (°C)	34	31.6–36	35	32.5–37	$p < 0.05$
Operation length (mins)	180	85–320	112	80–300	NS
Cross-clamp time (mins)	65	44–100	45	40–70	NS
Blood loss (ml)	5150	2000–12500	2035	840–10700	$p < 0.05$
Transfusion RCC (units) during first operation	12	6–18	6	1–16	NS

considerable experience in vascular surgery. All patients undergoing elective operation were given systemic heparin (5000 iu) prior to cross-clamping. Those undergoing operation for rupture were not given systemic heparin but heparinised saline (5000 iu/500 ml 0.9% saline) was used for irrigation and flushing.

Statistical analysis was carried out using Systat for windows v5.0®. Mann-Whitney U-test, χ^2 and Fisher's exact test were used where appropriate. Data are given as median and range unless otherwise stated.

Results

Operative findings

Mean operating time, cross clamp time, blood loss and transfusion requirements were all greater in patients who bled postoperatively than in the controls, although these differences were not statistically significant except for blood loss, $p < 0.05$, Mann-Whitney (see Table 1). The study group had a lower mean temperature at the end of operation ($p < 0.05$, Mann-Whitney). These data suggest that patients who bled post-operatively had more complicated primary operations.

At re-exploration a single bleeding point was found in only four patients. Of these two were bleeding from the proximal anastomosis, one from a lumbar artery and one from the median sacral artery. In two patients no active bleeding point was found although there was a considerable volume of blood in the peritoneal cavity. In the remaining 11 multiple minor bleeding points and/or diffuse oozing were found.

Clinical outcome

In total there were 10 deaths (58%) within 30 days in the study group compared with four deaths (23%) in the control group ($p < 0.05$, Pearson χ^2) (see Table 3) The mortality rate for the patients who had undergone an elective primary operation (4/5) was greater than for those who had undergone operation for rupture (6/12).

Seven of those who died in the study group followed a clinical course of progressive deterioration and death from continuing haemorrhage and multi-organ failure. Ten patients made a good initial recovery and were discharged from the intensive care unit back to the general ward. In this group there were three late deaths; two from cerebro-vascular accident (CVA), both at ten days postoperatively and one from cardiac arrest on the fourteenth postoperative day, subsequent to a myocardial infarction diagnosed on electrocardiogram findings earlier that day. In addition to these deaths a further patient suffered a non-fatal CVA 19 days after operation. In two of the three CVA cases the diagnosis was made on clinical grounds and in the other the diagnosis was confirmed by CT scanning. There were no late deaths in the control group but one patient suffered a non-fatal MI on the second postoperative day.

Table 2. Haematological and operative findings in patients who required re-operation after elective operation and control patients undergoing elective repair

	Study Group		Controls		
	Median	Range	Median	Range	
Preoperative platelet count ($\times 10^9$/l)	163	120–271	260	124–395	NS
Platelet count after first operation ($\times 10^9$/l)	81	63–177	187	93–220	NS
Fibrinogen after first operation (g/l)	1.80	1.1–2.2	2.05	1.7–2.4	NS
Prothrombin ratio after first operation	1.6	1.0–1.8	1.2	1.0–1.4	NS
Temperature at the end of operation (°C)	35.5	35–36.1	36.5	36.5–37	NS
Operation length (mins)	170	160–240	125	110–220	NS
Cross-clamp time (mins)	75	44–100	45	40–70	NS
Blood loss (ml)	1960	390–3665	1500	935–2000	NS
Transfusion RCC (units) during first operation	2.5	0–6	2.0	0–3	NS

Table 3. Outcome and site of bleeding in patients who required re-operation for control of haemorrhage

	Type	Outcome		Operative findings
1	Elective	Died	Early death	Proximal anastomosis
2	Elective	Died	MI day 12	Diffuse bleeding
3	Elective	Survived		Lumbar artery
4	Elective	Died	Early death	Diffuse bleeding
5	Elective	Died	CVA day 10	Proximal anastomosis
6	Rupture	Died	CVA day 10	Diffuse bleeding
7	Rupture	Survived	Renal failure	Diffuse bleeding
8	Rupture	Survived		Median sacral artery
9	Rupture	Died	Early death	Diffuse bleeding
10	Rupture	Died	Early death	Diffuse bleeding
11	Rupture	Survived	Renal failure	Diffuse bleeding
12	Rupture	Survived		Diffuse bleeding
13	Rupture	Survived	CVA day 19	Diffuse bleeding
14	Rupture	Survived		Diffuse bleeding
15	Rupture	Died	Early death	No active bleeding
16	Rupture	Died	Renal failure and MI	No active bleeding
17	Rupture	Died	Early death	Diffuse bleeding

Coagulation results

All but one patient who required re-operation had abnormal results on coagulation screening following the first operation (see Tables 1 and 2). The only patient who did not have a coagulopathy had bled because of a technical error — failure to under-run a lumbar artery. Of the five elective patients who required re-laparotomy for haemorrhage, three had platelet counts of less than 100×10^9/l and one had a platelet count less than 150×10^9/l following the first operation (normal range 150–450 $\times 10^9$/l). Only one of the elective patients in the group developed post-operative thrombocytopenia with a platelet count of 93×10^9/l.

Discussion

This study confirms that operation for control of haemorrhage after aortic aneurysm repair is an uncommon but serious complication which is nearly always associated with a coagulopathy and thrombocytopenia (Tables 1 and 2). The importance of coagulopthy has not been emphasised in previous reviews on this subject.[11] From the data available in this study it is impossible to determine whether the coagulopathy is the cause of the haemorrhage or vice versa. However, the fact that patients who bled had lower platelet counts prior to the first operation (Tables 1 and 2) and that very often no significant bleeding point could be found suggests that coagulopathy is an important causal factor in most patients. It should also be noted that many of the patients with ruptured aneurysms received platelet transfusions (median 5 units, range 0–20 units) and it may be that without them the severity of postoperative thrombocytopenia would have been greater.

The role of coagulopathy as a causal factor of haemorrhage is easily understood in patients with ruptured aneurysms, as it is well established that many of them have coagulopathy on admission,[12] and it may be that coagulopathy is a causal factor in elective patients as well. Coagulopathy is an established complication of elective aortic aneurysm repair. It was first reported in 1955[13] and subsequent studies have revealed that laboratory evidence of abnormal coagulation following aortic surgery is common. [14–16] In this study four out of five patients who required re-operation after elective operations had thrombocytopenia following the primary operation. Examination of the records of 20 consecutive patients undergoing elective aneurysm repair revealed that only one had postoperative thrombocytopenia. This is a significantly lower incidence of postoperative thrombocytopenia than in the study group, $p = 0.016$ by Fisher's exact test.

It is interesting to note that many patients were hypothermic at the end of operation. It may be advisable that greater efforts are made to avoid heat loss in these patients although all normal precautions, such as heated water blankets, polythene bags around the lower legs, the use of a blood warmer and placing the small intestine in a "gut-bag", were undertaken routinely. The hypothermia in the patients who bled may simply reflect the fact that they had undergone longer and more complicated operations but it should be noted that hypothermia potentiates the activation of platelets by plasmin and may thus contribute directly to the generation of a coagulopathy.[17]

The reason for the high incidence of stroke in this study is a matter for conjecture. In one case it is known that the stroke was thrombo-embolic in nature rather than haemorrhagic but in the other two cases this information is not available. If one assumes that these events were thrombotic and also that the post-operative MI was also a thrombotic episode then it may be that these patients develop a "rebound" prothrombotic state. This is supported by the fact that at the time of these events the platelet count was rising; in two cases the platelet count had more than doubled in the preceding 48 hours. The presence of a prothrombotic state after ruptured aneurysm has been suggested in a recent publication.[18] An association between coagulopathy and postoperative thrombotic events is also suggested by data published in another study of ruptured aortic aneurysms. Davies *et al.* reported that in a group of 12 patients who developed a coagulopathy in association with a ruptured aneurysm there were three postoperative deaths which might be attributed to thrombotic events; two from MI on days 1 and 9 and one from stroke on day 14. In contrast, in the group of 23 patients who had no evidence of coagulopathy there were no deaths from MI or stroke ($p = 0.05$ by Fisher's exact test).[12] The presence of a prothrombotic state may account for the high incidence of MI in the early postoperative period found in most published series of aneurysm repairs. Further studies are required to investigate this hypothesis.

The findings at second operation indicate that in most cases defective operative technique was not the cause of bleeding. The evidence suggests that the development of a coagulopathy was responsible. Greater understanding of the pathological process which leads to coagulopathy in these patients is required, in order to develop more effective therapeutic strategies and thus reduce the mortality associated with aortic surgery.

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References

1 Castleden WM. Abdominal aortic aneurysms in Western Australia: descriptive epidemiology and patterns of rupture. *Br J Surg* 1986; **73**: 551–553.
2 Naylor AR, Webb J, Fowkes FGR, Ruckley CV. Trends in abdominal aortic aneurysm surgery in Scotland (1971–1984). *Eur J Vasc Surg* 1988; **2**: 217–221.
3 Jenkins AML, Ruckley CV, Nolan B. Ruptured aortic aneurysm. *Br J Surg* 1986; **73**: 395–398.
4 Melton LJ, Bickerstaff LK, Hollier LH, *et al.* Changing incidence of abdominal aortic aneurysms: a population based study. *Am J Epidemiol* 1984; **120**: 79–86.
5 Chalmers RTA, Stonebridge PA, John TG, Murie JA. Abdominal aortic aneurysm in the elderly. *Br J Surg* 1993; **80**: 1122–1123.
6 Fowkes FGR, Macintyre CCA, Ruckley CV. Increasing incidence of aortic aneurysms in England and Wales. *BMJ* 1989; **298**: 33–35.
7 Creech O. Endoaneurysmorraphy and treatment of aortic aneurysm. *Ann Surg* 1966; **164**: 935.
8 Diehl JT, Cali RF, Hertzer NR, Beven EG. Complications of abdominal aortic reconstruction. *Ann Surg* 1983; **197**: 49–56.
9 Goldstone J. Aneurysms of the aorta and iliac arteries. In: Moore WS, ed. *Vascular Surgery. A Comprehensive Review.* 3rd ed. London: WB Saunders, 1991: 315–316.
10 Mutirangura P, Stonebridge PA, Clason AE, *et al.* A ten year review of non-ruptured aortic aneurysms. *Br J Surg* 1989; **76**: 1251–1254.
11 Crawford ES. Symposium. Prevention of complications of abdominal aortic reconstruction. Introduction. *Surgery* 1983; **93**: 91–96.
12 Davies MJ, Murphy WG, Murie JA, *et al.* Preoperative coagulopathy in ruptured abdominal aortic aneurysms predicts poor outcome. *Br J Surg* 1993; **80**: 974–976.
13 Krevans JR, Jackson DP. Hemorrhagic disorder following massive whole blood transfusions. *JAMA* 1955; **159**: 171–177.

14 MULCARE RJ, ROYSTER TS, WEISS HJ, PHILLIPS LL. Disseminated intravascular coagulation as a complication of abdominal aortic aneurysm repair. *Ann Surg* 1974; **180**: 343–349.
15 MULCARE R, ROYSTER T. PHILLIPS L. Intravascular coagulation in surgical procedures on the abdominal aorta. *Surg Gynecol Obstet* 1976; **143**: 730–734.
16 MASHIAH A, THOMSON JM, POLLER L, CHARLESWORTH D. Changes in the coagulation of blood during resection of the abdominal aorta. *Surg Gynecol Obstet* 1979; **149**: 214–216.
17 LU H, SORIA C, CRAMER EM, *et al.* Temperature dependence of plasmin-induced activation or inhibition of human platelets. *Blood* 1991; **77**: 996–1005.
18 BRADBURY AW, BACHOO P, MILNE AA, DUNCAN JL. Platelet count and the outcome of operation for ruptured abdominal aortic aneurysm. *J Vasc Surg* In press.

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Disseminated intravascular coagulation after aortic aneurysm repair, intraoperative salvage autotransfusion, and aprotinin

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Disseminated intravascular coagulation after aortic aneurysm repair, intraoperative salvage autotransfusion, and aprotinin

SIR—Intraoperative salvage autotransfusion and high-dose aprotinin have each been reported to reduce homologous blood transfusion in patients undergoing aortic aneurysm repair and each modality appears to be safe is isolation.[1-3] We describe a case of disseminated intravascular coagulation (DIC) with a fatal outcome after an elective aortic aneurysm repair during which intraoperative autotransfusion and aprotinin were used.

A 60-year-old male Jehovah's Witness was admitted for elective repair of an abdominal aortic aneurysm. He had type II hyperlipidaemia with widespread vascular disease. Coronary angiography one year previously had shown a severe stenosis in the left anterior descending coronary artery and an occluded right coronary artery. Preoperative haemoglobin was 140 g/L, platelet count 197×10^9/L, coagulation screen normal, urea 7·3 mmol/L, sodium 132 mmol/L, potassium 4·2 mmol/L, creatinine 126 μmol/L, normal chest radiograph, and a left ventricular ejection fraction of 47% by isotope scanning. An electrocardiogram showed inferolateral ischaemia.

Aprotinin 500 000 IU was given intravenously at induction and a further 1500 000 units were given during the operation. Heparin 5000 IU was given intravenously before aortic cross-clamping. Autotransfusion was carried out using a Solcotrans system after heparin had been given. The operative blood loss was 2100 mL of which 1400 mL was salvaged by autotransfusion. Immediately postoperatively all four limbs were extensively mottled. The patient subsequently developed diarrhoea and haemoglobinuria. Investigations 5 hours after the end of operation revealed platelet count 75×10^9/L, prothrombin ratio 1·25, activated partial thromboplastin ratio 1·4, fibrinogen 1·8 g/L, and D-dimers 1–2 (normal range <0·25). Urine output decreased overnight despite a central venous pressure of 5–10 cm H_2O. The next day the patient suddenly developed tachypnoea and tachycardia with evidence of anterolateral ischaemia. Cardiac arrest followed and, despite attempts at resuscitation, he died. At necropsy there were new areas of myocardial infarction around an old posterior infarct. The left ventricle was dilated and pulmonary oedema was present. The right kidney was infarcted; histology showed foci of cortical infarction with multiple fibrin microthrombi in small arteries, arterioles, and glomerular tufts. Microthrombi were also found in the liver and heart.

Intraoperative autotransfusion and aprotinin used alone are apparently safe, but there are no published data on their combined use. Activation of the clotting system occurs in the Solcotrans reservoir, presumably via the contact pathway, with associated fibrinolysis and generation of D-dimers.[3] The plasma concentration of aprotinin in this patient is unlikely to have inhibited D-dimer formation. However, systemic antifibrinolytic therapy may accentuate the clinical features of severe DIC and, although reinfusion of reservoir blood usually causes no systemic adverse effects, in this patient aprotinin could have contributed to deposition of fibrin microthrombi from the reinfused blood in the microvasculature and subsequent failure to clear such microthrombi by fibrinolysis. Although additional causes, such as mild perioperative hypotension and a pre-existing subclinical prothrombic state from clotting activation in the aneurysm sac, may have contributed to the clinical picture, fatal DIC following elective aneurysm repair is rare.[4] We suggest that caution should be exercised when considering the use of Solcotrans and aprotinin for blood saving at surgery.

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1 Thompson JF, Roath OS, Francis JL, Webster JHH, Chant ADB. Aprotinin in peripheral vascular surgery. *Lancet* 1990; **335:** 911.
2 Duchateau J, Nevelsteen A, Suy R, et al. Autotransfusion during aorto-iliac surgery. *Eur J Vasc Surg* 1990; **4:** 349–54.
3 Clifford PC, Kruger AR, Smith A, Chant ADB, Webster JHH. Salvage autotransfusion in aortic surgery: initial studies using a disposable reservoir. *Br J Surg* 1987; **74:** 755–57.
4 Gibney EJ, Bouchier-Hayes D. Coagulopathy and abdominal aortic aneurysm. *Eur J Vasc Surg* 1990; **4:** 557–62.

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Endothelial cell ultrastructure after aortic aneurysm rupture: an electron microscopy study

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Endothelial cell activation as an early response to hypovolaemic shock may be important in the pathogenesis of coagulopathy and multiple organ failure after aortic aneurysm rupture. The aim of this study was to determine whether any evidence could be found of changes in endothelial cell ultrastructure in patients undergoing operation for ruptured aneurysm and to establish a model for future studies of endothelial cells in these patients. At the start of operation, fat samples from the anterior abdominal wall were taken from five patients with ruptured abdominal aortic aneurysms and in an identical fashion from five control patients undergoing elective surgery. Endothelial cells lining capillaries were examined by transmission electron microscopy. Photomicrographs were taken of all capillaries present and these were assessed blindly by three independent observers. When compared with controls it was found that the luminal surface of the endothelial cells in patients with aneurysm rupture was more convoluted, with more frequent processes projecting into the lumen of the capillary. Budding of the luminal surface of the endothelial cell was seen in 11 of 45 capillaries in the rupture group and in none of 44 capillaries in the control group ($P < 0{\cdot}005$). Budding was seen in at least one capillary of all five patients presenting with rupture but in none of the control group ($P < 0{\cdot}05$). In conclusion, at the ultrastructural level there are changes within endothelial cells in capillaries at an early stage after ruptured abdominal aortic aneurysm.

The development of coagulopathy is a well recognized complication of ruptured abdominal aortic aneurysm which carries a very poor prognosis[1]. The coagulopathy may be present at the time of admission or may develop during the operation[2]. This condition has not been extensively investigated and the pathogenesis is as yet poorly understood. Although the pathogenesis of coagulopathy in this clinical situation remains obscure, there has been a considerable increase in understanding of the physiology and pathology of haemostatic systems in recent years. Disseminated intravascular coagulation associated with septicaemia is one of the few acquired coagulopathies which is well understood; it appears that endothelial cells, activated by endotoxin via tumour necrosis factor α (TNF-α), play a pivotal role in its pathogenesis[3]. It may be that endothelial cells, activated by hypoxia secondary to hypovolaemic shock, could play a similar role in the early pathogenesis of coagulopathy associated with ruptured aortic aneurysm.

This study was performed to determine whether there were morphological changes in endothelial cell ultrastructure at an early stage following ruptured aneurysm that could be associated with endothelial cell injury, and to establish a model for further studies of endothelial cell morphology and function after ruptured aneurysm or other causes of severe hypovolaemic shock.

Materials and methods

Fat samples were taken from the anterior abdominal wall immediately after skin incision at the start of operation in five patients undergoing operation for ruptured abdominal aortic aneurysm and from five control patients undergoing elective surgery for abdominal aortic aneurysm. The diagnosis of ruptured aneurysm was confirmed in all cases at the time of operation. The fat samples were cut into small blocks and immediately fixed in 2·5 per cent glutaraldehyde per 0·1 mol/l cacodylate buffer for 2–18 h. The samples were then post-osmicated (1 per cent osmium in cacodylate buffer) and embedded in Araldite (Ciba Geigy, Cambridge, UK). Two blocks of tissue were taken from each sample and sections (0·5–1·0 μm) were cut and stained with a mixture of 0·8 per cent toluidine blue and 0·2 per cent pyronin B. The sections were viewed under light microscopy to select appropriate areas from which ultrathin sections could be prepared subsequently. These were stained with uranyl acetate and lead acetate and viewed on a Philips (Eindhoven, The Netherlands) 301 electron microscope. Electron micrographs were taken of all capillaries present. The median number of capillaries examined in each patient was nine (ranging from six to 12).

Each electron micrograph was assessed blindly by three independent observers who graded each capillary for the presence or absence of luminal processes, budding, irregularity of the basement membrane and the presence of intracellular vesicles. Luminal processes were defined as broad-based processes projecting into the lumen. Processes which occurred at the site of cellular junction were excluded as these processes, known as marginal folds, are normal findings[4]. Budding was defined as the presence of three or more circular structures, approximately 0·1–0·5 μm in diameter, occurring on or near the luminal surface of the cell. Intracellular vesicles were defined as three or more cytoplasmic vesicles approximately 0·1–0·5 μm in diameter[4,5].

All patients having ruptured aneurysms were hypotensive on admission and one patient had an established coagulopathy at the start of the operation. No patient had received any blood products before operation. Two patients, both of whom had intraperitoneal rupture, died on the table. The patient with the coagulopathy on admission died in the early postoperative period with lower limb ischaemia and multiple organ failure. The two remaining patients had an uncomplicated recovery.

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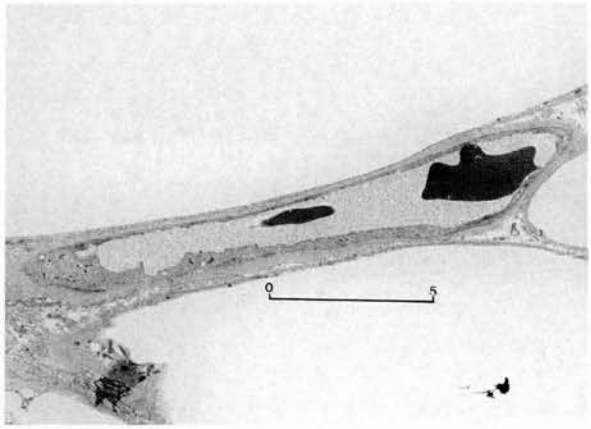

Fig. 1 Capillary from abdominal wall adipose tissue in a patient undergoing elective aortic surgery. Scale bar indicates 5 μm. A red blood cell is present in the lemen of the capillary

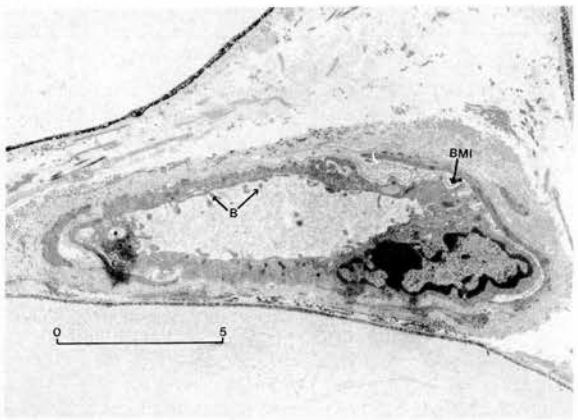

Fig. 2 Capillary from abdominal wall adipose tissue in a patient with ruptured aortic aneurysm. Endothelial cell budding (B) and basal membrane irregularity (BMI) are present. Scale bar indicates 5 μm

Results

Typical electron micrographs from patients in the control and study groups are illustrated in *Figs 1–3*. Examples of luminal processes, budding and irregularity of the basement membrane are shown. The numbers of capillaries in the control and study patients with each feature are shown in *Table 1*. Process formation on the luminal surface was significantly more common in patients who had suffered rupture than in the control group. Budding of the luminal surface was found in 11 of 45 of the capillaries from patients with ruptured aneurysms but in none of the capillaries from control patients ($P < 0{\cdot}005$, Fisher's exact test). Budding was found in at least one capillary from all patients with rupture but in none of the control patients ($P < 0{\cdot}05$, Fisher's exact test). The presence of intracellular vesicles, a sign of normal cellular ultrastructure[5], was more frequent in control patients, although this difference was not statistically significant.

Discussion

This study has demonstrated that there are ultrastructural differences between endothelial cells in patients undergoing operation for ruptured aortic aneurysm and patients undergoing elective operation for aortic aneurysm. The differences were observed in tissue samples taken at the start of operation and are thus unlikely to be attributable to artifacts generated by surgery or anaesthesia. The most marked difference observed was the presence of budding which was found in at least one capillary from all patients with rupture but in none of the capillaries from the patients in the control group. Although the authors have termed these structures 'budding' it must be remembered that transmission electron microscopy produces two-dimensional images and these structures may be cylindrical processes rather than spherical buds.

The ultrastructural changes in endothelial cells found in this study are similar to those described in heart capillaries of dogs subjected to a period of hypoxia produced by ligation of a coronary artery[6–8]. These studies describe loss of cytoplasmic vesicles after 10–30 min, and the production of blebs on the luminal surface of the cell after more prolonged ischaemia (60–180 min), although the blebs described are of greater size than the budding found in the present study. The endothelial cell budding observed has the appearance of shedding of membrane-bound vesicles[9]. Shedding is the release of cell surface constituents either in soluble form or in association with plasma membrane. Tissue factor, being an integral membrane glycoprotein, can be expressed in the plasma in this way[10,11].

The cause of the endothelial cell changes found in these patients has not been determined in this study. Several stimuli are known to cause endothelial cell activation:

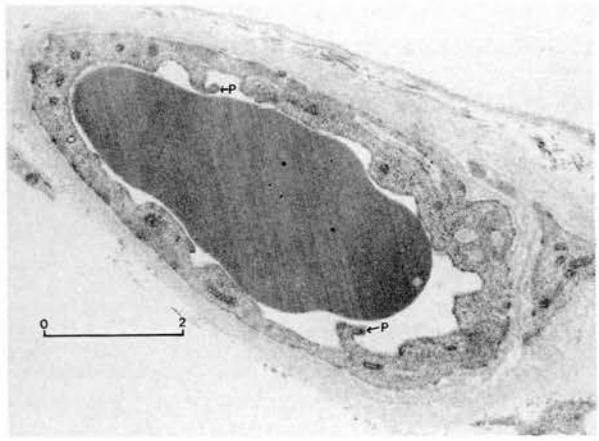

Fig. 3 Capillary from abdominal wall adipose tissue in a patient with ruptured aortic aneurysm. Processes (P) on the luminal surface are present. Scale bar indicates 2 μm

Table 1 Distribution of normal and abnormal ultrastructural features in capillaries in patients with ruptured aneurysms and controls

	Rupture group ($n = 45$)	Control group ($n = 44$)	P*
Luminal processes	37	26	$<0{\cdot}05$
Irregularity of basal membrane	12	11	n.s.
Budding	11	0	$<0{\cdot}005$
Intracellular vesicles	9	15	n.s.

*Fisher's exact test. n.s., Not significant

hypoxia, cytokines, endotoxin, activated leucocytes, thrombin and histamine[12]. In septic shock the trigger for endothelial cell activation[3] is endotoxin via TNF-α, but while patients with ruptured aneurysm may have some degree of endotoxaemia at the time of admission, it seems more likely that in these patients the stimulus is direct endothelial cell hypoxia. All patients studied were hypotensive on admission and it is not practice in this department to resuscitate aggressively to achieve normal blood pressure before operation but rather to proceed as quickly as possible to operation[13,14]. Hypovolaemic shock results in widespread tissue hypoperfusion and hypoxia which should be most marked in tissues such as subcutaneous fat. Such hypoxia has been shown to cause endothelial cell activation in cell culture and animal models[15].

The functional significance of these structural changes is a matter for speculation. No correlation has been shown in human studies between activation (expression of activation antigens) and the ultrastructural cell appearance. As stated above, it has been shown that hypoxia induces structural changes in endothelial cells in canine models and in one study it was shown that such cells demonstrate abnormal function in terms of vasodilatory reserve[16]. It has been shown in cell culture models that hypoxia can induce endothelial cells to express tissue factor[17–19]. This has not yet been demonstrated to be of direct clinical relevance in human studies but it does indicate that a mechanism exists whereby endothelial cells, activated by hypoxia, could stimulate thrombin production and trigger disseminated intravascular coagulation. It is of interest that only one of the patients in this study had evidence of an established coagulopathy; this suggests that the ultrastructural changes precede the development of coagulopathy in hypotensive shock, and that they may be an essential part of the process of development of disseminated intravascular coagulation in this clinical setting. If such functional changes can be demonstrated, these would have considerable clinical significance. However, further work, using techniques such as immunogold electron microscopy to examine cellular activity and function, is required to confirm this hypothesis.

In conclusion, this study shows that there are ultrastructural changes present in endothelial cells in adipose tissue at the start of operation in patients admitted with ruptured aortic aneurysm that warrant further investigation. In addition, the study has demonstrated that electron microscopic examination of tissue obtained at the start of operation is a feasible method for the detailed examination of pathological processes affecting endothelial cells in small vessels.

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References

1 Davies MJ, Murphy WG, Murie JA *et al.* Preoperative coagulopathy in ruptured abdominal aortic aneurysm predicts poor outcome. *Br J Surg* 1993; **80**: 974–6.
2 Getaz EP, Louw JH. The coagulopathy associated with aortic aneurysms. *Postgrad Med J* 1977; **53**: 668–71.
3 Levi M, ten Cate H, van der Poll T, van Deventer SJ. Pathogenesis of disseminated intravascular coagulation in sepsis. *JAMA* 1993; **270**: 975–9.
4 Bloom W, Fawcett DW. *A Textbook of Histology.* 10th ed. Philadelphia, Pennsylvania: WB Saunders, 1975: 386–425.
5 Cormack DH. *Ham's Histology.* 9th ed. Philadelphia, Pennsylvania: JB Lippincott, 1987: 439–49.
6 Armiger LC, Gavin JB. Changes in the microvasculature of ischemic and infarcted myocardium. *Lab Invest* 1975; **33**: 51–6.
7 Kloner RA, Ganote CE, Jennings RB. The 'no-reflow' phenomenon after temporary coronary occlusion in the dog. *J Clin Invest* 1974; **54**: 1496–508.
8 Kloner RA, Ellis SG, Lange R, Braunwald E. Studies of experimental coronary artery reperfusion. Effects on infarct size, myocardial function, biochemistry, ultrastructure and microvascular damage. *Circulation* 1983; **68**(Suppl I): 8–15.
9 Black PH. Shedding from the cell surface of normal and cancer cells. *Adv Cancer Res* 1980; **32**: 75–199.
10 Bona R, Lee E, Rickles F. Tissue factor apoprotein: intracellular transport and expression in shed membrane vesicles. *Thromb Res* 1987; **48**: 487–500.
11 Robinson RA, Worfolk L, Tracy PB. Endotoxin enhances the expression of monocyte prothrombinase activity. *Blood* 1992; **79**: 406–16.
12 Prydz H, Pettersen KS. Synthesis of thromboplastin (tissue factor) by endothelial cells. *Haemostasis* 1988; **18**: 215–23.
13 Crawford ES. Ruptured abdominal aortic aneurysm: an editorial. *J Vasc Surg* 1991; **13**: 348–50.
14 Westaby S. Resuscitation in thoracic trauma. *Br J Surg* 1994; **81**: 929–31.
15 Michiels C, Arnould T, Houbion A, Remacle J. Human umbilical vein endothelial cells submitted to hypoxia–reoxygenation *in vitro*. Implication of free radicals, xanthine oxidase and energy deficiency. *J Cell Biol* 1992; **153**: 53–61.
16 Forman MB, Puett DW, Bingham SE *et al.* Preservation of endothelial cell structure and function by intracoronary prefluorochemical in a canine preparation of reperfusion. *Circulation* 1987; **76**: 469–79.
17 Gertler JP, Welbe DA, Ocasio VH, Abbott WM. Hypoxia induces procoagulant activity in cultured human venous endothelium. *J Vasc Surg* 1991; **13**: 428–33.
18 Shreeniwas R, Ogawa S, Cozzolino F *et al.* Macrovascular and microvascular endothelium during long-term hypoxia: alterations in cell growth, monolayer permeability, and cell surface coagulant properties. *J Cell Physiol* 1991; **146**: 8–17.
19 Ogawa S, Geriach H, Esposito C, Pasaglan-Macaulay A, Brett J, Stern D. Hypoxia modulates the barrier and coagulant function of cultured bovine endothelium. Increased monolayer permeability and induction of procoagulant properties. *J Clin Invest* 1990; **85**: 1090–8.

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Randomized double-blind placebo-controlled trial of early octreotide in patients with postoperative enterocutaneous fistula

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Somatostatin and octreotide both enhance closure of gastrointestinal fistulas. The present trial was undertaken to test whether early combined treatment with parenteral nutrition and octreotide 100 μg every 8 h by subcutaneous injection had a beneficial effect compared with parenteral nutrition plus placebo. Thirty-one patients with postoperative gastrointestinal or pancreatic fistula were randomly assigned to receive parenteral nutrition plus octreotide (14 patients) or placebo (17) within 8 days of fistula onset. The percentage reduction in output and rate of spontaneous closure within 20 days were analysed. Mean(s.d.) reduction in output was similar after octreotide and placebo at 24 h (66(43) *versus* 68(47) per cent, $P = 0{\cdot}9$), 48 h (60(46) *versus* 57(43) per cent, $P = 0{\cdot}8$) and 72 h (62(50) *versus* 66(49) per cent, $P = 0{\cdot}9$) after starting the combined treatment. Closure within 20 days was observed in eight of 14 fistulas in patients given octreotide and in six of 17 in those receiving placebo ($P = 0{\cdot}4$). Administration of octreotide, within 8 days of fistula onset, associated with parenteral nutrition does not significantly increase the spontaneous fistula closure rate compared with parenteral nutrition plus placebo.

The powerful inhibitory effects of somatostatin on gastrointestinal secretion and motility fostered research on its use in the treatment of gastrointestinal fistula with encouraging initial results[1–3]. Three major limitations of native somatostatin are its very short plasma half-life (which makes continuous intravenous administration mandatory), the hormonal rebound phenomenon after stopping administration and its inhibitory effects on pancreatic insulin secretion, which may cause glucose intolerance[4]. Octreotide is a synthetic octapeptide analogue of somatostatin with a plasma half-life[5] of 90 min and a prolonged duration of action that makes subcutaneous administration possible[6].

Uncontrolled observations of reduced fistula output and enhanced closure after somatostatin or octreotide administration have been published[7–9]. In a placebo-controlled clinical trial octreotide effectively reduced small bowel fistula output in patients without sepsis[10] and a pilot clinical study demonstrated octreotide shortening the period of closure of well established intestinal fistulas[11]. The only open clinical trial published to date suggests a reduction in fistula output and shortening of closure interval with native somatostatin[12]. None of these studies, however, was designed as a double-blind trial. In a small placebo-controlled study, octreotide did not increase the spontaneous fistula closure rate or reduce fistula output[13]. In this study the time interval between fistula appearance and octreotide administration was not evaluated; the timing of administration may be a critical factor for octreotide to show any benefit. A multicentre, prospective, randomized, placebo-controlled, double-blind clinical trial was therefore undertaken to compare early administration of octreotide *versus* placebo in the treatment of postoperative enterocutaneous fistula.

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Patients and methods

Inclusion criteria

Between October 1988 and May 1992, all patients with postoperative enterocutaneous fistula of less than 8 days in duration with a daily output greater than 50 ml diagnosed at the surgical departments of any of the five participating centres were considered candidates to enter the trial.

Fistula classification

The origin of the fistula was proved in all patients by fistulography and/or upper gastrointestinal contrast studies and the lesion classified according to the anatomy and output as previously described[14]. Fistulas considered for the study belonged to group 1 (stomach and small bowel origin) either in the 1a (output less than 1000 ml per 48 h) or 1b (output more than 1000 ml per 48 h) categories. Pure pancreatic fistula was also included.

Exclusion criteria

Patients with a fistula arising from neoplastic or irradiated tissues were excluded from the trial. No fistula originating from gross anatomical abdominal wall defects and/or mucocutaneous continuity (type 2) or from colonic anatomoses (type 3) was included.

Ethics

The protocol and consent forms were approved by the Clinical Trials National Board of the Spanish Ministry of Health. The protocol was also approved by the clinical trial and ethics committee of the Hospital Universitari del Mar and other participating institutions. Informed consent was obtained from

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Fibrin Sealant Reduces Suture Line Bleeding During Carotid Endarterectomy: A Randomised Trial

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Objectives: To determine whether topical fibrin sealant reduced suture line bleeding during carotid endarterectomy with polytetrafluoroethylene (PTFE) patch closure.
Design: Prospective randomised non-blinded control trial.
Setting: Regional vascular surgery unit.
Materials: Seventeen patients undergoing carotid endarterectomy were randomised either to receive fibrin sealant as a topical haemostatic agent at the arteriotomy suture line or to act as control.
Outcome measures: Time taken to achieve haemostasis at the suture line. Intraoperative blood loss. Total operative time.
Results: The median time to achieve haemostasis was 5.5 min (range 4–31 min) in the treatment group and 19 min (range 10–47 min) in the control group. This difference was statistically significant $p < 0.005$ by Mann-Whitney test. There was no statistical difference in total operative time. Operative blood loss was lower in the treatment group (median 420ml, range 300–500ml) than in the control group (median 550ml, range 350–1200ml) but this difference was not statistically significant. One patient in the control group suffered a perioperative thrombo-embolic event.
Conclusion: Fibrin sealant is an effective topical haemostatic agent for arteriotomy suture lines involving PTFE material.

Key Words: Controlled randomised trial; Polytetrafluoroethylene; Carotid surgery; Fibrin sealant; Haemostasis.

Introduction

Suture line bleeding at vascular anastomoses is a problem which can increase operative blood loss and lengthen operating time. This is especially a problem in anastomoses involving polytetrafluoroethylene (PTFE) grafts and patches and may be exacerbated by the use of intraoperative heparin.

Fibrin sealant is a topical haemostatic agent which consists of two principal components, fibrinogen and thrombin, which are mixed, in the presence of Factors VIII and XIII, fibronectin and calcium to produce insoluble fibrin. Thus the main components of the final stage in the physiological pathway of coagulation are present.

Fibrin sealant of this form was first described in 1972 as a tissue adhesive for nerve repair.[1] Since then it has been used in a wide variety of clinical settings: otolaryngology,[2] plastic surgery,[3,4] neurosurgery,[5–7] thoracic surgery,[8–12] hepatobiliary surgery,[13,14] pancreatic surgery,[15,16] colorectal surgery,[17] gynaecology[18,19] and cardiac surgery.[20–26]

The possibilities for using fibrin sealant in cardiovascular surgery were recognised by Spangler early on during its development.[27] Borst and Haverich (1982) further developed and promoted the use of fibrin sealant in this field describing its use for sealing knitted Dacron aortic grafts, puncture holes and suture lines in vascular anastomoses.[20,21]

The success of fibrin sealant in cardiac surgery would suggest that it may also be useful in peripheral vascular surgery. However there have been few studies of fibrin sealant in this setting. In randomised trials using an animal model of peripheral vascular surgery with PTFE grafts, fibrin sealant was shown to be more effective than oxidised regenerated cellulose in controlling anastomotic bleeding.[28,29] However there are no reports of the clinical use of fibrin sealant

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in peripheral vascular surgery. Carotid endarterectomy using a PTFE patch is associated with prolonged anastomotic bleeding and the aim of this study was to determine whether fibrin sealant could significantly reduce suture line bleeding in these patients.

Material and Methods

The study was a prospective, randomised non-blinded trial. An initial pilot study involving eight patients was carried out in order to familiarise surgeons and theatre staff with the use of fibrin sealant prior to the commencement of the trial.

Patients and operative details

Patients were recruited from the vascular surgery unit of the Royal Infirmary of Edinburgh. All patients gave informed written consent and the trial was approved by the local ethical committee. Past medical history and drug use within the previous 14 days was recorded. To allow assessment of the viral safety of the sealant (as part of a larger safety study) patients who were known to be seropositive for anti-HIV, who had a history of hepatitis or whose liver function tests were outside the normal range were excluded. In addition, patients with a history of severe reactions to blood products or concurrent disease which might compromise their ability to be retained within the study were also excluded. A venous blood sample was taken for haemoglobin, haematocrit, platelet and white cell count, prothrombin time, activated partial thromboplastin time and liver function tests. A serum sample was stored at –40°C for later viral studies if required.

The mean age of patients was 64.5 years (range 48–75 years) and there was no difference in mean age between the patients in the treatment and control groups. Fifteen patients were taking regular aspirin; both the patients not taking aspirin were in the control group. No patient had any abnormality on preoperative coagulation screen.

Following entry into the trial the patients were randomised in a computer-generated sequence to treatment or control groups. In the treatment group, following completion of the vascular anastomosis, fibrin sealant was applied to the suture line using a dual syringe technique.[30] In the control group nothing was applied to the suture line. The clamps were released 2 min after application was complete. Pressure was applied with surgical swabs if there was significant bleeding. The surgeon was allowed to use haemostatic gauze at any time. The arteriotomy was inspected frequently and the time taken to achieve haemostasis from release of the clamps was recorded.

Surgical treatment was otherwise identical in the two groups. All operations were performed under general anaesthetic with subcutaneous local anaesthetic infiltration. The operations were all performed (6/9 control; 5/8 treatment) or supervised by one surgeon (C.V.R.). All patients received intraoperative heparin, 5000 I.U. before the artery was clamped. The heparin was not reversed. Arteriotomy closure was performed using a 0.5 mm PTFE patch (WL Gore) and 7.0 Coretex suture. In both groups any further manoeuvres, such as application of pressure or haemostatic gauze, required to achieve haemostasis were recorded. The length of operation, blood product usage were recorded and a personal assessment by the surgeon of effectiveness and ease of use was also recorded.

Patients were followed-up for 26 weeks. Blood was taken at 24 h and 5 days for haemoglobin, haematocrit, platelet count and white cell count; at 6 weeks and 12 weeks for liver function tests and at 26 weeks for virology testing. Virology testing consisted of determining the presence of hepatitis B surface antigen and enzyme immunoassay for antibodies to hepatitis A and hepatitis C, bioluminescence assay for antibodies to hepatitis B surface antigen, and radioimmunoassay for antibodies to hepatitis B core antigen.

Fibrin sealant kit was provided for the study by the Scottish National Blood Transfusion Service (SNBTS). Heat-treated human fibrinogen was manufactured at the SNBTS Protein Fractionation Centre from pooled donor plasma cryosupernatant. Solvent detergent treated human thrombin preparation was supplied by the Centre Regional de Transfusion Sanguine (CRTS) (Lille, France). The fibrin sealant supplied by SNBTS uses concentrations of thrombin between 200–500 I.U./ml and fibrinogen 29–39g/l.[31]

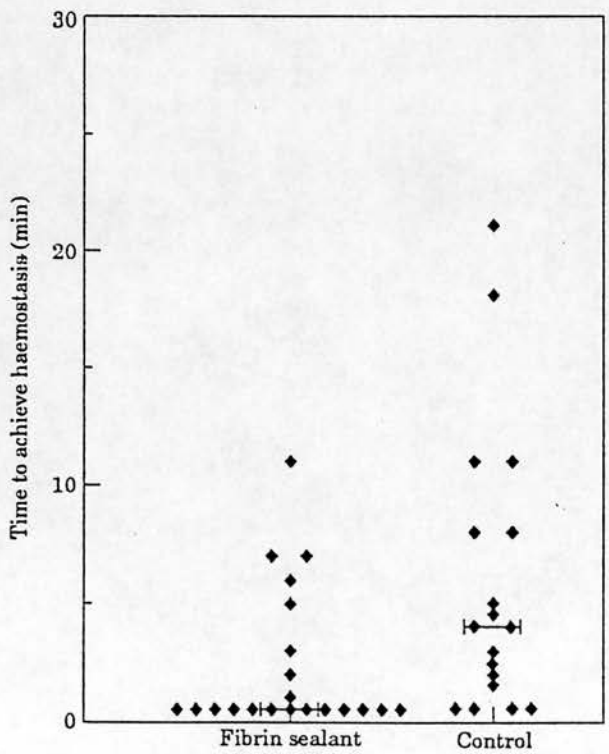
After 17 patients had been randomised it appeared to the surgeon and anaesthetist that there was a clinical benefit in patients treated with fibrin sealant and an interim analysis of the data was requested. This confirmed that there was a statistically significant benefit and the trial was stopped. Statistical analysis was carried out using a Mann-Whitney U test.

Results

The median time to achieve haemostasis was 5.5 min

(range 4–31 min) in the treatment group and 19 min (range 10–47 min) in the control group (Fig. 1). This difference was statistically significant $p < 0.005$ by Mann-Whitney test. There was no statistical difference in total operative time. Operative blood loss was lower in the treatment group (median:420ml, range:300–500ml) than in the control group (median 550ml, range 350–1200ml) but this difference was not statistically significant. All patients (9/9) in the control group and one patient (1/8) in the fibrin sealant group required the use of oxidised cellulose gauze to aid haemostasis. In no cases were further sutures required to achieve haemostasis. There were no significant differences in the drop in haemoglobin following operation. There was no serological evidence of hepatitis A, B or hepatitis C transmission at 6 months and no patients developed abnormal liver function tests during follow-up.

One patient in the control group suffered a post-operative cerebrovascular accident on the ipsilateral side to operation. There was no evidence of arterial embolism or thrombosis in the treatment group. No patients required reoperation for control of haemorrhage or evacuation of haematoma. There were no wound complications in any patient in either group.



Discussion

This study has shown that fibrin sealant is an effective topical haemostatic agent in vascular surgery. The only statistically significant benefit was a shorter time to achieve haemostasis. Blood loss was lower in the treatment group but this did not attain statistical benefit. This may be a type II statistical error: the trial was terminated after interim analysis was requested by the surgical team to confirm a clinical impression of haemostatic effectiveness.

It may be expected that fibrin-based adhesives and sealants are likely to be useful agents for surgical haemostasis and tissue adhesion since fibrin plays a central role in the physiological process of wound healing. Thus problems of foreign body reaction, delayed healing and fibrosis, which are associated with other haemostatic agents, might be avoided. The major advantages of fibrin-based glues over other tissue adhesives are tissue compatibility, biodegradability and the ability to adhere to wet surfaces. It may even promote healing since the first stage in normal wound healing is the production of a fibrin network into which fibroblasts and capillaries migrate. Evidence in support of this has been derived from several animal studies.[28,29,31,34]

Concern has been expressed that viral transmission could occur as a result of fibrin sealant use. Theoretically virus remaining in the preparation could be adsorbed and retained by the patient and there are reports in the literature which implicate cryoprecipitated fibrinogen as a mechanism for the transmission of HIV-1 and hepatitis.[35,36] Therefore the method of production has been designed to minimise the risk of viral transmission. The safety of fibrin sealant is further supported by this study in which no evidence of viral transmission could be found. The study of viral safety of this product is continuing, involving approximately 100 patients in detailed follow-up.

A further concern over the use of fibrin sealant on arterial suture lines is that it may promote intravascular thrombosis or embolism. No evidence to support such concerns was found in this study or has been reported elsewhere. There was only one embolic event and that was in a patient in the control group.

This study demonstrates that fibrin sealant is an effective and safe haemostatic agent for vascular suture lines involving PTFE.

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References

1 Matras H, Dinges H, Lassman H, Mammoli B. Zur nahtlosen interfaszikularen nerventransplantation im tierexperiment. (Suture-free interfascicular nerve transplantation in animal experiments). *Wien Med Wochenshr* 1972; 122: 517–523.

2 Staindl O. Tissue adhesion with highly concentrated human fibrinogen in otolaryngology. *Ann Otol* 1979; 88: 413–418.

3 Marchac D, Renier D. Fibrin glue in craniofacial surgery. *J Craniofacial Surg* 1990; 1: 32–34.

4 Vobel A, O'Grady K, Toriumi DM. Surgical tissue adhesives in facial, plastic and reconstructive surgery. *Facial Plastic Surg* 1993; 9: 49–57.

5 Pomeranz S, Constantini S, Umansky F. The use of fibrin sealant in cerebrospinal fluid leakage. *Neurochirurgia* 1991; 34: 166–169.

6 Lee KC, Park SK, Lee KS. Neurosurgical application of fibrin adhesive. *Yonsei Med J* 1991; 32: 53–57.

7 Cain JE, Dryer RF, Barton BR. Evaluation of dural closure techniques. Suture methods, fibrin adhesive sealant and cyanoacrylate polymer. *Spine* 1988; 13: 720–725.

8 Mouritzen C, Dromer M, Keinecke HO. The effect of fibrin glueing to seal bronchial and alveolar leakages after pulmonary resections and decortications. *Eur J Cardio-Thorac Surg* 1993; 7: 75–80.

9 Thetter O. Fibrin adhesive and its application in thoracic surgery. *Thorac Cardiovasc Surg* 1981; 29: 290.

10 McCarthy PM, Trastek VF, Bell DG *et al.* The effectiveness of fibrin glue for reducing experimental pulmonary air leak. *Ann Thorac Surg* 1988; 45: 203–205.

11 Goldman CD, Blocker SH, Ternberg JL, Crouch EC. Management of experimental pneumothorax in weanling rabbits with the use of fibrin glue sclerosant. *Arch Surg* 1986; 121: 565–568.

12 Jessen C, Sharma P. Use of fibrin glue in thoracic surgery. *Ann Thorac Surg* 1985; 39: 521–524.

13 Chisholm RA, Jones SN, Lees WR. Fibrin sealant as a plug for the post liver biopsy needle track. *Clin Radiol* 1989; 40: 627–628.

14 Kohno H, Nagasue N, Chang YC, Tantura H, Yamanoi A, Nakamura T. Comparison of topical haemostatic agents in elective hepatic resection: a clinical prospective randomized trial. *World J Surg* 1992; 16: 966–969.

15 Marczell AP, Stierer M. Partial pancreatiocoduodenectomy (Whipple procedure) for pancreatic malignancy: occlusion of a non-anastomosed pancreatic stump with fibrin sealant. *HPB Surg* 1992; 5: 251–259.

16 Kram HB, Clark SR, Ocampo HP, Yamaguchi MA, Shoemaker WC. Fibrin glue sealing of pancreatic injuries, resections and anastomoses. *Am J Surg* 1991; 161: 479–481.

17 Hjortup A, Moesgaard F, Kjaergard J. Fibrin adhesive on the treatment of perineal fistulas. *Dis Colon Rectum* 1991; 34: 752–754.

18 Feichtinger W, Barad D, Feinman M, Barg P. The use of fibrin sealant for embryo transfer. *Fertil Steril* 1990; 54: 733–734.

19 Adamyan LV, Myinbayev OA, Kulakov VI. Use of fibrin glue in obstetrics and gynaecology: a review of the literature. *Int J Fertil* 1991; 36: 81–88.

20 Borst HG, Haverich A, Walterbusch G, Maatz W. Fibrin adhesive: An important hemostatic adjunct in cardiovascular operations. *J Thorac Cardiovasc Surg* 1982; 84: 548–553.

21 Borst HG, Haverich A. Fibrin seal in cardiovascular surgery. Proceedings of a workshop. *Thorac Cardiovasc Surg* 1982; 30: 195–224.

22 Rousou J, Gonzalez-Lavin L, Cosgrove D *et al.* Randomized clinical trial of fibrin sealant in patients undergoing resternotomy or reoperation after cardiac operations. *J Thorac Cardiovasc Surg* 1989; 97: 194–203.

23 Rousou JA, Engelman RM, Breyer RH. Fibrin glue: an effective hemostatic agent for non-suturable intraoperative bleeding. *Ann Thorac Surg* 1984; 38: 409–410.

24 Sponitz WD, Dalton MS, Baker JW, Nolan SP. Reduction of perioperative hemorrhage by anterior mediastinal spray application of fibrin glue during cardiac operations. *Ann Thorac Surg* 1987; 44: 529–531.

25 Seguin JR, Frapier J-M, Colson P, Chaptal P. Fibrin sealant improves surgical results of type A acute aortic dissections. *Ann Thorac Surg* 1991; 52: 745–749.

26 Stark J, de Leval M. Experience with Fibrin Seal (Tisseel) in operations for congenital heart defects. *Ann Thorac Surg* 1984; 38: 411–413.

27 Spangler H. Gewebeklebung und lokale blutstillung mit fibrinogen, thrombin und blutgerinnungsfaktor XIII. *Wien Klin Wochenschr* 1976; 88 (suppl 49): 1–18.

28 Kram H, Nugent P, Reuben B, Shoemaker W. Fibrin glue sealing of polytetrofluoroethylene vascular graft anastamoses: comparison with oxidosed cellulose. *J Vasc Surg* 1988; 8: 563–568.

29 Jakob H, Campbell C, Qui Z, Pick R, Repogle R. Use of fibrin sealant for reinforcing arterial anastomoses. *J Vasc Surg* 1984; 1: 171–179.

30 Matras H. Fibrin seal: the state of the art. *J Oral Maxillofac Surg* 1985; 43: 605–611.

31 Byrne D, Hardy J, Wood R, McIntosh R, Cischieri A. Effect of fibrin glues on the mechanical properties of healing wounds. *Br J Surg* 1991; 78: 841–843.

32 Harris DM, Siedentop KH, Ham KR, Sanchez B. Autologous fibrin tissue adhesive biodegradation and systemic effects. *Laryngoscope* 1987; 97: 1141–1144.

33 Jonas R, Schoen F, Ziemer G, Britton L, Castaneda A. Biological sealants and knitted dacron conduits: Comparison of collagen and fibrin glue pretreatments in circulatory models. *Ann Thorac Surg* 1987; 44: 283–290.

34 Jonas R, Schoen F, Levy R, Castaneda A. Biological sealants and knitted dacron: porosity and histological comparisons of vascular graft materials with and without collagen and fibrin glue pretreatments. *Ann Thorac Surg* 1986; 41: 657–663.

35 Wilson SM, Pell P, Donegan EA. HIV-1 transmission following the use of cryoprecipitated fibrinogen as a gel/adhesive. *Transfusion* 1991; 31: 515.

36 Bove JR. Fibrinogen — Is it worth the risk? *Transfusion* 1978; 19: 129–136.

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