NEW PERSPECTIVES IN ABDOMINAL AORTIC ANEURYSM MANAGEMENT

By

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MB ChB (Leic 1991), FRCS (Edin 1996)

A thesis submitted to the University of Leicester for the Degree of Doctor of Medicine (MD)

Department of Surgery, University of Leicester, UK.

March 2000
The work on which this thesis is based, is my own independent work except where acknowledged.

Jonathan Boyle

March 2000
Dedicated to Patricia and Anna

'A pint, why that's very nearly an armful!'

Abstract: New perspectives in abdominal aortic aneurysm management

Jonathan Robert Boyle MB ChB, FRCS

The prevalence of abdominal aortic aneurysms is rising in the Western World with rupture accounting for approximately 10,000 deaths per annum in England and Wales. The condition commonly affects elderly men, who are offered elective surgical repair if they are anaesthetically fit and the aneurysm is greater than 5.5cm diameter. Conventional surgical repair carries an operative mortality rate of 5% in most centres. There are two sub-populations of patients who pose management problems, firstly those patients who have small aneurysms, in whom the risk of surgical intervention is greater than that of rupture and secondly elderly patients or those with severe co-morbidity with aneurysms greater than 5.5cm, in whom mortality rates may reach 60% after elective repair.

A better understanding of the pathophysiology of abdominal aortic aneurysms has recently been established paving the way for potential targeted pharmacotherapy aimed at inhibiting the growth of small aneurysms. In particular the matrix metalloproteinase enzymes have been implicated in the destruction of the aortic wall. To this end the first part of this thesis investigates the potential therapeutic role of doxycycline, a non-specific metalloproteinase inhibitor, in an established model of aneurysmal disease. Subsequently the role of Amlodipine a calcium antagonist and metalloproteinase potentiator is investigated in the same model.

Endovascular AAA repair is a new minimally invasive technique that allows treatment of aortic aneurysms without major abdominal surgery. The feasibility of this technique has been established, however a number of important questions remain unanswered. The second half of this thesis investigates the invasiveness of endovascular repair in comparison to conventional surgery. In particular the impact both procedures have on respiratory, cardiac, renal and metabolic responses is studied in comparative cohorts undergoing both conventional and endovascular AAA repair. Finally the implications of offering a tertiary referral service for AAA treatment is investigated.

The results presented in this thesis demonstrate that doxycycline inhibits MMP activity and thus elastin destruction in a porcine model of aneurysmal disease. In the same model however, Amlodipine potentiates MMP activity and accelerates elastin degradation. There may be a therapeutic role for doxycycline in reducing the growth rate of small aneurysms. The clinical investigations of this thesis show that endovascular AAA repair attenuates the respiratory, cardiovascular, renal and metabolic responses associated with conventional aneurysm surgery. There were however still considerable insults from endoluminal surgery. Endovascular AAA repair may reduce morbidity and mortality rates after elective AAA surgery. The last experimental chapter illustrates that offering endovascular AAA repair as a tertiary centre has considerable clinical and financial implications. Finally a number of problems remain with endovascular AAA surgery which require evaluation by randomised controlled trial before its widespread use.
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The clinical research presented in this thesis would not have been possible without close collaboration between the Departments of Surgery, Anaesthesia, Radiology and Pathology. In particular I would like to thank Dr Jonathan P Thompson and Professor Graham Smith for anaesthetising all the patients, and further thank Jonathan for his collection and analysis of overnight oxygen saturation recordings, cardiovascular indices and his supervision of the catecholamine assays. I would like to acknowledge the help of Dr Guy Fishwick and Dr Aman Bolia for the radiological assessment of all the patients and would also like to thank the radiographers in the angiography suite and CT scanner, who always managed to fit in patients, despite a heavy workload. I also extend my thanks to Professor Nick JM London and Mr A. Ross Naylor for allowing their patients to participate in the study. My thanks also go the departments of Haematology and Chemical Pathology for full blood count and clotting analysis and the albumin/creatinine ratios respectively.

The in vitro experimental work presented in this thesis was based on the model created by Dr Andy D Wills and further developed by Dr Edward McDermott, my thanks go to both, who patiently taught me the experimental techniques needed to complete this work. I would also like to acknowledge Mr M Crowther for his help with the Western blotting, Mr Ewan MacCauley for the NAG assay technique and particularly Mr Steve Goodall for his help with all the assays. I would also like to thank Dawkins International (Nuneaton, UK) for the provision of tissue samples and Pfizer (Sandwich, UK) for providing the doxycycline and amlodipine.

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Lastly I thank my wife, Patricia, for her constant and unfailing support and encouragement during the preparation of this thesis.
**ABBREVIATIONS**

<table>
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<tbody>
<tr>
<td>AAA</td>
<td>Abdominal aortic aneurysm</td>
</tr>
<tr>
<td>ACR</td>
<td>Albumin creatinine ratio</td>
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<tr>
<td>AD</td>
<td>Anno domini</td>
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<tr>
<td>AOD</td>
<td>Atherosclerotic occlusive disease</td>
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<tr>
<td>AP</td>
<td>Anteroposterior</td>
</tr>
<tr>
<td>ARDS</td>
<td>Adult respiratory distress syndrome</td>
</tr>
<tr>
<td>ARF</td>
<td>Acute renal failure</td>
</tr>
<tr>
<td>ASA</td>
<td>American Society of Anesthesiology</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BAPN</td>
<td>Beta-aminopropionitrile fumarate</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary artery bypass grafting</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CCO</td>
<td>Continuous cardiac output</td>
</tr>
<tr>
<td>CFA</td>
<td>Common femoral artery</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval or Cardiac index</td>
</tr>
<tr>
<td>CIA</td>
<td>Common iliac artery</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>COAD</td>
<td>Chronic obstructive airways disease</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<tr>
<td>cm</td>
<td>Centimetre</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<td>CVP</td>
<td>Central venous pressure</td>
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<tr>
<td>DI</td>
<td>Desaturation index</td>
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<tr>
<td>DTS</td>
<td>Dipyridamole thallium scintigraphy</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EIA</td>
<td>External iliac artery</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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et al. et alia (and others)
EVG Elastin van Gieson
EVT Endovascular Technologies
FCS Fetal calf serum
FDPs Fibrinogen degradation products
FEV1 Forced expiratory volume in one second
FVC Forced vital capacity
GI Gastrointestinal
GP General practice
H Hour
H+E Haemotoxylin and eosin
HR Heart rate
IA-DSA intra-arterial digital subtraction angiography
ICAM Intercellular adhesion molecule
ICU Intensive care unit
IDDM Insulin dependant diabetes mellitus
IIA Internal iliac artery
IL Interleukin
IMS Industrial methylated spirits
ISCVS International Society of Cardiovascular Surgery
IVC Inferior vena cava
IVUS Intravascular ultrasound.
KDa Kilodaltons
l Litre
LVSWI Left ventricular stroke work index
M Molar
MAP Mean arterial pressure
MEM Minimal essential medium
MHC Major histocompatibility complex
MI Myocardial infarction
min Minute
<table>
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<th>Description</th>
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<tbody>
<tr>
<td>ml</td>
<td>millilitre</td>
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<tr>
<td>mm</td>
<td>millimetre</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinases</td>
</tr>
<tr>
<td>MMPI</td>
<td>Matrix metalloproteinase inhibitor</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic resonance</td>
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<tr>
<td>MRA</td>
<td>Magnetic resonance angiography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>4-MU</td>
<td>4-methylumbelliferyone</td>
</tr>
<tr>
<td>MUGA</td>
<td>Multigated acquisition scan</td>
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<tr>
<td>MW</td>
<td>Molecular Weight</td>
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<tr>
<td>NAG</td>
<td>N-acetyl glucosamidase</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCA</td>
<td>Patient controlled analgesia</td>
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<tr>
<td>PCWP</td>
<td>Pulmonary capillary wedge pressure</td>
</tr>
<tr>
<td>PMSF</td>
<td>Phenylmethylsulphonyl fluoride</td>
</tr>
<tr>
<td>PTCA</td>
<td>Percutaneous transluminal coronary angioplasty</td>
</tr>
<tr>
<td>PVRI</td>
<td>Pulmonary vascular resistance index</td>
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<tr>
<td>RT-PCR</td>
<td>Reverse transcriptase polymerase chain reaction</td>
</tr>
<tr>
<td>RVSWI</td>
<td>Right ventricular stoke work index</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulphate polyacrylamide gel electrophoresis</td>
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<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
</tr>
<tr>
<td>SpO₂</td>
<td>Oxygen saturation</td>
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<tr>
<td>SVRI</td>
<td>Systemic vascular resistance index</td>
</tr>
<tr>
<td>SVS</td>
<td>Society for Vascular Surgery</td>
</tr>
<tr>
<td>TBS-T</td>
<td>Tris-buffered saline</td>
</tr>
<tr>
<td>TIMP</td>
<td>Tissue inhibitor of metalloproteinases</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>------------------------------</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>V</td>
<td>Volts</td>
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<tr>
<td>WBC</td>
<td>White blood cell</td>
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<td>yrs</td>
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PUBLICATIONS ARISING FROM THIS THESIS

PUBLISHED PAPERS


**PRESENTATIONS TO LEARNED SOCIETIES**


7. JR Boyle, MM Thompson, A Nasim, RD Sayers, C Maltezos, G Fishwick, PRF Bell. 
A comparison of CT and Duplex scanning in assessing aortic morphology following 
endovascular aneurysm repair.
International Congress X on Endovascular Interventions, Scottsdale, Arizona 9th -13th 
February 1997.

8. JR Boyle, MM Thompson, T Hartshorne, C Maltezos, A Nasim, RD Sayers, 
G Fishwick, PRF Bell.
The role of Duplex in assessing morphology following endovascular aneurysm repair.
Midland Vascular Surgical Society, Burton on Trent, 14th March 1997.

9. JR Boyle, MM Thompson, RD Sayers, A Nasim, P Healy, PRF Bell.
The changes in referral practice, workload and operative mortality following the 
establishment of endovascular abdominal aortic aneurysm repair.
Winner of the Midland Vascular Surgical Society Registrar's Prize.
Midland Vascular Surgical Society, Burton on Trent, 14th March 1997.

10. JR Boyle, MM Thompson, RD Sayers, A Nasim, P Healy, PRF Bell.
The change in referral practice following the establishment of endovascular abdominal 
aortic aneurysm repair.
Association of Surgeons of Great Britain and Ireland, Annual Meeting, Bournemouth, 9th - 
11th April 1997.

11. JR Boyle, JP Thompson, MM Thompson, RD Sayers, G Smith, PRF Bell.
Respiratory function after endovascular and conventional AAA surgery.
Association of Surgeons of Great Britain and Ireland, Annual Meeting, Bournemouth, 9th - 
11th April 1997.

12. JR Boyle, S Goodall, IM Loftus, M Crowther, E McDermott, AD Wills, PRF Bell, 
MM Thompson.
Doxycycline inhibits and Amlodipine potentiates elastin degradation and 
metalloproteinase activity in a model of aneurysmal disease.
ESVS and EAVST Prize Presentation. European Society for Vascular Surgery XI 

13. JR Boyle, IM Loftus, S Goodall, M Crowther, PRF Bell, MM Thompson.
Calcium antagonists accelerate elastin destruction in a model of aneurysmal disease.
Patey Prize Presentation. Surgical Research Society, St Thomas’ Hospital, London, 

14. JR Boyle, MM Thompson, RD Sayers, A Nasim, G Fishwick, PRF Bell.
The Long-term results of aortomonoiliac endovascular grafting are superior to aortoaoortic 
tube grafts.
International Congress XI on Endovascular Interventions, Scottsdale, Arizona 8th -12th 
February 1998.
15. JR Boyle, S Goodall, JP Thompson, PRF Bell, MM Thompson.
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Endovascular abdominal aortic aneurysm repair attenuates the renal dysfunction associated with conventional surgery.

PUBLISHED ABSTRACTS

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A comparison of CT and Duplex scanning in assessing aortic morphology following endovascular aneurysm repair.

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Development of an endovascular aorto-unii-iliac graft: difficult solutions for difficult aneurysms.

3. JR Boyle, E McDermott, M Crowther, A Wills, PRF Bell, MM Thompson.
Doxycycline inhibits elastin degradation and metalloproteinase production in a model of aneurysmal disease.

4. JR Boyle, E McDermott, M Crowther, A Wills, PRF Bell, MM Thompson.
Doxycycline inhibits elastin degradation and metalloproteinase production in a model of aneurysmal disease.

5. JR Boyle, MM Thompson, RD Sayers, A Nasim, P Healy, PRF Bell.
The change in referral practice following the establishment of endovascular abdominal aortic aneurysm repair.
Br J Surg 1997;84: Suppl. 1 43.

6. JR Boyle, JP Thompson, MM Thompson, RD Sayers, G Smith, PRF Bell.
Respiratory function after endovascular and conventional AAA surgery.
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Pathophysiology of Abdominal Aortic Aneurysms

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<td>1.10 Summary</td>
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1.1 Definition

The term "aneurysm" is derived from the Greek word *aneurynein*, meaning to widen or dilate (Gosling 1994). With respect to abdominal aortic aneurysms (Figure 1.1 and Figure 1.2), three definitions have been proposed (Sterpetti et al. 1987; Collin 1990a; Johnston et al. 1991; Moher et al. 1992). The most commonly used (Moher et al. 1994) is that reported by the Society for Vascular Surgery (SVS), who with the International Society of Cardiovascular Surgery (ISCVS) proposed that an aneurysm can be defined as a "permanent localised dilation of an artery having at least a 50% increase in diameter compared to the expected normal diameter of the artery in question" (Johnston et al. 1991).

The ISCVS also recommended that aneurysms be classified by a combination of characteristic factors such as site (e.g. abdominal aorta, popliteal), aetiology, histological features (morphology e.g. fusiform or saccular), and clinico-pathological manifestations (pulsatile mass, thromboembolism, pressure effects, rupture, fistula) (Johnston et al. 1991). An aetiological classification of arterial aneurysms may include one of a variety of "specific" causative factors affecting the integrity of the arterial wall, such as congenital disorders of connective tissue (e.g. Marfan and Ehlers-Danlos syndromes) (Johnston et al. 1991), blunt trauma ("false" aneurysm) (Johnston et al. 1991; Cook et al. 1994), cystic medial necrosis (resulting in a "dissecting" aneurysm) (Johnston et al. 1991; Ponraj and Pepper 1992), infectious agents such as *treponema pallidum* (i.e. mycotic aneurysms) (Johnston et al. 1991) (Pasic et al. 1992), anastomotic aneurysms (Johnston et al. 1991), various "inflammatory" diseases (Takayasu's, Behcet's and Kawasaki's disease) and autoimmune reactions (Johnston et al. 1991; Rijbroek et al. 1994). All of the above represent distinct pathologies manifesting themselves as an aneurysm. However, it is the "non-specific" aneurysm, commonly referred to as an atherosclerotic aneurysm (Johnston et al. 1991), which is the most prevalent (Johnston et al. 1991; Reed et al. 1992). The site most often affected is the abdominal aorta just distal to the renal arteries (Reed et al. 1992; Gillespie et al. 1992). With this epidemiology in mind, this
Figure 1.1: Operative photograph of an abdominal aortic aneurysm.

Figure 1.2: Operative photograph of a conventional repair of an AAA.
project will concentrate only on non-specific aneurysms of the infrarenal abdominal aorta.

1.2 Histology

The surgical management of abdominal aortic aneurysms has remained relatively unchanged over the past forty years. The morbidity and mortality from surgery, however, have been improved by the advent of new graft technology and advances in anaesthetic and post-operative intensive care. The development of endovascular techniques may improve results further. Despite improvement in outcome, the pathogenesis of aneurysms remains unclear. Over the past decade the understanding of the disease process has changed, from that of a simple weakening of the vessel wall, to a complex and extensive circumferential remodeling involving both synthesis and degradation of matrix proteins by native vascular cells and migrating leukocytes.

Arteries consist of three layers. The tunica intima is bound by a monolayer of endothelial cells on its luminal surface and the internal elastic lamina on its outer aspect. The tunica media lies between the internal and external elastic laminae and the external elastic lamina and the outer surface of the vessel delineate the tunica adventitia.

The ability of the arterial wall to counter the force exerted by the blood is dependent on maintenance of the structural proteins of the media and adventitia (Baxter et al. 1992). Collagen and elastin are the most abundant structural proteins of the aorta, imparting both strength and distensibility, resulting in uniform stresses and appropriate viscoelastic responses to pulsatile oscillations. Smooth muscle cells (SMCs) are the major cell types within the aorta (Galis et al. 1994a). In conjunction with the adventitial population of fibroblasts they synthesize the important connective tissue components of the extracellular matrix (ECM) including collagens, elastin and proteoglycans.

The media of the normal infrarenal aorta is arranged in a series of well-defined concentric elastic lamellae. Each layer is bound by relatively thick elastin bands, and contains circumferentially orientated collagen bands, a network of fine elastin fibres and a layer of SMC's. The close association between these three components of the aortic
media is responsible for the viscoelastic properties of the aorta (Wolinsky and Glagov 1967b).

In abdominal aortic aneurysms the regularly arranged elastic lamellae are disrupted. The disease is characterised by degeneration, destruction and remodeling of the aortic wall, with thickening of the tunic intima and tunica adventitia and a marked loss of extracellular components from the tunica media, most notably elastin (Baxter et al. 1992). The elastin and SMC's concentrations are reported to have decreased by 91% in a stereological study of aortic aneurysms (He and Roach 1994). The decrease in concentration of both elastin and SMC's are accompanied by an increase in collagen concentration and a ubiquitous inflammatory cell infiltrate (Koch et al. 1990).

1.3 Changes in the Extracellular Matrix

Abdominal aortic aneurysms are characterised by an overall thickening of the aortic wall, an increase in total protein, microfibrillar protein and collagen content together with a marked reduction in elastin concentration, and in the number of smooth muscle cells (Koch et al. 1990; Baxter et al. 1992; Baxter et al. 1994). The depletion in elastin in aneurysmal tissue was first described by Sumner et al (Sumner et al. 1970) and this has been confirmed by numerous subsequent studies (Campa et al. 1987; Powell and Greenhalgh 1989; Gargiulo et al. 1993; Gandhi et al. 1994). The concentration of elastin may however be misleading, as although it has been reported to be as low as 5-8% in aneurysmal aorta (Campa et al. 1987; Sakalihasan et al. 1993) (Powell and Greenhalgh, 1989), compared to 15-35% in aged matched controls (Powell and Greenhalgh 1989; Sakalihasan et al. 1993), when taken in the context of the increased overall thickness of the aortic wall and aortic circumference, the total elastin content is increased (Minion et al. 1994; Baxter et al. 1994). Despite increased elastin content its concentration falls in the presence of an increased in total protein content (Minion et al. 1994; Baxter et al. 1994).

The tensile strength of the aortic wall is provided, in the main, by the fibrillar collagen network, which contains both type I and type III collagen (Powell and Greenhalgh 1989), with type III contributing the tensile characteristics (Menashi et al.
Elastin is the principle load-bearing component under normal conditions (Dobrin 1988) and its depletion in aneurysmal tissue leads to the progressive "recruitment" of collagen fibres (MacSweeney et al. 1994b) resulting in weakening and dilation of the aortic wall.

Collagen is the principle component of the adventitia of any AAA. Several studies have demonstrated both an increase in total collagen content and collagen concentration within the aneurysmal aorta. Minion et al and Baxter et al (Minion et al. 1994; Baxter et al. 1994) showed a 5 fold and 3 fold increase in collagen content respectively, when compared to age matched controls. Menashi et al (Menashi et al. 1987) showed an increase from 62% to 84%, as a percentage of dry weight, in collagen content. Similarly He and Roach (He and Roach 1994) demonstrated a 77% increase in collagen as a total volume fraction.

The high degree correlation between increasing collagen content and increasing aneurysm size has led some authors to hypothesize that there is a causal relationship between collagen synthesis and aneurysm formation (Minion et al. 1994). Others have however suggested that the increase in collagen content be in compensation to increased wall stress (White et al. 1993). Wall stress is known to stimulate connective tissue synthesis by vascular SMC's (Leung et al. 1976) and McGee et al have demonstrated accelerated collagen synthesis and deposition in the walls of non-ruptured aneurysms (McGee et al. 1991). This study showed increased mRNA levels of alpha1-procollagen chains in aneurysm tissue extracts. Since elastin gene expression is unaltered in the wall of aortic aneurysms (Mesh et al. 1992), these data suggest that disordinate gene expression may contribute to the relative decrease in elastin concentration in AAA's.

The ratio of types I to type III collagen in aneurysmal aorta is unchanged when compared to normal aorta (Menashi et al. 1987). As the relative amount of elastin is markedly diminished in aneurysmal media, selective degradation of elastin in the aneurysmal media gives an apparent dilutional increase in collagen concentration. It seems likely, however, that a combination of increased collagen synthesis and enhanced elastin degradation is responsible for the matrix changes seen in the aneurysmal aorta.

These matrix abnormalities are not solely confined to the aneurysmal aortic segment but are also found in the aorta proximal to the aneurysm (Baxter et al. 1994).
Ward (Ward 1992a) also demonstrated significantly greater mean diameters for carotid, femoral, brachial and popliteal arteries in patients with AAAs when compared to aged matched controls, suggesting that infrarenal aneurysmal disease may be a localised manifestation of a systemic disease.

In addition to its association with collagen, elastin and SMC's, the medial elastic lamellae are closely related to microfibrillar proteins, a family of glycoproteins defined by their proximity to amorphous elastin and their fibre diameter on electron microscopy. A number of studies have shown an increase in content of approximately 20% of an unknown protein, most likely to be a microfibrillar such as fibrillin in aneurysmal tissue (Baxter et al. 1992; Minion et al. 1994; Gandhi et al. 1994). The reasons for this increase in microfibrillar protein are currently unclear.

The increased turnover of the extracellular matrix observed in the aneurysmal aorta leads to a relative imbalance in structural proteins. It is not known whether this is imbalance is an important aetiological factor in aneurysmal disease or whether it results from changes such as wall tension or chronic inflammation. However, it is likely that the abnormal mechanical properties of AAAs are due to a decrease in the ratio of elastin to collagen, resulting in a functionally compromised aortic wall.

1.4 Aneurysm Genetics

Inherited mutations of fibrillin and type III collagen are associated with Marfan's syndrome and Ehlers-Danlos type IV disease respectively. Although these diseases lead to aortic fragility and rupture they are distinct clinical entities which do not often manifest in true infrarenal AAAs.

Since Clifton reported AAA rupture in three siblings of a family in 1977 (Clifton 1977), a number of studies have identified an increased incidence of aneurysms in blood relatives, suggesting the existence of a hereditary component predisposing to aneurysmal disease.

The mode of inheritance remains controversial; Verloes et al (Verloes et al. 1995) examined 313 pedigrees and indicated a preeminence of genetic factors on multifactorial
or environmental effects in the pathogenesis of abdominal aortic aneurysm. They concluded that the major genetic defect was a dominant trait, with penetrance dependant on both age and sex. Majumder et al (Majumder et al. 1991) also utilised a segregation analysis to study 91 families, and concluded that a major autosomal recessive gene could explain the mechanism of inheritance. The discrepancies in these series may be explained by differences in methodology, but both support the concept of a genetic element in the pathogenesis of aneurysms.

There are several mechanisms by which genetic variables may predispose to AAA development. Increased enzymatic degradation of the ECM or decreased enzymatic inhibition of this destruction may result in aortic dilatation. Initial studies suggested that the serine protease inhibitor, alpha 1-antitrypsin, might play an important role. A deficiency of this enzyme leads to elastic tissue destruction and emphysema in the lung. Cohen et al (Cohen et al. 1990) described an increased frequency of the poorly inhibitory heterozygous MZ alpha-1 phenotype in patients with AAA. These findings have however not been confirmed by larger series. In Sweden (Elzouki and Eriksson 1994) and Ireland (Ramsbottom et al. 1994) and Cohen et al (Cohen et al. 1990), themselves, identified more than 85% of patients with aneurysms to have the common homozygous MM phenotype.

Other theories suggest inherited defects in elastin and collagen structure. Very little variation in the elastin gene has been identified, and it has not been demonstrated in aneurysm patients as previously stated (Baxter et al. 1992). However some patients with cerebral aneurysms have been identified to have, impaired type III collagen synthesis (Pope et al. 1981). Subsequently Kontusaari et al (Kontusaari et al. 1990) identified the single base mutation, at position 619, in the coding gene for type III collagen was associated with AAA disease in a single family in the U.S.A. These patients, however, suffered aortic dissections, similar to those seen in Ehlers-Danlos with the anomaly having surprisingly little effect before the fourth to sixth decade of life. The mutation has not been identified in other patients presenting with abdominal aortic aneurysms (Powell et al. 1993a).

This more recent study however found a specific polymorphism in the type III collagen gene, detected with the restriction enzyme Ava II, to be associated with AAA.
This is may potentially be significant, because as the elastin concentration in the aneurysmal aortic media falls, the mechanical load of the pulse pressure is shifted from elastin to collagen (Campa et al. 1987). In such a situation subtle changes in collagen structure may alter the mechanical properties of the aorta. Powell et al also demonstrated that the rate of aneurysm growth was proportional to the polymorphic variation (Powell et al. 1993a). However despite these findings a recent study using detailed DNA sequencing of the type III procollagen domain demonstrated that mutations of this gene were responsible for only 2% of aortic aneurysms (Tromp et al. 1993).

It seems likely that the development of aneurysmal disease is a multifactorial process, involving a complex interaction between genetic susceptibility and environmental influences (Powell and Greenhalgh 1989). Despite the familial clustering of AAAs the precise genetic basis for aneurysm formation remains unresolved (MacSweeney et al. 1994b).

2.5 Haemodynamic Influences

The strength of the normal aortic wall is conferred by the medial lamellae of smooth muscle cells, tensile collagen and, particularly, the concentric elastic connective tissue. The infrarenal aorta contains a lower concentration of elastic lamellae than the thoracic aorta (Wolinsky and Glagov 1967b; Baxter et al. 1994). This segment of aorta is therefore stiffer and less compliant. In addition the whole aorta demonstrates a natural age-related decrease in distensibility and elasticity, owing to the effects of haemodynamic stress imparted during the cardiac cycle (Sonesson et al. 1994).

The predisposition of aortic aneurysms to occur in the infrarenal aorta has been said to result from the unique haemodynamic conditions in this region (Henney et al. 1993). The aorta tapers and becomes less compliant as it descends from the thorax into the abdomen, causing the pulse pressure to reach its maximum in the infrarenal aorta. In addition, the higher pressure waves reflecting off the iliac and other arteries contribute to the pulsatile stress within the abdominal aorta, potentially fracturing the elastic lamellae and possibly precipitating the development of an aneurysm in the weakened wall. Interestingly amputees demonstrate asymmetric flow dynamics and are accordingly at
increased risk of aortic aneurysms (Vollmar et al. 1989). Recent improvements in magnetic resonance imaging have allowed blood velocity profiles to be calculated for the normal aorta. Oyre et al (Oyre et al. 1998) reported that peak wall shear stress is lower in the infrarenal than suprarenal aorta and that the magnitude and duration of negative shear stress is higher at the posterior than anterior wall in the infrarenal segment.

Although the infrarenal aorta is associated with low shear stress and high pulse pressure, the haemodynamic conditions become increasingly deranged with aortic dilatation. Bluth et al (Bluth et al. 1990) validated early experimental studies (Schrader et al. 1992; Peattie et al. 1994) by demonstrating that abdominal aortic aneurysms may exhibit both laminar and turbulent flow in vivo. At low flow rates, the flow is laminar with a core of swiftly moving fluid being surrounded by an annulus of slower moving fluid circulating as a rotating vortex (Sugimoto et al. 1994). Low et al (Low et al. 598) confirmed these observations and showed that intra-aneurysmal flow is slower than in the non-dilated vessel and that stagnation of flow may occur in the dome of the aneurysm. In contrast at higher flow rates the flow becomes turbulent (Laustsen et al. 1995), and the threshold Reynolds number determining turbulence is reduced as the aneurysm expands (Asbury et al. 1995). These observations have important implications for shear stress within aortic aneurysms. In small aneurysms, laminar flow may predominate, and the resulting shear stress is lower than recorded in the normal calibre vessel, and may even become negative. However, in larger aneurysms, the likelihood of turbulent flow is greater, and in turbulence, the peak shear stress will be much greater than in the non-dilated vessel (Asbury et al. 1995).

The process of aneurysmal dilatation will itself perpetuate continued expansion. Laplace's Law: \( T=\frac{P \times r}{2} \) (where \( T \) is wall tension, \( P \) is transmural pressure, and \( r \) is mean vessel radius) states that the wall tension required to maintain equilibrium is elevated as the radius increases. Therefore, an increase in aortic radius, or an increase in blood pressure will require an increase in wall tension to maintain the equilibrium. This vicious circle will lead to continued and accelerated aneurysm expansion and eventual rupture.
1.6 Proteolysis

Extracellular matrix (ECM) degradation contributes to the initial phase of tissue remodeling inherent to the physiological processes of morphogenesis, angiogenesis, bone resorption and wound healing (Agren 1994). This delicate balance of degradation and remodeling is deranged in several pathological processes such as rheumatoid arthritis, reactive arthritis (Lauhio et al. 1994b), tumour metastasis (Stetler-Stevenson et al. 1993) and atherogenesis (Galis et al. 1994b), as well as aneurysm formation. Since the initial work into the nature of the matrix degrading enzymes involved in aneurysmal disease (Busuttil et al. 1980) there has been much controversy over their precise identity. There is now, however overwhelming evidence that the family of enzymes responsible for the ECM degradation, observed in aneurysmal disease, are the matrix metalloproteinases (MMPs). These are a group of enzymes that together digest all the individual components of the ECM. There still, however, remains some controversy over which of the cell types known to synthesize these enzymes (smooth muscle cells, fibroblasts and inflammatory cells) plays the most significant role in vivo.

The normal vascular ECM is a complex network of proteins and proteoglycans, which exist in a constant state of flux. Homeostasis within the ECM is achieved by an intricate balance between synthesis and degradation of the matrix proteins under the control of the native mesenchymal cells. Under normal physiological conditions a balance is achieved between the matrix degrading enzymes and their natural inhibitors. The matrix metalloproteinases, so called because their catalytic mechanism relies on the presence of zinc at the active site (Woessner, Jr. 1991), are inhibited under physiological conditions by the endogenous family of tissue inhibitors of metalloproteinases (TIMP-1, 2 and 3) (Woessner, Jr. 1991). The MMPs themselves are divided into three main groups according to their substrate specificity; the collagenases (e.g. MMP-1) which selectively degrade the fibrillar collagens (Type I, II and III) at a single site; the gelatinases (MMP-2 and MMP-9) which degrade denatured type IV and V collagen (i.e. gelatin) and elastin (Senior et al. 1991; Katsuda et al. 1994); and the stromolysins (e.g. MMP-3)
considerable MMP-1 activity in aneurysmal aortas using a specific antibody. Irizarry et al (Irizarry et al. 1993) also found that AAAs homogenised in urea contained increased amounts of material immunoreactive with MMP-1 when compared to controls. Despite these findings both Herron et al and Webster et al were unable to immunoprecipitate MMP-1 from normal aorta or aneurysmal tissue. Tilson has suggested (Tilson and Newman 1995) that MMP-1 may bind to its natural inhibitor TIMP-1, forming a high molecular weight species under non-reducing conditions, which mask its detection in activity assays. The controversy was resolved by Irizarry et al (Irizarry et al. 1993) who identified increased MMP-1 activity in aneurysmal extracts when compared to controls by running immunoblotts, after polyacrylamide gel electrophoresis under reduced conditions.

Stromelysin-1 (MMP-3) has also been implicated in aortic matrix degradation both directly (Okada et al. 1986; Woessner, Jr. 1991), and indirectly through MMP-9 and MMP-3 activation (Suzuki et al. 1990). A number of authors have identified increased levels of MMP-3 in aneurysm homogenates (Vine and Powell 1991; Newman et al. 1994c; Newman et al. 1994d).

Freestone et al (Freestone et al. 1995) have recently demonstrated that MMP-2 is the principle proteolytic enzyme in early aortic aneurysms, with later progression of disease characterised by a pronounced inflammatory infiltrate and principally increased MMP-9 activity. Recent work in our own centre (Crowther et al. 1996) has demonstrated increased MMP-2 production by aneurysmal smooth muscle cells (SMC) when compared to normal aged matched aorta. Similar results have recently been described by Patel et al (Patel et al. 1996) who identified that medial SMCs isolated from aneurysmal tissue produce significantly higher levels of both MMP-2 and MMP-9 than SMCs from isolated from control arterial tissue. In summary there is now good evidence for increased activity of MMPs-1, 2, 3 and 9 in aneurysmal tissue when compared to atherosclerotic controls.

The normal aortic ECM is maintained in constant flux by the delicate balance between the proteolytic and anti-proteolytic systems. It would follow therefore that under activity of TIMPs might lead to an imbalance in favour of proteolysis. Recent work by Brophy et al has demonstrated decreased TIMP-1 in AAA tissue when compared to
which are capable of degrading elastin, fibronectin, collagen IV and V, and proteoglycans (Woessner, Jr. 1991).

The activity of MMPs is tightly regulated at several levels. Many growth facts, cytokines, hormones and tumour promoters influence MMP transcription. All the MMPs are secreted as inactive proenzymes that require proteolytic cleavage by other proteinases (such as plasmin, trypsin and even other MMPs) for activation. Once cleaved the potential to degrade matrix proteins is determined by the relative balance between the MMPs and the endogenous TIMPs (Woessner, Jr. 1991). The pathological changes seen in the ECM in aneurysmal disease result from an imbalance between these enzymes. Many studies have now identified increased elastase, collagenase and gelatinase activity within the wall of aortic aneurysms (Busuttil et al. 1980; Busuttil et al. 1982; Dubick et al. 1988; Vine and Powell 1991; Herron et al. 1991; Newman et al. 1994b; Newman et al. 1994c).

In separate studies both Busuttil et al and Cannon et al (Busuttil et al. 1982; Cannon and Read 1982) identified increased elastase activity in aneurysmal disease. Despite initial controversy as to the identity of the elastase, with serine proteases, circulating pancreatic elastase and leukocyte elastase all suggested, (Dubick et al. 1988; Herron et al. 1991) (Cannon and Read 1982) the overwhelming evidence now points to a member of the metalloproteinase family (Vine and Powell 1991; Newman et al. 1994b; Newman et al. 1994c). Vine et al found the elastolytic activity in aneurysmal tissue to be significantly increased when compared to homogenates from both atherosclerotic or normal aorta, with the principle enzyme identified as a MMP with a molecular weight of 92 kDa (Vine and Powell 1991) that was suggested to be proMMP-9. Both Brophy et al and Newman et al identified an 80 kDa band by gel enzymography that Newman et al confirmed as the active form of MMP-9 by immunoblotting (Brophy et al. 1991c; Newman et al. 1994c; Newman et al. 1994d). On the basis of these observations and the known elastolytic properties of MMP-9 (Senior et al. 1991), this proteinase is believed to play a central role in aneurysm pathogenesis.

A number of authors have also suggested a role for interstitial collagenase, MMP-1, in aneurysmal disease after Busuttil et al reported increased collagenolytic activity (Busuttil et al. 1980). Vine and Powell (Vine and Powell 1991) demonstrated
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normal aorta (Brophy et al. 1991a). However, Tilson et al (Tilson et al. 1993) have demonstrated that this is not result from a primary genetic defect.

Despite proving that the 80 kDa proteinase band isolated from AAA homogenates contained active MMP-9, Newman et al also observed a portion of the activity could not be bound to a recombinant TIMP-1 affinity column. This activity was subsequently inhibited by PMSF, a serine proteinase inhibitor, suggesting some serine proteinase activity within the extract. The serine proteinase, plasmin, has been demonstrated in higher levels in aneurysmal aorta when compared to controls by Jean -Claude et al(Jean-Claude et al. 1994), whilst Reilly et al(Reilly et al. 1994) showed tissue type plasminogen activator to be abundant in AAA tissue compared to normal or atherosclerotic aorta. Plasmin may not only be important for its ability to degrade the ECM, but it is also known to activate latent pro-MMP-1, pro-MMP-3 and pro-MMP-9 (HE et al. 1989). Plasmin may therefore have an important role in the cascade of enzyme activation within the aortic wall.

Recent work has identified the importance of the metalloproteinases not only in aortic aneurysms but also in occlusive disease. Interestingly Thompson et al identified a six-fold increase in MMP-9 in AOD and a ten-fold increase in aneurysmal tissue when compared to control aorta (Thompson et al. 1995). They also demonstrated a marked increase in TIMP-1 in AAA but not AOD specimens. McMillan et al however could demonstrate no difference in TIMP-1 mRNA levels between AAA, AOD and normal aortic tissue (McMillan et al. 1998). Knox et al (Knox et al. 1997) suggested the large increases in MMP activity in both AAA and AOD seen using in situ zymography overwhelmed a lesser increase in TIMPs. The AAA tissue in this study was taken from older patients than the AOD specimens and it may be that higher MMP levels are present earlier in aneurysmal change.

The synergistic actions of the MMPs within the aortic wall in elevated levels, could both initiate and maintain the ECM degradation seen in the aneurysmal aortic media. MMP-9 is capable of degrading denatured collagen as well as elastin (Senior et al. 1991; Katsuda et al. 1994) thus facilitating the action of MMP-1 on type I and type III collagen, which are major constituents of the vascular wall (Powell and Greenhalgh 1989). In addition Okada et al have shown that MMP-9 may degrade native fibrillar
collagens which are also reduced in the aneurysmal wall (Okada et al. 1986). The evidence points to a cascade of MMP activation that results in a concerted degradation of elastin, fibrillar collagens and other ECM components in the aortic wall during aneurysm development and progression (Newman et al. 1994d). Brophy et al (Brophy et al. 1991a) have also identified a relative deficiency of TIMP-1 in aneurysmal tissue, supporting the hypothesis that the destruction of matrix components reflects an imbalance of the MMPs and their natural inhibitors.

1.7 Inflammation

In addition to the proteolytic activity seen in AAA, the disease process is also characterised by a marked chronic inflammatory infiltrate throughout the affected aortic segment (Beckman 1986; Koch et al. 1990; Newman et al. 1994c). The inflammatory response is either initiated by an exogenous antigen (oxidised LDL) or an endogenous antigen (elastin fragments) (Senior et al. 1980; Cid et al. 1989). Once the inflammatory response is initiated it may lead to ECM digestion either directly, through phagocytosis, cell lysis and metalloproteinase release, or indirectly through cytokines and growth factors, which in turn control native aortic mesenchymal cells.

Normal aortic tissue has been shown to contain few, if any, inflammatory cells (Koch et al. 1990; Newman et al. 1994c). In contrast many investigators have reported gross inflammatory changes in AAA tissue extracts. Koch et al (Koch et al. 1990) found that aortic inflammation appeared as a progressive continuum from the non-inflammatory AAA (NIAAA) to inflammatory AAAs (IAAA). They also showed that the majority of inflammatory cells present in the adventitia and media were CD3+ T-lymphocytes. These T-lymphocytes were found predominantly in the adventitia, in contrast to aortic occlusive disease where only 25% were found in the adventitia, the majority being present in the media. Koch et al (Koch et al. 1990) also identified that the CD19+ B-lymphocyte accounted for 25% of the inflammatory infiltrate, also preferentially aggregating in the adventitia, with the remainder of the inflammatory infiltrate being CD11c+ macrophages. These findings are at odds with more recent studies, which have found greater numbers of B-lymphocytes within the
adventitia (Brophy et al. 1991b; Pasquinelli et al. 1993). This discrepancy may be explained by the fact that during B cell maturation, both the CD19 and CD22 surface antigens are lost with conversion to the plasma cell and would therefore remain unrecognised by the anti-CD19 antibody.

Newman et al (Newman et al. 1994c) phenotyped the various cell types using fluorescence-activated cell sorting (FACS). They identified a fifty-fold increase in CD15+ macrophages/granulocytes, a fifteen-fold increase in CD3+ T-lymphocytes, and a ten-fold increase in CD22+ B-lymphocytes. The predominant cell type labelled with the CD15 antibody was identified as the macrophage by immunohistochemistry.

Koch et al. (Koch et al. 1990) also proposed that the T-cell population was mature and did not appear to be proliferating. In order to further evaluate the T-cell response they calculated the CD4+: CD8+ T cell (helper, inducer / cytotoxic, suppressor) ratio. The CD4+ helper cells predominated with ratios ranging from 3:1 to 20:1. The high ratio of CD4+ cell to CD8+ cells suggests an unabated B-cell type response. The CD4+ subset of the CD3+ T-lymphocytes are helper cells and release IL-2, which causes activation and proliferation of CD3+ T- and CD19+ B-lymphocytes. They also have the ability to interact with activated macrophages or smooth muscle cells in aortic tissue bearing the class II major histocompatibility determinants. These macrophages and smooth muscle cells can process foreign or autologous altered antigen, perhaps products of elastin degradation, perpetuating the aberrant immune response (Koch et al. 1990). The relatively low proportion of the CD8+ suppressor T-lymphocytes may also contribute to the net activation of the immune system, perhaps ultimately leading to the destruction of the aortic wall (Koch et al. 1990).

Koch et al. (Koch et al. 1990) also identified clusters of lymphocytes within the infiltrate consisting predominantly of B-lymphocytes, expressing CD22+ antigens, surrounded by CD3+ T-cells and macrophages. In addition to focal accumulation, macrophages and lymphocytes were found throughout the tissue often clustering around neovasa vasorum in the adventitia.

The importance of the inflammatory cell infiltrate in the pathogenesis of AAAs has been alluded to by a number of authors. Vine and Powell showed a positive
correlation between elastolytic activity and the presence of inflammatory infiltrates (Vine and Powell 1991). Recent work in our department by Wills et al. (Wills et al. 1996) has demonstrated enhanced elastin degradation in an organ culture model of aneurysmal disease, when co-cultured with leukocytes. These inflammatory cells have the potential to secrete all the MMP's responsible for ECM degradation. Further evidence highlighting the importance of the inflammatory response in aneurysm development comes from immunosuppression experiments. Ricci et al. (Ricci et al. 1996b) demonstrated that CD-18 monoclonal antibody significantly reduced aneurysmal change in normotensive and genetically hypertensive rats after initial exposure to elastase. Dobrin et al. (Dobrin et al. 1996) recently showed that prednisolone and cyclosporin inhibited aneurysmal change, also in an elastase induced rat model.

Macrophages are known to produce an array of degrading enzymes and the large increase in population that accompanies aneurysmal change suggests that they are a good candidate as the source of one or more of the metalloproteinases observed. Shapiro et al. (Shapiro et al. 1991) have demonstrated that as monocytes migrate into tissue they differentiate into macrophages, at which time they lose the ability to produce serine proteinases. At the same time they show upregulated gene expression for a number of MMPs including MMP-1, MMP-3 and MMP-9. The same study also demonstrated the ability of types I and III collagen (native and denatured) to markedly enhance macrophage interstitial collagenase production via a cell matrix mechanism. This may be significant when considering the increased turnover of these two collagen types commonly seen in the aneurysmal aorta.

Furthermore, a recent immunohistochemical study attempted to delineate the in situ source of the enzymes present in AAA tissue using specific monoclonal antibodies (Newman et al. 1994b). This study identified the infiltrating inflammatory cells, specifically the CD14+ macrophage, to be responsible for the delivery of two of the MMP's present, namely MMP-3 and MMP-9 (Newman et al. 1994b). Again, this study reported an abundance of adventitial and medial macrophages in AAA tissue, underscoring the importance of their enzyme-secreting ability (Newman et al. 1994b). Furthermore, Unkeless (Unkeless 1980) showed that the monocyte/macrophage could
release plasminogen activators. Plasmin has the capability to activate several pro-MMP's, thus indirectly enhancing the potential for macrophages degradation.

In addition to the direct destruction of the ECM by releasing MMP's, the infiltrating macrophages, in association with lymphocytes, may be important in activating resident mesenchymal cells (Galas et al. 1994a). Regulation of metalloproteinase production during inflammatory processes is subject to cytokine control mechanisms. Cytokine modulation may regulate the immune response and also the metabolic function of the resident vascular SMC (Keen et al. 1994). A number of cytokines have been shown to be significantly increased in homogenates and explants from the aneurysmal aortic wall, in particular tumour necrosis factor-alpha (TNF-) (Newman et al. 1994a), interleukin-1 (IL-1) (Newman et al. 1994a), interleukin-6 (IL-6) (Szekanecz et al. 1994b), interferon-gamma (IFN-g) (Szekanecz et al. 1994b), monocyte chemoattractant protein-1 (MCP-1) (Koch et al. 1993), and interleukin-8 (IL-8) (Koch et al. 1993). Their presence further implicates the active involvement of the inflammatory cells in the development of AAA.

Newman et al. (Newman et al. 1994a), in examining AAA tissue homogenates, found levels of both IL-1 and TNF- to be significantly increased by comparison with cadaveric controls. These findings are consistent with the results of another study demonstrating in vitro that cells present in aneurysmal aortic tissue produce and secrete IL-1 in greater amounts than controls in organ culture (Pearce et al. 1992).

Interleukin-1, also known as lymphocyte activating factor, is typically secreted as a glycosylated protein of 17 kDa (Newman et al. 1994a). IL-1 enhances the proliferation of CD4+ T cells and the growth and differentiation of B cells. Macrophages are the principal source of IL-1b (Moyer et al. 1991), but B cells, endothelial cells, and fibroblasts have all been shown to produce it when stimulated (Moyer et al. 1991). This positive-feedback loop involving the resident vascular cells may act locally to amplify and propagate the inflammatory reaction (Pearce et al. 1992). Interleukin-1 is a versatile cytokine exerting further biological effects on resident endothelial and smooth muscle cells (Libby et al. 1988). Elias et al (Elias et al. 1990) found IL-1 to induce collagen synthesis in mesenchymal cells, which parallels the increases in collagen reported by numerous biochemical and genetic studies on the aneurysmal aortic wall.
Tumour necrosis factor- is a 17-kDa protein secreted primarily by activated macrophages/monocytes (Moyer et al. 1991), although other cells can produce it, including lymphocytes and vascular smooth muscle cells (Warner and Libby 1989; Rock and Lowry 1991). Similar to IL-1, TNF- induces its own gene expression in vascular smooth muscle cells (Warner and Libby 1989). Although significant levels of TNF- have also been detected in homogenates of aneurysmal aortas (Newman et al. 1994a), its continued secretion by aneurysmal aortic explants has not been observed (Pearce et al. 1992), suggesting phenotypic changes in the cells involved or disruption of the cytokinetic communication regulating its production.

In addition to its other inflammatory actions, TNF- has been shown to be angiogenic, stimulating endothelial cell growth and proliferation (Szekanecz et al. 1994a). Newly formed capillaries seem to arise from the vasa vasorum of the arterial wall (Kamat et al. 1987). Macrophages accumulate in large numbers around the vasa in the adventitia of the diseased vessel and the secretion of TNF-a, inducing the formation of new capillaries, provides the physical means for further recruitment of both macrophages and lymphocytes, thus amplifying the inflammatory loop.

Interleukin-11 and TNF- can induce the expression of endothelial cell adhesion molecules (Pober and Cotran 1991). Vascular adhesion molecules play an important role in the inflammatory process as they control the transmigration of leukocytes across the endothelial layer. Davis et al. (Davis et al. 1993) localised a significant increase in one of these particular adhesion molecules, intercellular adhesion molecule-1 (ICAM-1), to the endothelial cells of the vasa vasorum in AAA tissue, suggesting recruitment of monocytes and other inflammatory cells by means of the adventitial vasa. This induction of ICAM-1 expression on the endothelial cells of the vasa vasorum may induce a permissive state in which mononuclear cells enter the adventitial matrix. Once in place, aortic tissue macrophages further stimulate the expression of ICAM-1 via IL-1 and TNF-, which then enhance the attraction of additional inflammatory cells to the adventitia. Indeed, White et al. (White et al. 1993) have suggested that destruction of adventitial elastin may play a primary role in the pathogenesis of AAA formation, and may result from the recruitment of inflammatory cells by ICAM-1 expression and
neovascularisation. However, this hypothesis does not reveal causality and may be due to an overall heightened inflammatory state within the vessel wall (Davis et al. 1993).

Szekanecz et al. (Szekanecz et al. 1994b), using an enzyme-linked immunosorbent assay (ELISA) of explant culture supernatants, revealed a significant increase in IL-6 and IFN- production by AAA tissue compared to either occlusive or normal explants. Interleukin-6 is mainly produced by monocytes/macrophages, as well as fibroblasts, endothelial and smooth muscle cells (Loppnow and Libby 1990). It is a cytokine predominately involved with T and B lymphocyte activation during inflammation (Szekanecz et al. 1994b). Interleukin-1 has the capacity to induce vascular SMC's to secrete copious amounts of IL-6, which stimulates lymphocyte proliferation and antibody production by B-lymphocytes (Loppnow and Libby 1990). The accumulation of immunoglobulin in aneurysmal walls may be the result of increased local levels of IL-6 (Brophy et al. 1991b). Interferon- has the ability to both stimulate lymphocytes and induce MHC class II antigen expression in smooth muscle and endothelial cells. The enhanced levels of IL-6 and IFN- in AAA tissue strongly suggests they play an important regulatory role in AAA development. Their release from macrophages results in further T- and B-lymphocyte participation thus perpetuating continued macrophage mobilisation and activation, which, although meant to be protective could lead to continued destruction rather than repair.

While it seems likely that aortic aneurysmal disease partially represents an immune-mediated disease, the signals that initially attract leukocytes and further propagate the immune-mediated mechanisms remain to be fully elucidated. Several chemotactic soluble products of ECM degradation have been suggested, including peptides generated from elastin degradation (Senior et al. 1980). Analogies have been drawn between the processes involved in aneurysm formation and those involved in other pathologies (Koch et al. 1990). Cid et al. (Cid et al. 1989) have postulated that the putative antigenic substance responsible for temporal arteritis might be an inert substance, such as elastin derived peptides. Elastin could potentially degenerate and have its structure altered by the aging process, or its degradation could be initiated by some proteolytic imbalance defined by a genetic predisposition. Mononuclear phagocyte
migration into the diseased aorta may be mediated by such factors, thus initiating and propagating the inflammatory response (Koch et al. 1990).

However, increased levels of known inflammatory cytokines with potent chemotactic activity have been identified in AAA tissue. Monocyte chemoattractant protein-1 (MCP-1) is expressed to a greater extent by explants of aneurysmal tissue (Koch et al. 1993). MCP-1 is a 76-amino-acid basic protein with selective chemotactic activity for mononuclear phagocytes (Matsushima and Oppenheim 1989). Koch et al. (Koch et al. 1993) showed the main cellular source of MCP-1 to be the tissue macrophage, thus exerting an autocrine positive feedback influence.

Interleukin-8 is also a monocyte/macrophage-derived peptide belonging to a novel cytokine family of 8- to 10-kDa molecular mass (Koch et al. 1993) and has been demonstrated to be chemotactic for neutrophils and lymphocytes (Larsen et al. 1989). Interleukin-8, like TNF-, has also been shown to have potent angiogenic effects (Szekanecz et al. 1994a). In addition, endothelial cells in vitro have been shown to produce IL-8 in response to cytokines such as IL-1 and TNF- (Strieter et al. 1989), as have smooth muscle cells (Wang et al. 1991). It may be that IL-1 and TNF- induce IL-8 expression in these cells, thereby creating a cytokine network within the diseased aortic aneurysmal wall, involving recruitment of chronic inflammatory cells through neovascularisation and increased levels of ICAM-1, further perpetuating the inflammatory response and subsequent destruction of the aortic wall (Koch et al. 1993).

Several studies have demonstrated the ability of the inflammatory infiltrate, through their cytokinetic secretions, to influence the expression of MMP's directly, at the transcriptional level (Mauviel 1993). The treatment of cultured rabbit aortic smooth muscle cells with IL-1 resulted in increased collagenase mRNA levels (Evans et al. 1991). Similarly, a study by Keen et al. (Keen et al. 1994), who cultured smooth muscle cell explants from human aneurysmal tissue with IL-1b, demonstrated a dose-dependent increase in interstitial collagenase (MMP-1) gene expression. Newman et al. (Newman et al. 1994b), in using immunohistochemical techniques on human AAA tissue, observed the antibody to MMP-1 to localise to mesenchymal cells (either smooth muscle cells or fibroblasts), thus correlating the results found in culture with those observed in vivo.
The study by Keen et al. (Keen et al. 1994) only examined for variations in interstitial collagenase. A more recent study by Galis et al. (Galis et al. 1994a) assayed for the whole spectrum of MMP's. Using human SMC's cultured in vitro with IL-1 or TNF- they showed de novo synthesis and secretion of MMP's -1, -2, -3 and -9 (Galis et al. 1994a). Galis et al. (Galis et al. 1994a) also showed that whilst SMC's stimulated with cytokines expressed several MMP's, mRNA and protein levels of TIMP's 1 and 2 appeared unaffected. These data further support the concept that cytokines alter the balance between molecules that promote or inhibit matrix degradation (Galis et al. 1994a). If SMC's exhibit such properties in vivo as has been shown in vitro (Galis et al. 1994a), the local secretion of cytokines could sway the balance between the production of MMP's and TIMP's to favour ECM degradation by SMC's.

Although SMC's, fibroblasts, and macrophages can all potentially produce interstitial collagenase in vitro, the capacity for mesenchymal cells (SMC and fibroblasts) to produce MMP-1 appears to be much greater than that found in macrophages (Keen et al. 1994). Welgus et al. (Welgus et al. 1990) observed that stimulated fibroblasts produced six fold greater amounts of collagenase than an equal number of stimulated macrophages. If IL-1 produced by adventitial macrophages does activate aortic SMC's to synthesize collagenase; it would be expected that collagenase activity to be greatest in the region of the aortic wall where IL-1 produced by these macrophages could act on medial SMC in a paracrine manner. The observation by Vine and Powell (Vine and Powell 1991) that collagenase activity in aneurysms is greatest in the outer aspect of the media is consistent with the results obtained by Keen et al. (Keen et al. 1994) and Newman et al. (Newman et al. 1994b), suggesting that the SMC is the source of the elevated collagenase found in AAA's.

Thus, it is likely that the cascade of events resulting in the destruction of extracellular matrix structure is highly complex, with both cell-matrix and cell-cell interactions contributing to the processes in vivo that lead to progressive matrix degradation (Newman et al. 1994a). The importance of the inflammatory cellular infiltrate found in aortic aneurysmal disease lies in both the potential paracrine modulation of adventitial endothelial and vascular smooth muscle cell function, and autocrine activation and recruitment of migrating leukocytes through the cytokine
network (Pearce et al. 1992). Further studies of metalloproteinase production and regulation of their expression by both infiltrating macrophages and lymphocytes of the aneurysmal aortic wall will clarify the undoubted importance of these cells during AAA development. In addition the exact nature of the antigen initiating the inflammatory response remains unknown.

1.8 Atherosclerosis and Abdominal Aortic Aneurysm Formation

Abdominal aortic aneurysms have historically been ascribed to atherosclerosis because of the almost universal finding of calcified atherosclerotic degeneration in the walls of aneurysms (Campa et al. 1987). This assumed "causative" relationship is based on common risk factors such as hypertension, smoking and cholesterol (Powell and Greenhalgh 1989; Reed et al. 1992); other regional manifestations of atherosclerotic occlusive disease (AOD) such as coronary artery, cerebral vascular, and peripheral vascular disease in patients with AAA; histological features of marked atherosclerotic lesions in AAA tissue; and localisation of aneurysms to the atherosclerosis-prone infrarenal aorta. The fact that aneurysmal disease develops at a later age has led some to propose that it is a delayed manifestation of the atherosclerotic process (Auerbach et al 1980; DePalma et al 1982; Zarins et al 1982; Crawford and Levine 1953).

Zarins et al. (Zarins et al. 1990) were able to induce AAA formation experimentally in monkeys fed on a lipid-rich atherogenic diet. They found that 5 of 443 monkeys who experienced prolonged exposure to an atherogenic diet (12 months) and who were then transferred to a regression regimen developed aneurysms. They hypothesised that the elaboration of fibrous tissue characteristic of atherosclerotic plaque evolution may provide structural support to the aortic wall where the media is eroded, and that during the period of regression of the atherogenic diet the plaques receded removing the support that these lesions could have afforded to the underlying thinned media, resulting in AAA formation (Zarins et al. 1990).

In contrast to the thoracic aorta, the infrarenal abdominal aorta contains relatively few vasa vasorum, with appropriate levels of oxygenation and nutrition being provided to those outer layers by pressure filtration from the lumen (Wolinsky and Glagov 1967a).
This paucity of vasa has been suggested as a factor that may explain the particular propensity of this segment of the human aorta to develop early and severe atherosclerosis (Reed et al. 1992). Consequently, advanced thickening of the intima by atherosclerotic lesions and thrombus could further impede the only source of nutrients to a faltering media, and theoretically exacerbate deterioration of the elastic and collagen architecture of the aortic wall, initiating aneurysm formation. Moreover, accumulation of the atherosclerotic material may occlude the ostia of the vessels supplying what little vasa vasorum originally existed in the infrarenal aorta.

However, separating "cause" and "effect" has proven far more problematical than a superficial examination would lead to believe. A differing school of thought began to develop in the late 1970's (Martin 1978) with the premise of explaining how the deposition of atheromatous plaque results in occlusive disease in some individuals, and aneurysm formation in others. Tilson (Tilson 1990; Tilson 1992) and others have argued that aneurysms may become atherosclerotic as a secondary phenomenon to dilatation, since atheromatous plaque is preferentially formed in regions of turbulence and low shear stress, possibly as a result of prolonged contact between blood borne atherogenic factors and the vessel wall(Glagov et al. 1988). Tilson(Tilson 1992) has also proposed that the effects of smoking and hypertension, risk factors in both atherosclerotic occlusive, and aneurysmal disease, may mediate the promotion of either disease through unique disease-specific mechanisms, dependent on the constitutional susceptibilities to both diseases.

Reports of familial clustering of aneurysmal disease (Clifton 1977; Darling et al. 1989) has left little doubt that there must be an important genetic susceptibility factor associated with the hereditary nature of AAA formation. In addition, Ward (Ward 1992b) observed that AAA patients demonstrated systemic arterial dilatation in peripheral arteries such as the brachial artery at the elbow and the carotid artery beyond its first branch, which are seldom, if ever, involved in atherosclerosis. Such clinical observations and the results of various biochemical studies of possible genetic causes suggest that atherosclerosis is an "effect" of AAA's and that AAA's may be a manifestation of a systemic abnormality. These findings add further support to the view that aneurysmal disease has specific determinants that may be unrelated to the atherosclerotic process and distinguish it from occlusive disease as a unique pathogenetic entity.
The considerable controversy surrounding the aetiological role that atherosclerosis plays in AAA formation necessitates further detailed studies on a molecular level to reveal precisely which disease is the prerequisite for the other. Further insights will then be obtained on whether specific conditions operating during the atherosclerotic process act to potentiate the divergent pathological outcomes of aneurysm formation in some individuals and obstructive disease in others.

1.9 Pharmacotherapy for Aneurysms

A greater understanding of the pathophysiology of aneurysmal disease may allow a targeted therapeutic approach to reduce or inhibit the growth of small AAAs and therefore reduce the mortality from rupture or the progression to elective surgery.

In previous studies a number of pharmacological agents have been proposed to inhibit aneurysm growth. The Beta 2 adrenergic antagonist, dl-propranolol, and reserpine both reverse the B-aminopropionitrile mediated reduction in aortic strength and the risk of aortic dissection in a turkey model, whereas the Beta 1 antagonists sotalol and prolactol have very little or no effect on tensile strength (Simpson et al. 1976; Simpson and Boucek 1983). It has been suggested that propranolol acts independently of its blood pressure and heart rate to promote collagen and elastin crosslinking, by stimulating lysyl oxidase. Propranolol has also been suggested to delay the formation of aneurysms in the genetically susceptible blotchy mice (Brophy et al. 1988) and in elastase induced aneurysms in rats (Slaiby et al. 1994), via a direct action on the aortic connective tissue. A retrospective study, in humans, has also shown that beta blockers may decrease the growth rate of small aneurysms (Leach et al. 1988), however a recent prospective study has not confirmed this finding (Brown 1996).

Inhibition of the inflammatory response associated with aneurysm formation using the anti-CD 18 monoclonal antibody also slows the expansion of experimentally induced AAAs in a rat model (Ricci et al. 1996b). It may be hypothesised that the reduction in expansion resulted from a reduced activity of both inflammatory cell (monocyte) and aortic wall synthesized matrix metalloproteinases.
The apparent importance of the matrix metalloproteinases in the destruction of the arterial wall in aneurysmal disease makes these enzymes an attractive target for pharmacotherapy aimed at inhibiting aneurysm growth. The metalloproteinase inhibitory properties of doxycycline have been successfully utilised in clinical trials of periodontal disease and reactive arthritis with the aim of reducing tissue destruction (Lauhio et al. 1994a; Lauhio et al. 1994c; Golub et al. 1995). Recently doxycycline has been shown to prevent the development of AAAs in an elastase induced rat model when administered by subcutaneous injection. The authors also showed preservation of aortic medial elastin and suggested, that that this was as a result of reduced MMP-9 expression by the infiltrating inflammatory cells (Petrinec et al. 1996). Research into a possible therapeutic approach to aneurysmal disease is in its infancy, but holds much promise for the future.

1.10 Summary

Pathological and biochemical studies of human abdominal aortic aneurysms demonstrate a thinned media, fractured elastic lamellae, decreased concentrations of elastin, and a relative increase in collagen concentration. The synchronous appearance of macrophages and T- and B- lymphocytes with aneurysmal changes in the aortic wall suggests that production of matrix degrading proteinases is immune-mediated by these cells and appear to be important in the aetiology of AAA formation. Aneurysm development appears to be multifactorial in nature, with both a genetic predisposition and collective environmental influences acting in alliance, somehow setting the cascade of degradation in motion. The important role of the matrix metalloproteinases makes these enzymes an attractive target for pharmacotherapy.
CHAPTER TWO

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2.1 Demography

Abdominal aortic aneurysm is a disease of the elderly and is uncommon under 55 years of age (Bengtsson et al. 1992a). The incidence is greater in men for any given age and the onset of disease appears to be later in women (Bengtsson et al. 1992a; Scott et al. 1998). Scott et al quoted a prevalence of 4% in a large screened population of men and women aged 65-80 years (7.6% in men only). Similar figures have been reported from a number of other screening studies (Lucarotti et al. 1993; Morris et al. 1994b).

Other authors have based estimates of prevalence on post-mortem studies, Darling et al. found AAAs in 2% in a large consecutive series (Darling et al. 1977) and more recently Bengtsson et al described a rising frequency with increasing age to a maximum of 5.9% at 80-85 years in men (Bengtsson et al. 1992a). This increasing prevalence with age has also been demonstrated by Morris et al. who detected aneurysms greater than 4.5cm in 0.3%, 2.5% and 4.1% of men aged 50-64 years, 65-79 years and greater than 80 years, respectively in an ultrasound screening study (Morris et al. 1994b).

In addition to rising age and male gender a higher prevalence of AAA has been reported in patients with established cardiovascular risk factors. Smoking and systolic hypertension have been identified as risk factors for aneurysmal disease (Allen et al. 1987; O’Kelly and Heather 1989; Strachan 1991). Allen reported AAA prevalence of 5.3% in hypertensive patients. More recently MacSweeney et al. have demonstrated that smoking accelerates the growth rate of small aneurysms (MacSweeney et al. 1994a). Similarly hypercholesterolaemia and other cardiovascular disease are associated with an increased prevalence (Pleumeekers et al. 1995). Allardice et al. reported 20% of male patients with peripheral vascular disease to have ectatic or aneurysmal aortas of 30mm diameter or greater (Allardice et al. 1988) and Anton et al. have demonstrated an even greater association with popliteal artery aneurysms (Anton et al. 1986). Smith et al. showed significant associations with ischaemic heart disease and previous myocardial infarction though interestingly not hypertension (Smith et al. 1993). However the same group later demonstrated that the relative risk for developing an AAA was 2.7 in a subgroup of hypertensive patients aged between 60 and 64 years (Grimshaw et al. 1994). There still remains some doubt as to whether hypertension influences the development of
AAA or whether there is only an association between them, indeed some authors have not demonstrated any correlation between the diseases. Scott et al found no significant difference in the incidence of hypertension in a large screened cohort of patients with aneurysms (14%) and those without (11.3%) (Scott et al. 1991).

Genetic susceptibility to aneurysmal disease has been discussed in detail in chapter 1. In more general terms there is good evidence of increased prevalence in siblings and offspring of patients with aortic aneurysms. Bengtsson et al found 8 of 39 male offspring of patients who had died from rupture to have a dilated aorta (Bengtsson et al. 1992b). Similarly Adams et al identified aortic dilatation in 27% of sons and 17% of brothers of 100 consecutive aneurysm patients (Adams et al. 1993). There is also evidence that race influences prevalence with Lilienfeld et al identifying a higher rate among white relative to non-white Americans (Lilienfeld et al. 1987).

In summary established risk groups for AAA include males over the age of 65 years, smokers, hypertensives, claudicants, hyperlipidaemics and first-degree relatives of AAA patients. It is however, difficult to assess some these factors individually to decide whether they cause the development of aneurysms or are just associated with them.

There is little doubt however that the prevalence of aneurysmal disease is increasing in the Western world. This rise is of particular interest as it has occurred when rates of other forms of vascular disease, namely coronary artery disease, cerebrovascular disease and peripheral vascular disease are falling (Coggon D. et al. 1996). Samy et al reported an increase in diagnosis of both intact and ruptured AAA in Scotland from 5.5 per 100,000 in 1980 to 12.0 in 1991 (Samy et al. 1994). Budd et al also demonstrated an increase in rupture rate over a six-year period (Budd et al. 1989). The age standardised mortality for aortic aneurysms in England and Wales rose by 20-fold in men and 11-fold in Women between 1950 and 1984 mainly due to deaths from abdominal aneurysms (Fowkes et al. 1989). Although advances in diagnosis and surgery may have influenced these figures, the authors concluded that there had been a true rise in incidence of abdominal aortic aneurysm. Over a similar period Melton et al demonstrated a threefold rise in prevalence of AAA in the United States (Melton et al. 1984). Further evidence from the necropsy study in Sweden confirmed age and sex specific increased prevalence between 1958 and 1986. Ernst concluded that although in part, the aging
population, improved diagnostic methods and greater clinical awareness may explain increased prevalence, the magnitude of the rise suggested a genuine increase (Ernst 1993).

2.2 Clinical presentation

The majority of infrarenal abdominal aortic aneurysms are asymptomatic at initial diagnosis (Mansfield and Gilling-Smith 1990). Most are detected during routine physical examination (Kiell and Ernst 1993) or diagnosed incidentally radiologically or at laparotomy for an unrelated condition. In particular abdominal ultrasound has identified aneurysms in up to 4.9% of patients scanned for non-vascular reasons (Akkersdijk et al. 1992).

Abdominal pain and back pain, due to pressure on adjacent structures, are the commonest chronic symptoms of an abdominal aortic aneurysm, but still only occur in the minority of patients. Less common sites of pain include chest, flank and groin. Rarely patients may present with signs of peripheral embolisation from the aneurysm sac (Kiell and Ernst 1993) and even colonic ischaemia or paraplegia if mural thrombus occludes spinal or visceral vessels (Mansfield and Gilling-Smith 1990). Patients with inflammatory aneurysms are more likely to have symptoms (Goldstone et al. 1978) and may present with a systemic illness and complain of malaise, anorexia and weight loss.

Retroperitoneal fibrosis may cause symptoms and signs of ureteric obstruction in these patients (Pennell et al. 1985). The inflammation around the aorta may involve other adjacent structures producing a variety of symptoms (Crawford et al. 1985).

Occasionally intact aortic aneurysms may become acutely symptomatic. Patients may present with a short duration of severe abdominal pain, thought to result from rapid expansion of the aneurysm and subsequent stretch on the surrounding peritoneum and pressure on adjacent somatic nerves (Mansfield and Gilling-Smith 1990; Goldstone 1991). In these symptomatic aneurysms pain may be accompanied by nausea and vomiting (Hojer 1992) and patients require urgent surgical repair (Hollier et al. 1992).

Ruptured abdominal aortic aneurysms usually present with acute abdominal pain, hypotension and a pulsatile abdominal mass (Darke and Eadie 1973) and this classic triad is said to be diagnostic (Banerjee 1993). The diagnosis may be missed if the triad is not
present in its entirety (Hojer 1992). In the majority of patients who die from rupture this is the first presentation of the disease (Collin 1990b). Without treatment rupture results in a uniformly fatal outcome. Rupture most frequently occurs in the posterolateral aortic wall and is temporarily contained in the retroperitoneum (Hojer 1992; Banerjee 1993). It may produce symptoms characteristic of many other acute abdominal emergencies such as renal colic, pancreatitis, cholecystitis and diverticulitis (Moran et al. 1987; Kiell and Ernst 1993; Siegel and Cohan 1994). Less common is the intraperitoneal rupture, which results in rapid blood loss and commonly death.

Rupture may rarely occur into adjacent structures including the inferior vena cava, left renal vein and the duodenum (Sweeney and Gadacz 1984; Lanne and Bergqvist 1992; Thompson et al. 1993; Miani et al. 1994). Aortocaval fistulae may present with a pulsatile abdominal mass, continuous harsh abdominal bruit, lower limb oedema and a palpable thrill. These rare sites of rupture are seldom diagnosed before surgery.

There is some evidence that the type of AAA presentation is influenced by age. Mulak et al demonstrated that patients who presented under the age of 51 years were symptomatic 46% of the time compared to 6.7% for patients aged 65-75 years. They also showed that aneurysms were larger in the younger group and extended more proximally and suggested that a higher rate of cigarette smoking in this group was important in the pathogenesis of AAA (Muluk et al. 1994).

2.3 Diagnosis

Collin suggested that an aneurysm had to reach 5cm in size to be detectable on routine physical examination (Collin et al. 1988). However palpation is imprecise in determining aneurysm size (Goldstone 1991) and may lead to erroneous diagnosis of an AAA when an unrelated mass lies adjacent to the aorta (Kiell and Ernst 1993). The diagnosis can be confirmed and the aneurysm size accurately determined by ultrasound scanning (Hallett, Jr. 1992; Stevens 1993) which has a sensitivity of approaching 100% (Ernst 1993; Siegel and Cohan 1994). Ultrasound does however, have limitations in the obese patient, and those with excessive bowel gas or periaortic disease (Ernst 1993) and is highly operator dependent (Rubin et al. 1993). Views obtained
of visceral vessels are of insufficient accuracy to help plan surgery (Ernst 1993; Siegel and Cohan 1994).

Computed tomography is also highly sensitive and specific in the diagnosis of AAA (Figure 2.1) (Hojer 1992; Siegel and Cohan 1994). It is particularly useful in determining the proximal and distal extent of the aneurysm and defining the anatomical relations of the visceral and renal vessels preoperatively (Todd et al. 1991). With the advent of endovascular repair CT scanning is vital in determining patient suitability and accurate sizing of the endoluminal prosthesis (Thompson et al. 1995). CT is also useful at delineating AAA from other causes of abdominal pain or abdominal mass (Hallett, Jr. 1992) and is the investigation of choice for the stable patient in whom the diagnosis of ruptured AAA is suspected (Siegel and Cohan 1994). Conventional CT however also has its limitations, a recent study from our department demonstrated that up to 40% of renal artery origins were inadequately visualised with 3 mm slices (Nasim et al. 1998a). Conventional axial images may cut obliquely through a tortuous aorta and thus potentially over estimate aortic diameter.

Spiral CT has overcome some of these problems by producing multiplanar images and subsequent three-dimensional reconstruction. With the addition of dynamic intravenous contrast it is an accurate technique for displaying even the most complex aortic anatomy (Balm et al. 1994) and its has been demonstrated to be a very useful imaging modality in the selection of patients for endovascular repair (Armon et al. 1997).

Magnetic resonance imaging (MRI) accurately predicts aortic dimensions when compared to operative measurements (Figure 2.2) (Fox et al. 1996). It has been recently shown in our centre to be significantly better at assessing aortic morphology than both, axial CT and arteriography and avoids the use of ionizing radiation and iodinated contrast medium (Nasim et al. 1998a). At present no study has compared spiral CT and MR angiography in assessing aortic dimensions.

Finally the use of aortography is not justified in the routine evaluation of aortic aneurysms (Johnston and Scobie 1988a). It may underestimate size or even misdiagnose the presence of an aneurysm due to mural thrombus (Johnston et al. 1991; Pleumeekers et al. 1994). Arteriography however may be helpful when juxta- or supra-renal
Figure 2.1: A contrast enhanced CT image showing a large abdominal aortic aneurysm.
Figure 2.2: An image of the abdominal aorta obtained using MRA (gradient echo sequence) demonstrating the origin of the renal arteries, the infra-renal AAA, and tortuosity of the iliac arteries.
involvement is anticipated or in the presence of multiple renal arteries, horseshoe kidney and visceral and distal occlusive disease (Johnston et al. 1991; Ernst 1993). The advent of endovascular AAA repair has seen a return to the use of aortography in the assessment of patients prior to surgery. It has been demonstrated to be very useful for identifying visceral artery origins and assessing the state of the distal vessels, however accurate evaluation of neck lengths and arterial diameters remains difficult even with the addition of a calibrated angiographic catheter (Nasim et al. 1998a).

In summary AAA diagnosis may be easily made clinically or relatively non-invasively with abdominal ultrasound. Further advances in MRI and CT technology should provide highly accurate morphological information in the future.

2.4 Screening

Age standardised mortality from aortic aneurysm in England and Wales rose 20 fold in men and 11-fold in women between 1950 and 1984 (Fowkes et al. 1989). Similar trends have been reported in other Western countries with Bengtsson et al reporting a clear rise in prevalence when reviewing all autopsies between 1958 and 1986 (Bengtsson et al. 1992a). A significant proportion of the mortality occurs after rupture which caused 1.3% of all deaths in men older than 65 years in England and Wales in 1983 (Collin 1985), 1.4% of deaths in males over 55 years in the Netherlands in 1990 (Central bureau for statistics) and carries an overall mortality of 90% (Drott et al. 1992). In the majority of patients presenting with rupture the existence of the aneurysm was undiagnosed since the majority of AAAs remain asymptomatic until rupture (Webster 1994). For these reasons emphasis has been placed on early detection of asymptomatic aneurysms which permits timely elective surgical repair (Webster 1994). Mortality after elective surgical repair has been significantly reduced over the past thirty years, with various centres reporting rates between 2-8% (Fielding et al. 1981b; Naylor 1988; Greenhalgh 1990; Akkersdijk et al. 1994; Katz et al. 1994; Johnston 1994; Chen et al. 1996) and as a result is the treatment of choice for fit patients of suitable size.

With these facts in mind a number of authors have advocated the introduction of widespread screening programs aimed at detecting and treating AAAs before they
rupture (Scott et al. 1998). When considering the introduction of a screening program, its usefulness can be assessed by comparison with Wilson and Jungner's criteria of 1968 (Wilson and Junger 1968).

There is little doubt that aortic aneurysmal disease is an important health problem accounting for 10,000 deaths in the UK each year (Geroulakos and Nicolaides 1992). A four-fold increase in workload over a ten-year period has also been reported in an English district due to a rise in the incidence of rupture (Ingoldby et al. 1986).

The generally accepted treatment for asymptomatic aneurysms detected by screening is timely elective surgical repair based on size and patient risk. However mortality rates from elective repair range from 2-8%, a not insignificant risk (Fielding et al. 1981b; Greenhalgh 1990; Akkersdijk et al. 1994; Katz et al. 1994; Johnston 1994; Chen et al. 1996). In addition surgery is associated with significant morbidity, with Resnikoff et al reporting early post-operative non-fatal complications in 11% of patients in addition to a mortality rate of 2.4% (Resnikoff et al. 1996). Complications such as graft infection, aorto-enteric fistula and graft occlusion should also be considered when estimating long-term survival after elective repair. At present elective transperitoneal surgical repair remains the gold standard treatment, however the advent of endovascular aneurysm repair may play an important role in reducing morbidity and mortality in the coming years. The prospect of effective pharmacotherapy for small aneurysms, detected by screening, may however become the most significant advance in AAA treatment in the future.

Abdominal aortic aneurysms are easily detectable at a latent stage by ultrasound scanning, which provides a suitable screening test that is readily available. It is also relatively cheap, with sensitivity and specificity estimated to be better than 95% and is reproducible within a detection range of less than 2.2mm (Shapira et al. 1990). It does have some limitations however, in obese patients and those with peri-aortic disease. The other advantage of ultrasound is that it is painless, non-invasive and is therefore acceptable to the patients.

Despite abundant information on the risk factors associated with aneurysmal disease, the prediction of rupture in the individual patient remains difficult. More information about the natural course of the disease has now become available with the
recent publication of the UK small aneurysm trial (Anonymous 1998). The surgical strategy for non-ruptured AAA had previously been the subject of much controversy. A report from a subcommittee of the Joint Council for Vascular Surgery and the North American Chapter of the International Society for Cardiovascular Surgery suggested that all patients with aneurysms sized more than 4.0 cm should undergo surgery (Hollier et al. 1992). However many surgeons in this country were likely to treat patients conservatively until aneurysm size reaches 5.5 cm, a strategy reinforced by the small aneurysm trial.

The cost effectiveness of screening is also controversial. The expenditure on screening and elective surgery must be balanced against the overall enhanced life expectancy. At present we await a randomised controlled trial of outcome of a screened cohort versus that of an unscreened group. The long-term survival rate 5 years after elective surgery for AAAs was reported to be similar for age and sex matched controls by Rohrer et al (Rohrer et al. 1988), however Johnston reported a 60% 5 year survival, 18% lower than that of the controls (Johnston 1994). It was suggested that the difference might have been as a result of co-morbid factors in the AAA group.

In summary the technical feasibility of AAA screening appears to be proven. There remains doubts over it's effectiveness, with many authors emphasizing that although 5-8% of elderly males are affected by mostly small AAAs these aneurysms may not rupture before death from other causes. The mean age of aneurysm rupture is between 70-75 years approximately the same as life expectancy for males, many of whom die from other causes. Some have therefore suggested screening of high-risk groups such as hypertensives (O'Kelly and Heather 1989), who have a higher incidence of aneurysms and may rupture at a younger age. Other authors have demonstrated that screening of smokers (Muluk et al. 1994) and first degree relative of patients with AAA yield better results (Webster et al. 1991). Several studies have proposed the addition of an aortic scan to peripheral arterial or carotid investigations in the vascular laboratory (Wolf et al. 1995). Carty et al demonstrated an increased incidence of AAA in patients presenting with carotid artery disease (Carty et al. 1993) and Karanjia suggested that up to one fifth of patients with carotid stenosis had coexistent aortic aneurysmal disease (Karanjia et al. 1994). Collin suggested that such screening of patients presenting with other diseases
might achieve almost as much success as total population screening (Collin 1993). The detection of AAAs allows elective repair, the benefits of which must be taken in the context of a 5% risk of mortality and considerable morbidity.

There is now evidence that an effective screening program reduces the mortality from rupture. However the greatest benefit from screening may come with effective pharmacotherapy for small AAAs.

2.5 Indications for Surgery

Elective repair of abdominal aortic aneurysms aims to prevent rupture, which carries a high mortality. However, elective repair itself has a mortality of 5.4% (Johnston 1994). The rationale for treatment therefore may be based on the premise that the mortality after surgery is less than the risk of rupture within one year (Scott et al. 1998).

Aneurysm diameter remains the strongest predictor for rupture (Nevitt et al. 1989). Larger aneurysms are more likely to rupture than smaller ones. Absolute diameter itself may be misleading as the normal aortic diameter varies with the size of the patient and an aneurysm of a specific diameter may be more likely to rupture in a smaller patient (Ouriel et al. 1992). Chronic obstructive pulmonary disease and diastolic hypertension have also been associated with an increased risk of rupture (Cronenwett et al. 1985).

The recent publication of the UK small aneurysm trial has clarified the indications for elective surgical repair of abdominal aortic aneurysms (Anonymous 1998). This study randomised fit patients with asymptomatic infrarenal aneurysms of 4.0-5.5cm to early elective repair or ultrasonographic surveillance of aneurysm diameter. The results demonstrated that elective surgical repair was not associated with a long-term survival benefit and that ultrasound surveillance provided a safe alternative method of management in these patients. In both groups there was a 64% survival at 6 years. Based on this data most surgeons in the United Kingdom will offer elective surgical repair in asymptomatic abdominal aortic aneurysms with diameters of greater than 5.5 cm. A similar study is at present underway in the United States (Aneurysm Detection and Management) (Lederle et al. 1994) and its results will be reported in the future. At
present however, surgeons in the US have a lower threshold for operating. The subcommittee of the Joint Council of the Society of Vascular Surgery and the North American Chapter of the International Society for Cardiovascular Surgery recommended AAA repair in all patients with aneurysms greater than 4cm in diameter (Hollier et al. 1992).

The decision to offer elective AAA repair in the individual patient is not determined by aneurysm diameter alone. The age of the patient and the presence or absence of co-morbid risk factors are also important determinants. Careful preoperative assessment and preparation are therefore of paramount importance.

The indications for surgery in ruptured or symptomatic aneurysms are clearer with most surgeons recommending urgent repair, provided coexisting conditions do not preclude operation (Brown et al. 1992; Ernst 1993). The coexistence of a terminal illness, such as metastatic cancer, is sufficient cause to elect a conservative approach (Hollier et al. 1992).

2.6 Preoperative Assessment

Prior to elective repair of an asymptomatic AAA preoperative assessment of both the aneurysm and the patients general condition is required. Operative risk is largely determined by individual patient characteristics and must be carefully evaluated before surgery (Hallett et al. 1994). In common with any major surgical procedure, risk is closely associated with age, pulmonary, renal and cardiac function.

Mortality has been reported to be greater with increasing age (Katz et al. 1994). Despite this other authors have reported good results after surgery in-patients over 70 (Bernstein et al. 1988) and even 80 years of age (Paty et al. 1993). Both authors suggested that physiological rather than chronological age should aid in the selection of elderly patients for surgery.

Respiratory function need not be formally assessed in all patients undergoing AAA repair. Those, however who give a history of COPD or who demonstrate signs of respiratory disease should undergo formal lung function tests, such as spirometry, and arterial blood gas analysis. Hallett suggested that chronic obstructive pulmonary disease
should not be considered an absolute contraindication to surgery, but should identify the
patient who requires special postoperative care (Hallett, Jr. 1992). He suggested that
pulmonary complications could be minimised by preoperative cessation of smoking, the
use of bronchodilators and antibiotics for chronic bronchitis and early extubation and
mobilisation following surgery (Hallett, Jr. 1992).

Pre-existing renal insufficiency is linked with increased mortality, but likewise
should not preclude aneurysm repair, but identify the patient who needs special
perioperative management (Katz et al. 1994). Johnston and Scobie identified 6% of
patients undergoing elective AAA repair to have significantly elevated creatinine levels
(>2mg/dl) (Johnston and Scobie 1988a). These patients should have careful perioperative
fluid monitoring including pulmonary artery wedge pressure readings (Riles and
Pasternack 1990) and may be treated with dopamine to dilate renal arterioles and
mannitol to increase renal cortical flow. Nicholson at al have demonstrated mannitol to
have a protective effect on renal function, in a randomised-controlled trial of elective

Coronary artery disease (CAD) is, however, the major cause of perioperative
death (Johnston 1989). This multicentre study reported an overall mortality of 4.8% with
two-thirds of the operative deaths secondary to cardiac complications. Johnston and
Scobie also reported the prevalence of CAD to be 44.7% in patients undergoing elective
AAA surgery, of whom 26% had a postoperative cardiac event and 6.2% died of
myocardial infarction (Johnston and Scobie 1988a). Clearly aggressive preoperative
evaluation and optimisation of cardiac associated risks is desirable. The diagnosis of
CAD may be already apparent or may be diagnosed by careful history examination and
ECG interpretation (Riles and Pasternack 1990). Patients who present with angina at rest
or on mild exertion or who have a past history of myocardial infarction or congestive
cardiac failure should undergo further cardiac evaluation (Johnston 1989). These
investigations may include exercise stress testing, radionuclide ventriculography,
dipyridamole-thallium scintigraphy (DTS) and coronary angiography (Suggs et al. 1993;
Blackbourne et al. 1994). Hertzer recommended that patients should undergo cardiac
catheterisation prior to aneurysm repair and myocardial revascularisation, if required, as a
preliminary or synchronous procedure (Hertzer et al. 1984). Their initial work identified
only 6% of patients in this group to have normal coronary arteries and a subsequent publication identified 42% of patients with clinical disease and 19% with no clinical indications of CAD to have severe surgically correctable disease (>70% stenosis)(Young et al. 1986). However subsequently other authors have suggested that only 5-10% of patients require preliminary revascularisation via coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA) before AAA repair(Goldstone 1991; Hallett et al. 1994). Most surgeons do not routinely arrange coronary angiography prior to AAA surgery. In the US a selective policy is commonly practiced whereby angiography is reserved for patients with severe angina(Golden et al. 1990; Lachapelle et al. 1992). Patients are divided into three groups: those without any evidence of CAD (Group I), those with mild to moderate CAD (previous MI, class I and II angina [New York Heart Association Classification], abnormal ECG) (Group II) and those with severe CAD (congestive cardiac failure, class III-IV angina) (Group III). Based on these criteria, Lachapelle et al. reported an overall mortality of 1.8% and a cardiac mortality rate after AAA repair in-patients in group I(Lachapelle et al. 1992). They also confirmed that patients with severe CAD benefited from coronary angiography and if indicated coronary artery bypass before AAA repair(Lachapelle et al. 1992).

Those patients with mild to moderate CAD (Group III) pose a greater management problem. There is not a reliable non-invasive test to identify those patients who would benefit from myocardial revascularisation. Some authors have suggested that the left ventricular ejection fraction (LVEF) is a good predictor of postoperative cardiac complications. Pasternack et al reported a 17% incidence of cardiac complications in patients who had a LVEF of less than 40% as assessed by radio nuclide scan, compared to 3.4% for patients with LVEF of greater than 40%(Pasternack et al. 1984). Lachapelle et al however found no correlation between LVEF and the risk of a postoperative cardiac event(Lachapelle et al. 1992). LVEF is measured at rest and therefore is not an accurate measure of the stressed myocardium. Exercise tolerance testing (ETT) overcomes this problem, however it is not well-tolerated vascular patients(Cutler et al. 1981; McPhail et al. 1988) and a positive test does not always predict significant CAD on subsequent coronary angiography(Cutler et al. 1981).
More recently dipyridamole-thallium scanning has been identified as a useful alternative to ETT (Cutler and Leppo 1987). Eagle et al have demonstrated a dipyridamole-thallium scan can identify those patients, with intermediate cardiac risk, who are most likely to suffer postoperative cardiac complications (Eagle et al. 1989). Thus a dipyridamole-thallium scan can select those patients in Group II, who may benefit from coronary angiography.

The timing of coronary artery investigation and subsequent revascularisation complicates the management of the aortic aneurysm. Some authors have reported early postoperative deaths after CABG as a result of AAA rupture (Suggs et al. 1993; Blackbourne et al. 1994). Surgeons must therefore weigh the risk of delay of AAA repair and possible rupture against that of postoperative cardiac morbidity.

In deciding to proceed with elective AAA repair in the individual patient both aneurysm size and co-morbid risk factors must be considered. In most cases careful preoperative evaluation and perioperative monitoring and management allow safe aneurysm repair (Hollier et al. 1992; Hallett et al. 1994). In those patients with correctable risk factors rapid optimisation should be undertaken prior to surgery (Hollier et al. 1992; Bower et al. 1993)

2.7 Management

The publication of the UK small aneurysm trial has clarified the role of surgery for abdominal aortic aneurysm. Patients with asymptomatic aneurysms with a diameter of 5.5 cm or less should be treated conservatively and undergo regular ultrasound surveillance (Anonymous 1998). Prior to this study some had suggested that smaller aneurysms should be surgically repaired (Hollier et al. 1992) and others suggested conservative management until an aneurysm reaches 6 cm (Scott et al. 1998). A further trial is required determine whether aneurysms of greater than 5.5 cm can be treated conservatively. Until this time most surgeons will offer surgical repair to fit patients with aneurysms of greater than 5.5 cm.

It is not my intention to give a detailed description of conventional surgical techniques; however, the general principles are discussed below. The importance of good
perioperative anaesthetic management should not be underestimated and is discussed in
greater detail in view of its importance in later experimental chapters. Aneurysm patients
are typically elderly with limited function and reserve in many organ systems. Aneurysm
repair imposes substantial physiological and haemodynamic stresses, with large fluid
fluxes, aortic cross-clamping and unclamping, bleeding and renal compromise. The aims
of anaesthesia should be to provide a pain and stress free environment for the patient,
provide relaxation to facilitate surgery and minimise the incidence of perioperative
morbidity and mortality(Hessel 1989). After induction of anaesthesia the following
procedures should be performed prior to the start of surgery; insertion of at least one,
large gauge, venous cannula, radial artery cannulation, central venous catheterisation and
right atrial pressure monitoring, insertion of a temperature probe and urinary bladder
catheterisation(Aitkenhead and Smith 1990). There is also some evidence that epidural
anaesthesia may reduce cardiac and metabolic stress(Kiell and Ernst 1993). Three
specific stimuli may give rise to cardiovascular instability during aneurysm repair. Firstly
tracheal intubation may cause a considerable rise in systemic arterial pressure and should
be minimised to avoid myocardial ischaemia. This may be achieved by the intravenous
administration of a -blocker or a lipid soluble opioid such as alfentanil before intubation.
Secondly cross clamping of the aorta causes a sudden rise in systemic vascular resistance
(afterload). This increases cardiac work and may result in myocardial ischaemia,
arrhythmias and left ventricular failure. It may be necessary to administer short acting
vasodilators such as sodium nitroprusside or glyceryl trinitrate during this period to
obviate these problems. Finally aortic declamping causes a sudden decrease in afterload
and reperfusion of the lower part of the body. Acid metabolites from the limbs enter the
circulation and may cause vasodilatation and metabolic acidosis. Bleeding may be a
problem throughout the operation but may be particularly important at this time as the
vascular anastomoses are tested. All these problems may combine to cause severe
hypotension unless circulating volume has been well maintained and transfusion is
continued to maintain an adequate CVP. This can be avoided by producing relative
hypervolaemia during the period of clamping by infusing fluid to maintain CVP at 10-12
cmH$_2$O and perhaps the use of sodium nitroprusside. Aneurysm patients generally have a
low metabolic rate and do not tolerate heat loss well. All possible measures to minimise heat loss should be taken (Aitkenhead and Smith 1990).

In the more compromised patient with poor left ventricular function, the use of a pulmonary artery catheter is indicated. This allows measurement and preservation of optimum left ventricular preload at crucial points of the operation (Aitkenhead and Smith 1990).

Charles Dubost of Paris reported the first successful infrarenal abdominal aortic aneurysm repair in March 1951 (Dubost et al. 1952) using a cadaveric aortic homograft. There have since been considerable modifications in surgical technique. The major problem with aneurysm resection and subsequent graft replacement was the risk of injury to adjacent structures, particularly the inferior vena cava and the iliac veins. Consequently transabdominal repair of abdominal aortic aneurysm with endoaneursymorraphy and inlay grafting has become the gold standard to which all other modes of contemporary aneurysm repair are compared since it was first described by Creech in 1966 (Creech, Jr. 1966). On going surgical advances, based on this technique, combined with improvements in peri-operative anaesthetic care have led to dramatic reductions in morbidity and mortality following elective aneurysm repair. Critics of this technique have, however, voiced concern over the prolonged ileus, post-operative respiratory dysfunction and increased third space fluid losses (Johnson et al. 1986; Sicard et al. 1989). An alternative retroperitoneal approach with inlay-grafting, has its proponents who believe this technique offers better aortic exposure and reduces peri-operative morbidity (Johnson et al. 1986; Darling et al. 1992; Sicard et al. 1995). Some have therefore advocated the use of the retroperitoneal approach for high-risk patients, believing it to be better tolerated and less physiologically stressful. Several retrospective and prospective randomised studies have supported the premise that the retroperitoneal approach is associated with fewer post-operative complications (Johnson et al. 1986; Darling et al. 1992; Sicard et al. 1995). The most recent randomised study demonstrated a higher incidence of prolonged ileus, small bowel obstruction and total complications after transabdominal surgery and shorter stays on the intensive care unit after a retroperitoneal incision. There was however no difference in pulmonary complications between the two groups. The retroperitoneal group reported more incisional pain at
follow-up (Sicard et al. 1995). There are some disadvantages to the retroperitoneal approach, it is technically demanding and access to the right iliac and renal vessels may be difficult. It also does not allow adequate assessment of the abdominal viscera in a group of patients who have a substantial risk of other pathology, particularly in those who present with abdominal symptoms not clearly due to the aneurysm (String 1984). Despite the evidence above, the transperitoneal approach for uncomplicated infra-renal AAA remains the most popular; with the indications for retroperitoneal surgery, limited to supra-renal, juxta-renal or inflammatory aneurysms, cases of 'hostile abdomen' and high anaesthetic risk patients (Butler et al. 1993). The choice of incision for transperitoneal repair is either transverse or midline and there is some evidence to suggest that hospital stay is significantly shorter after a transverse incision (Lord et al. 1994).

Briefly after access has been obtained the aneurysm is isolated, by preferably; infra-renal and iliac clamping and inlay grafting performed. Straight segmental grafts can be implanted into the majority of patients undergoing AAA repair with significantly reduced operating time and lower blood loss than the one third that require bifurcated grafts (Wilson et al. 1993).

The use of intraoperative heparin in uncomplicated surgery is controversial and remains a matter of individual surgical preference. There is evidence from a randomised controlled trial that heparin significantly reduces the rate of perioperative myocardial infarction but does not influence thrombotic complications or the degree of blood loss during surgery (Thompson and Clyne 1994).

Complications of aneurysm repair are numerous and have considerable relevance to latter experimental chapters, and are detailed below.

**2.8 Complications of AAA surgery.**

**Early complications**
Procedure related

**Haemorrhage**

The incidence of operative bleeding in the Canadian multicenter prospective study was 4.8% with lower rates for postoperative bleeding requiring transfusion, 2.3%, or
repeat operation 1.4% (Johnston 1989). Operative and postoperative haemorrhage most commonly results from injury to surrounding venous vasculature, the proximal aortic anastomosis, the lumbar arteries and from coagulopathies (Barnhard and Towne 1983). The infrarenal aorta is surrounded by a halo of veins and anomalies of venous anatomy are common, particular care must be taken to avoid iatrogenic injury to these structures (Bartle et al. 1987).

Haemorrhage from the proximal anastomosis is more likely to occur with high lying short-necked aneurysms and when there is severe degeneration of the aortic wall. Bleeding from lumbar arteries occurs either as a result of iatrogenic laceration or avulsion during aortic neck dissection or into the opened aneurysm sac and requires suture ligation (Hermreck 1989).

Postoperative bleeding is associated with volume of intraoperative blood loss and increases in early and late morbidity. In particular postoperative bleeding is associated with cardiac events and arrhythmias, prolonged ventilation, deterioration in renal function and coagulopathy (Johnston 1989). This study demonstrated no association however with mortality, however previous work by Diehl et al identified large intraoperative blood loss an important variable in predicting death (Diehl et al. 1983). In the long term intraoperative blood loss has been associated with a predisposition to incisional hernia (Lord et al. 1994).

In summary most intraoperative bleeding results from arterial or venous injury and may be prevented in many cases by adhering to established technical principles (Johnston 1989). Operative haemorrhage influences morbidity and mortality.

**Arterial embolisation and thrombosis**

Intraoperative limb ischaemia occurs in 3.5% of cases and is strongly associated with pre-existing distal arterial occlusive disease (Johnston 1989). The majority of cases are secondary to thrombosis with a significant minority embolic in origin. The presence of large amounts of atheromatous debris and clot in most aneurysms puts virtually all structures distal to the diaphragm at risk of embolisation (Hermreck 1989). Starr et al suggested that distal embolisation could be reduced by distal clamping prior to applying the proximal aortic clamp (Starr et al. 1979). Embolisation of renal and mesenteric
vessels leads to renal failure and intestinal ischaemia respectively which are discussed in greater detail below.

Interestingly embolisation has been reported after endovascular AAA repair. In particular massive microembolisation has been described by a number of authors, a serious complication with a mortality approaching 90%. Three of Parodi’s first 50 patients suffered this complication all of whom died (Parodi 1995). Marin et al also reported three deaths due to diffuse embolisation during endograft placement (Marin et al. 1995) and Mialhe and Amicabile four cases of isolated lower limb embolisation in 81 patients (Mialhe and Amicabile 1995). Recent work in our department using modified transcranial Doppler technology has demonstrated significantly greater numbers of both particulate and gaseous emboli in the superficial femoral artery during endovascular AAA surgery in comparison with conventional repair (Thompson et al. 1997d; Thompson et al. 1997e). The mechanism of embolisation during endovascular repair is presumably due to the manipulation of the endograft within the thrombus-lined sac.

**Declamping hypotension.**

Careful anaesthetic management as discussed above and good communication between surgeon and anaesthetist can avoid hypotension on restoration of blood flow to the lower limbs. It may lead to myocardial ischaemia, infarction, or cardiac arrest in the presence of severe coronary artery disease (Rutherford 1984).

**Renal Failure**

Acute renal failure requiring dialysis is a rare complication of elective conventional aneurysm surgery. Johnston demonstrated an incidence of 0.6%, however 5.4% of patients showed renal damage, measured as a rise in creatinine or blood urea nitrogen by greater than 20% of the pre-operative value. He also showed that deterioration of renal function had a mortality rate of 27.8% (Johnston 1989). Other authors have demonstrated mortality of acute renal failure of between 50 and 90% (Porter et al. 1966; McCombs and Roberts 1979). The mechanism of renal injury is usually ischaemic in nature and results from hypovolaemic or cardiogenic shock, an extended period of suprarenal clamping or embolisation. Less commonly nephrotoxic injury results
from radiographic contrast agents, perioperative antibiotics, haemoglobinuria secondary to transfusion reactions, or myoglobinuria secondary to skeletal muscle necrosis (Miller and Myers 1987).

Early experience with endoluminal surgery has also identified renal failure as a complication of this technique. May et al reported renal insufficiency in 7 of 121 patients after endovascular repair, five of which were related to contrast media, and the other two caused by stents covering renal vessels. Two of these patients died (May et al. 1997).

More recently the EUROSTAR collaborators reported renal complications in 32 of 899 patients undergoing endovascular repair with various devices (Cuypers et al. 1999).

Prevention of renal failure requires recognition of risk factors, preoperative preparation, and careful operative monitoring and management. In addition to adequate intravenous hydration there is evidence that mannitol given prior to aortic cross clamping during conventional surgery reduces subclinical glomerular and tubular renal damage (Nicholson et al. 1996). Low dose dopamine may also improve cardiac output and dilate renal vasculature.

Preoperative renal insufficiency is the only independent risk factor for postoperative acute renal failure after conventional surgery (Miller and Myers 1987). Renal damage is also more likely if the aorta is clamped above the renal vessels and/or the renal vein is ligated (Johnston 1989). Existing renal impairment is an important variable in predicting death after conventional repair; Diehl et al identified an increased mortality with creatinine greater than 2mg/dl (Diehl et al. 1983) and similarly Johnston 1.8mg/dl. These patients require careful perioperative management.

**Colonic Ischaemia**

Ischaemic colitis is a recognised complication of aorto-iliac surgery. The incidence of clinically detectable ischaemia is 0.6-3% (Lannerstad 0 1985, Schroeder T 1985, Johnston 1989, Brewster DC 1991) (Bjorck et al. 1996) with higher rates among patients operated for ruptured AAA (Bjorck et al. 1996). This large Swedish study reported an incidence of 7.3% in shocked patients who underwent surgery for rupture. Similarly the incidence appears to be greater in those patients receiving large blood transfusions after rupture (Farooq et al. 1996). The incidence of mucosal ischaemic colitis
as assessed by sigmoid tenometry is greater with Bjorck et al reporting rates of 7.4% after elective and 29% after emergency aortic surgery (Bjorck and Hedberg 1994). The incidence of intestinal gangrene is much lower, and the clinical significance of mucosal injury is therefore controversial (Bjorck et al. 1997). Although a sigmoid pH below 7.1 for more than 2 hours was highly predictive of all major complications and death after aortoiliac surgery (Bjorck and Hedberg 1994).

The commonest presenting symptoms are early postoperative passage of stool, early diarrhoea and early bloody diarrhoea. Only 12% present with peritonitis and abdominal pain (Bjorck et al. 1996). Patients are at greater risk of colonic ischaemia with increased intraoperative blood loss, increased blood transfusion, increased operating and clamp time and if one or both of the internal iliac vessels are ligated (Bjorck et al. 1997). They did not however demonstrate an association with inferior mesenteric artery patency. Interestingly endovascular aneurysm repair commonly involves the occlusion or ligation of one internal iliac artery (Thompson et al. 1997b). However a recent small study comparing sigmoid pH in comparative cohorts undergoing conventional and endovascular AAA repair demonstrated the lowest perioperative pH to be significantly lower in the conventional group (Syk et al. 1998). Cuypers et al recently reported only one case of colonic ischaemia in 899 cases of endovascular repair (Cuypers et al. 1999).

As the diagnosis of colonic ischaemia is not always evident, a high degree of suspicion and willingness to perform flexible sigmoidoscopy and if indicated second look laparotomy is important (Bjorck et al. 1996).

**Internal iliac artery insufficiency.**

Ligation of the internal iliac vessels during conventional aneurysm repair may not only cause colonic ischaemia and has been associated with buttock claudication, impotence and even paraplegia and buttock necrosis (Hermreck 1989). Unilateral occlusion of the internal iliac artery during aneurysm repair causes severe buttock claudication in a significant number of patients (Iliopoulos et al. 1987). The more severe buttock necrosis is an exceedingly rare complication (Iliopoulos et al. 1987) and is usually associated with infarction of the anorectum and prostate, and paraplegia; it is almost always fatal (Hermreck 1989). Spinal cord injury is also extremely rare after
elective conventional surgery but is more common after rupture (Szilagyi et al. 1978; Hands et al. 1991). Reduction in the blood supply to the anterior spinal artery may be due to interruption of an anomalous greater radicular artery of Adamkiewicz, which is said to originate from a lumbar artery in 10% of cases. However Johnston suggested that the one reported case in the 666 patients of the Canadian study resulted from ligation of both internal iliac arteries reducing collateral flow to the lower spinal cord or cauda equina (Johnston 1989). Impotence after aortoiliac reconstruction is thought to be secondary to diversion of pelvic blood flow and or autonomic nerve injury during dissection (Depalma 1982). Preservation para-aortic and superior hypogastric nerve plexuses and internal iliac artery blood flow decreases the incidence of sexual dysfunction after aortoiliac reconstruction (Queral et al. 1979; Depalma 1982; Depalma 1982). The incidence of the internal iliac artery related complications after endovascular repair are yet to be accurately established. We have reported 2 cases of non-disabling buttock claudication after internal iliac ligation in our first 25-aortomoiliac endovascular repairs. The importance of maintaining the patency of at least one internal iliac artery during conventional aneurysm surgery may well have equal importance for endovascular repair.

Systemic complications
Cardiac complications

Cardiac events following conventional AAA repair are the commonest cause of both early and late mortality (Crawford et al. 1981; Diehl et al. 1983). Crawford et al reported a thirty-day mortality of 4.76% in 860 patients with more than half the deaths attributed to a cardiac cause. The same study also identified cardiac death as the commonest cause of late mortality (Crawford et al. 1981). The Canadian Study reported 15.1% of patients had a perioperative cardiac event, defined as the occurrence of myocardial infarction, arrhythmia requiring treatment, new arrhythmia and/or congestive cardiac failure. Cardiac events accounted for 25 of 32 deaths in this series (Johnston 1989). Similar figures have been reported from our centre with cardiac events causing or contributing to mortality in 66% of deaths (Sayers et al. 1997). More recent evidence has suggested that multisystem organ failure (MSOF) is currently the leading cause of death.
after elective aneurysm surgery. Huber et al reported 6.1% mortality in a population of 722 aortic reconstructions of whom 56.8% died of multiple organ failure and 25% secondary to a cardiac event. They suggested that the rise in incidence of MSOF might have resulted from performing more complex procedures on older sicker patients. Multivariate analysis identified age, history of myocardial infarction/congestive cardiac failure, left ventricular ejection fraction less than 50%, increasing operative time and performance of additional procedures to be associated with increased mortality (Huber et al. 1995).

Preoperative cardiac assessment and optimisation is of paramount importance as described above as a history of congestive heart failure or perioperative myocardial infarction have been identified as independent variables predictive of death (Chen et al. 1996). Patients with clinically severe CAD appear to benefit from previous coronary artery bypass and therefore should be assessed prior to AAA repair with coronary angiography (Lachapelle et al. 1992).

Inevitably cardiac complications have been reported after endovascular repair. May et al reported an incidence of 8% compared with 14% after conventional surgery in a concurrent comparison of endoluminal versus open repair (May et al. 1997a). Myocardial infarction accounted for 50% of the deaths in this study (May et al. 1997a). The EUROSTAR Collaborators recently reported cardiac events and multiple system organ failure as the leading two causes of death in 899 patients undergoing endovascular repair and significantly correlated death with ASA grade (Cuypers et al. 1999). One might predict however that the absence of aortic cross clamping during endovascular repair may reduce cardiac morbidity in comparison to conventional repair and this hypothesis is tested in subsequent experimental chapters.

Respiratory complications

Pulmonary complications are a significant cause of both early and late mortality after conventional AAA repair (Crawford et al. 1981). Diehl et al reported postoperative pulmonary insufficiency in 5.1% of 350 elective cases but this did not correlate with preoperative pulmonary function (Diehl et al. 1983). Johnston described respiratory failure in 8.4% and was more common if the patient had pre-existing pulmonary disease,
a large volume of blood transfused and/or other postoperative complications (Johnston 1989). More recently Huber et al reported primary respiratory failure caused 4.5% of deaths but more significantly postoperative pneumonia alone or complicated by aspiration or adult respiratory distress syndrome caused MSOF in 36% of fatal cases (Huber et al. 1995).

The absence of a large upper abdominal incision during endovascular AAA surgery may reduce postoperative respiratory complications; this hypothesis is examined in greater detail in later experimental work. Early series have reported respiratory complications, Balm et al described three cases of respiratory failure, two requiring ventilation (Balm et al. 1996), and Yusuf et al reported one death from pneumonia (Yusuf et al. 1997). The recent EUROSTAR data recorded 26 pulmonary complications in 899 patients without more detailed description, equating to 2.9% (Cuypers et al. 1999). Other large series have however not commented on respiratory complications (May et al. 1997a; Blum et al. 1997b).

**Cerebrovascular complications**

Stroke is a less common complication of AAA surgery. Crawford et al reported 8 cases of CVA in 860 elective AAA repairs, three of which subsequently died (Crawford et al. 1981). Similarly Johnston and Diehl et al identified low incidence of cerebrovascular events in 0.6% and 0.9% of cases respectively (Diehl et al. 1983; Johnston 1989).

The endoluminal manipulation involved in the deployment of an endovascular prosthesis and the use of intra-arterial catheters and guidewires might lead to an increased risk of cerebrovascular complications after endovascular AAA surgery. Interestingly May et al have reported a 3% stroke and TIA rate after endoluminal repair compared to 1% after conventional surgery (May et al. 1997a). Cuypers et al recently described cerebral complications in 10 (1.1%) patients causing three deaths (Cuypers et al. 1999).

**Ischaemia reperfusion injury**

Reperfusion of lower limb skeletal muscle after conventional AAA repair leads to the production of oxygen-derived free radicals and cytokines and neutrophil activation. These products of reperfusion overcome the normal cellular protective pathways to cause
distant organ injury, in particular damage to the vascular endothelium, increasing its permeability and leading to pulmonary and renal dysfunction.

Smith et al demonstrated increased albumin secretion from the kidneys after AAA surgery and correlated the degree of microalbuminuria to postoperative pulmonary dysfunction (Smith et al. 1994). The same group have also shown a fall in antioxidant concentrations during clamping and after clamp release during conventional AAA surgery and thus indirectly a rise in oxygen-derived free radicals (Khaira et al. 1996).

Interestingly work from our centre measuring oxygen free radicals directly has demonstrated significantly lower levels when comparing endovascular with conventional AAA repair (Thompson et al. 1996a).

Late Grafted Related Complications

Infected aortic prosthesis

The incidence of aortic graft infection is approximately 1% (Fry and Lindenauer 1967; Szilagyi et al. 1972; Kaiser et al. 1978; Lorentzen et al. 1985). It is an extremely serious complication of aortic surgery with a reported mortality ranging from 24% (Hannon et al. 1996) to 83% in severe graft infections (Jacobs et al. 1991). The condition is also associated with a prolonged inpatient stay; Lorentzen et al reported a mean of 90 days and high amputation rates (Lorentzen et al. 1985). Hannon et al reported nine major limb amputations in fifty patients treated for aortic graft infection (Hannon et al. 1996) similar to the 27% of patients quoted by O’Hara et al, of whom greater than a third required bilateral amputations (O’Hara et al. 1986).

A number of different organisms have been implicated in aortic graft infection, most commonly staphylococcus aureus, epidermidis and escherichia coli have been isolated (Hannon et al. 1996; Speziale et al. 1997). The source of infection is usually the skin, staphylococcus epidermidis being abundant in normal skin flora. The predominance of this organism has been blamed on the inability to sterilize the skin completely (Bandyk et al. 1984; Schmitt et al. 1986; Bergamini et al. 1988). Schmitt et al suggested that mucin produced by this organism facilitated its adhesion to a vascular graft (Schmitt et al. 1986). Other potential avenues of infection include infected lymphatics, aneurysm sac contents, the gastrointestinal and genitourinary systems, and haematogenous spread.
Symptoms of graft infection include intermittent fever, gastrointestinal bleeding, weight loss, groin sinus or abscess and abdominal pain (O’Hara et al. 1986; Speziale et al. 1997). These symptoms may be accompanied by abnormal erythrocyte sedimentation rates and white cell counts (Speziale et al. 1997). CT or MRI can confirm the diagnosis in the majority of patients, but a labeled white cell scan appears to be more sensitive. Hannon et al demonstrated 64% of patients to have abnormalities on CT suggesting infection, compared with 79% with abnormalities on indium-labeled white cell scan (Hannon et al. 1996). Speziale et al similarly showed positive CT, MRI and technetium-labeled scans confirmation in 72%, 75% and 100% respectively (Speziale et al. 1997).

Treatment of graft infection is controversial, the gold standard approach has been total graft excision, aortic stump closure and extra-anatomic bypass to the lower limbs (O’Hara et al. 1986). However some authors have proposed a more conservative approach placing gentamicin beads inside a fenestrated tube drain in the retroperitoneum or irrigating this space with gentamicin solution (Reilly et al. 1989; Quick et al. 1990). Morris et al recently reported an 80% one-year survival in a group of ten patients treated with prolonged, high dose local antibiotic irrigation therapy, systemic antibiotic treatment, surgical debridement and graft conservation (Morris et al. 1994a). More recently in situ graft replacement has been described. Jacobs et al reported good results using this technique in the presence of low-grade graft infection but poor results in severe graft infection (Jacobs et al. 1991). This technique has been used in our centre using a rifampicin-bonded prosthesis with promising early results (Naylor et al. 1995).

To date there are no reports of significant graft infection after endovascular AAA repair. Parsons et al however demonstrated that endovascular grafts are more susceptible to infection than standard retroperitoneal grafts in a canine model (Parsons et al. 1996). We have experienced a minor staphylococcus aureus graft infection localised to the groin, which responded to conservative management (Thompson et al. 1997b).

Aorto-enteric fistula

A fistulous communication between an aortic graft and the gastrointestinal tract is a serious and life threatening complication of AAA and AOD surgery. The precise
incidence of this complication is difficult to determine with certainty as most large series include patients from a number of different sources. However Champion et al demonstrated an incidence of 1.5% in a group of 1,376 abdominal aortic graft reconstructions (Champion et al. 1982). Other authors have quoted an overall aortic graft complication rate of 2% (Plate et al. 1985). Greater than 75% of aortoenteric fistulae involve the duodenum and nearly all the small intestine, although communication with the stomach, colon and appendix have been reported (Connolly et al. 1981). Aetiological factors predisposing to aortoenteric fistula include graft infection (Busuttil et al. 1979) which is present in most cases (McCann et al. 1993), false aneurysm formation (Treiman et al. 1988) and failure to interpose adequate viable tissue between the graft and the gastrointestinal tract. Graft infection is present in the majority of aortoenteric fistulae, however whether infection caused the fistula or resulted from it is not always clear. Mechanical factors are also likely to play a role, primary aortoenteric fistulae occur spontaneously between an unoperated abdominal or iliac aneurysm and the gut implying that communication is secondary to a repetitive mechanical pulsatile force (Connolly et al. 1981).

The diagnosis of aortoenteric fistula commonly but not always presents with gastrointestinal haemorrhage, McCann et al reported gastrointestinal bleeding in 12 (69%) of seventeen patients with the remainder demonstrating evidence of infection (McCann et al. 1993). Champion et al however demonstrated melaena and/or haematemesis in all of 22 patients with aortoenteric fistula (Champion et al. 1982). Reilly et al reported haemorrhage in 61.5% of aortoenteric fistulae and suggested a spectrum of gastrointestinal tract involvement in prosthetic graft infection. The pathological interaction subdivided into direct (fistula formation and GI bleeding), indirect (GI bleeding with no fistula formation), or occult (fistula formation with no GI bleeding) (Reilly et al. 1985). A definitive diagnosis of fistula is achieved in a minority of cases; Peck et al attained a preoperative diagnosis in only 36% (Peck and Eidemiller 1992).

Further investigation of these patients may be inappropriate in the presence of ongoing haemorrhage but in the stable patient may include upper GI endoscopy. This may successfully identify the fistula itself in a proportion of cases (Reilly et al. 1985;
McCann et al. 1993) and also may diagnose other causes of upper GI haemorrhage(Connolly et al. 1981). The presence of a bleeding peptic ulcer however does not exclude an aortoenteric fistula and both pathologies have been described concurrently(Champion et al. 1982). Other investigations include upper GI contrast studies, ultrasound and CT, none of which have high diagnostic sensitivities(Reilly et al. 1985; McCann et al. 1993). Angiography is of little diagnostic value but may help in planning lower extremity revascularisation(McCann et al. 1993). In the absence of a reliable diagnostic test, early diagnosis requires a high index of suspicion in-patients with an aortic aneurysm; a previously placed aortic prosthesis or a previously removed infected aortic graft(Connolly et al. 1981).

Surgery for aortoenteric fistula is extremely hazardous and carries a high mortality. Various authors describe 30 day mortality between 33% and 80%(O'Donnell et al. 1985; O'Hara et al. 1986; Moulton et al. 1986; McCann et al. 1993). Surgery involves separation of the involved segment of gut from the prosthesis and subsequent management of infection as described above.

To date no case of aortoenteric fistula has been described after endovascular AAA repair.

Anastomotic aneurysm

Para-anastomotic aneurysms of the abdominal aorta occur in between 1% and 15% after AAA or AOD reconstruction(Mehigan et al. 1985; Mikati et al. 1990; Gautier et al. 1992). After six years follow up the Canadian multicentre prospective study reported an incidence of false aneurysm rupture of 1.5%(Johnston 1994). The true incidence may however be higher as few studies have performed serial routine radiological follow up. Edwards et al identified 11 (10%) para-anastomotic aneurysms by ultrasound in a group of 111 patients with aortic grafts(Edwards et al. 1992). Similarly Mikati et al described a 15% false aneurysm rate, in 52 asymptomatic patients, 5-10 years after aorto-bifemoral grafting as imaged by computed tomography(Mikati et al. 1990).

Para-anastomotic aneurysms are either true or false, true aneurysms are more likely to occur after AAA repair, whereas false aneurysms are seen more commonly after surgery for occlusive disease(Curl et al. 1992; Edwards et al. 1992; Allen et al. 1993). True
aneurysms may result from inadequate resection at the initial operation or from continued aneurysmal degeneration of the native vessel. False aneurysms result from suture failure, prosthetic dilatation, arterial wall weakness, vessel to graft compliance mismatch and aortic graft infection (Gaylis 1981; McCann et al. 1993).

Para-anastomotic aneurysms may be asymptomatic or present with abdominal or back pain, abdominal mass, claudication and GI haemorrhage (Edwards et al. 1992; Allen et al. 1993). Both CT and ultrasound may confirm the diagnosis, which have sensitivities of up to 100% (McCann et al. 1993). Most para-anastomotic aneurysms are detected late and therefore are large at diagnosis; Allen et al reported a mean diameter of 7.1cm (Allen et al. 1993). These aneurysms can therefore also present with rupture requiring urgent treatment (McCann et al. 1993) which is associated with a very high mortality (Plate et al. 1985; Treiman et al. 1988).

Elective surgical repair of para-anastomotic aneurysms is the treatment of choice. Operative morbidity and mortality is high, although better than for aortoenteric fistula. McCann et al reported a thirty-day mortality rate of 25% and an 18-month survival of 50% (McCann et al. 1993). Operative complications and mortality increase in the urgent setting and in the presence of other aortic graft complications (Plate et al. 1985; Treiman et al. 1988; Curl et al. 1992). The high morbidity and mortality associated with aortic graft complications has led some authors to call for mandatory life-long surveillance for the patient with a synthetic aortic graft (McCann et al. 1993).

As yet no reports of para-anastomotic aneurysm have been reported after endovascular repair other than false aneurysms of the common femoral artery secondary to arterial access procedures (Cuypers et al. 1999). Interestingly however work from our centre has demonstrated continued expansion of the juxta-renal aorta after successful endovascular AAA repair (Thompson et al. 1998).

Graft Thrombosis

Graft thrombosis is rare following aneurysm repair and in the perioperative period usually results from technical error (Moore 1982) (Moore 1982) (Tchirkow and Beven 1978). Patients should be heparinised and returned to theatre for thrombectomy. Thromboses that occur months or years after surgery is usually attributable to progressive
distal occlusive disease, false aneurysm formation, neointimal hyperplasia or excessive build-up of graft pseudointima (Moore 1982). Angiographic assessment is mandatory before reconstruction.

Interestingly a number of authors have described graft thrombosis after endoluminal AAA repair. Chuter et al described five cases of contralateral limb thrombosis in their bifurcated Z-stent device. The problem was ascribed to kinking of the fairly rigid Dacron fabric and necessitated conversion to conventional repair (Chuter et al. 1997). We have also experienced one case of iliac limb occlusion with a bifurcated device secondary to torsion during deployment; this patient was treated by femoro-femoral bypass (Boyle et al. 1998). Cuypers et al also recently reported 8 cases of device or limb occlusion in their large series (Cuypers et al. 1999) and May et al described iliac artery thrombosis in 4% of patients undergoing endoluminal repair (May et al. 1997a). These early reports suggest that graft limb occlusion may be a greater potential problem for endovascular AAA repair than for conventional surgery.

2.9 Late Survival after AAA Surgery

The long-term survival after conventional aortic aneurysm repair is well documented. Johnston et al reported survival rates of 90.7% at one year, 67.7% at five years and 60.2% at 6 years (Johnston 1994). This late survival rate was significantly less than age- and sex-matched normal population. Previous data from the UK has demonstrated similar long-term survival, Fielding et al quoted 68.1% in 142 elective cases (Fielding et al. 1981b) and more recently Stonebridge et al reported an 8 year survival of 45.2% (Stonebridge et al. 1993).

Cardiac and cerebrovascular related late mortality has been demonstrated to occur more frequently after AAA repair than in an age- and sex-matched population (Johnston 1994). The key to late survival is the absence of coronary heart disease at the time of surgery. Roger et al predicted an 8-year survival of 59% in-patients with no clinically recognised CAD compared to 34% who had overt or uncorrected CAD. The latter group having an almost fourfold increased risk of cardiac event (Roger et al. 1989). The long-term survival after CABG was however good and led the authors to conclude that patients
undergoing AAA repair should have aggressive life-long management of their coronary artery disease (Roger et al. 1989).

2.10 Endovascular Aortic Aneurysm Surgery

Endovascular aortic aneurysm repair involves the transfemoral placement of an intraluminal prosthetic graft into the infra-renal aorta, with the aim of excluding the aneurysm sac from the circulation. The prosthetic vascular graft is anchored to the aortic wall by one or more metallic stents. The concept of endovascular AAA repair was conceived by Juan Carlos Parodi in 1976, whilst he was a resident in vascular surgery at the Cleveland Clinic. His initial device consisted of compressible stainless steel wire and a polyester graft, however results in canine experiments were terribly disappointing and the project was abandoned. Parodi however re-initiated the project in 1988 after meeting Julio Palmaz and obtaining some Palmaz stents. After further canine experiments the first human implant was performed in 1990 (Parodi 1997a).

Historical Aspects

The history of endovascular AAA repair can be traced back to 1864 when Hewitt Moore attempted to cause aneurysm thrombosis by inserting 75 feet of wire at the Middlesex Hospital (Haeger 1988). Subsequently Corradi attempted coagulation, by passing electric current through a wire inserted into an aneurysm, a technique that was subsequently practiced widely with sporadic success until the 1950s. The risk of aneurysm rupture after thrombosis is, however, well described (Kwaan and Dahl 1984; Hollier 1986). The relatively poor results of thrombosis led to the development of aneurysm resection, as we know it today.

Conventionally aortic grafts have been sutured in place. Recently, however, some have advocated the use of sutureless intraluminal grafts that are inserted through an open surgical approach (Dureau et al. 1978; Ablaza et al. 1978; Oz et al. 1989). The grafts incorporate polypropylene rings at each end that are secured into the proximal and distal neck of the aneurysm with ligatures. Another device utilises an elastic end ring removing the need for a tie (Matsumae et al. 1988). The technique has the advantages of reduced
aortic 'clamp time', blood loss and total operating time in comparison with the standard surgical repair (Lemole et al. 1984; Goddard et al. 1985; Oz et al. 1989). Despite these advantages the technique still requires a laparotomy and is not in common use.

An endovascular prosthesis consists of a vascular graft (Dacron or expanded polytetrafluoroethylene (ePTFE)) which is attached to one or more metallic stents. Dotter was the first to describe the deployment of a metallic stent from a distant site when he reported the insertion of stainless steel coils into canine popliteal arteries. However despite good early patency, long term results were poor (Dotter 1969). It was then not until 1983 that both Dotter et al and Cragg et al demonstrated the successful placement of Nitinol wire stents, delivered to the canine aorta from a remote site under fluoroscopic control (Dotter et al. 1983; Cragg et al. 1983). Both authors used the unique thermal memory of the nickel titanium alloy to facilitate deployment. During the past decade several stent designs have been developed and tested in animal experiments (Dotter et al. 1983; Cragg et al. 1983; Wright et al. 1985; Palmaz et al. 1985; Palmaz et al. 1986; Rousseau et al. 1987; Strecker et al. 1987). These stent designs fall into three main categories, the memotherm alloy stents, the balloon expandable stents and the self-expanding stainless steel stents. Much of the preliminary evaluation of these devices was performed in the peripheral vasculature and it was not until 1986 that Balko et al demonstrated that AAAs could be treated by the transfemoral placement of an intraluminal device. He performed the procedure in three sheep and used polyurethane graft material supported by memotherm (Nitinol) or stainless steel stents (Balko et al. 1986). At approximately the same time Lawrence et al performed similar work in dogs using multiple self-expanding (Gianturco) stents and a Dacron graft with good immediate results for the abdominal aorta (Lawrence et al. 1987). These early results were later confirmed by Mirich et al who inserted a semi-porous graft supported by a Gianturco stent into canine aneurysms with only one technical failure and good long-term patency (Mirich et al. 1989).

Later still Parodi et al (Parodi et al. 1991) and Laborde et al (Laborde et al. 1992) described an endovascular graft consisting of a Dacron graft attached to balloon expandable stents at either end. This design proved successful in several other canine studies (Boudghene et al. 1993; Sayers et al. 1994). It was, however, Parodi and his
colleagues who performed the first human implantation on 7th September 1990 and went on to describe his preliminary experience with such a device in clinical practice (Parodi et al. 1991). Parodi's device consisted of a specially designed thin-walled Dacron graft sutured to a balloon expandable Palmaz stent. He allowed the graft to partially overlap the stent, so that after stent deployment the graft was positioned against the aortic wall creating an impermeable seal. The device was delivered through a Teflon sheath with the stent mounted co-axially on a balloon angioplasty catheter. All of this early work concentrated on straight endovascular prosthesis, and it was not until 1993 that Chuter et al first described the deployment of a bifurcated device again in a canine model (Chuter et al. 1993). He successfully placed 8 prostheses consisting of a proximal Gianturco stent and combination thin-walled woven polyester graft, 4 of the devices also had distal self-expanding stents to secure the limbs of the prostheses in the iliac vessels.

Since these early animal experiments and the first successful human procedures reported by Parodi et al (Parodi et al. 1991) several other authors have published their early results with this technique (Yusuf et al. 1994; Moore and Vescera 1994; Scott and Chuter 1994; May et al. 1994b; Nasim et al. 1996a). A variety of different devices have been investigated, a detailed description of each prosthetic design is discussed below.

2.11 Endovascular Prosthetic Designs

Following Parodi's initial report of the technical feasibility of endovascular aneurysm repair in humans (Parodi et al. 1991), several other authors have reported their initial results with a variety of different devices. At the outset of this thesis the number of such devices was small and detailed description below is limited to the early endografts and those that were subsequently deployed in the following experimental chapters.

Parodi device

The Parodi device is constructed with a Palmaz balloon expandable stent (4.6mm in diameter and 3.5cm in length) and a specially designed thin-walled, knitted Dacron graft (Barone Industries, Buenos Aires, Argentina) (Parodi et al. 1991). The graft is sutured to the stent so that two thirds of the stent is covered, allowing the graft to be
compressed against the arterial wall on stent expansion. The endograft is then mounted on a 9 Fr polyurethane-shafted valvuloplasty balloon catheter with either one or two 3.5 cm long balloons of variable diameter. The device is introduced transfemorally through a 21 Fr Teflon sheath over a 0.038 super stiff guidewire. Once at the level of the proximal neck the Teflon sheath is withdrawn and the cephalic balloon inflated. The balloon is kept inflated under low pressure to expand the folded graft. The catheter is then moved caudally to deploy the distal stent at the aortic bifurcation.

After his initial clinical experience Parodi modified this device (Parodi 1995). The graft now overlaps half the stent, the distal stent is deployed separately after anchoring the device with a single proximal stent and the Teflon delivery sheath has been reduced in size to 18 Fr.

Between September 1990 and December 1996, Parodi treated 109 patients with 51 tube grafts as described above, 46 aorto-iliac grafts and 12 bifurcated devices (Parodi et al. 1997b). The stent-graft was fixed with only a single proximal stent in eight of the early patients. Successful deployment (complete exclusion of aneurysm without endoleak or procedural death) was achieved in 81 patients (74%). There were 20 early endoleaks, of which half were corrected by further endovascular intervention and the remainder left untreated. All three patients with untreated proximal leaks died within 7 months of their procedure, one from aneurysm rupture at two months and the other two from unrelated causes. One of the seven patients with a distal leak also died of congestive heart failure 8 months after surgery and in one further patient the endoleak sealed spontaneously. Four patients with large tortuous aneurysms died from massive microembolisation following difficult device delivery. Other procedural complications included three minor embolic phenomena treated successfully with intra-arterial prostaglandins, one case of iliac artery rupture treated surgically and two groin haematomas.

At a mean follow-up of 18 months a number problems were identified. In four patients with only proximal stent-graft insertion distal reflux into the aneurysm sac was observed. Late distal endoleaks were also observed in two aorto-aortic grafts and one aortoiliac graft.
Leicester Device

The development of the Leicester aortomoiliac and aortomonofemoral devices has been ongoing since 1993. Akhtar Nasim, Robert Sayers, Matthew Thompson and Peter Bell have played integral parts in its development and design and I was responsible for its construction and further modification as part of the work for this thesis.

The basic design of the aortomonofemoral and aortomoiliac endografts, long, tapered grafts sutured to a balloon expandable stent (Figures 2.3-2.6), was adapted from the endovascular prostheses described by Parodi et al (Parodi et al. 1991) and Richter et al (Richter et al. 1994) and is similar to that deployed by Marin et al (Marin et al. 1995). The devices were constructed as previously described, using a Palmaz stent (Johnson and Johnson, UK) sutured to a thinwalled 8mm pre-expanded ePTFE graft (Impra UK) which was serially dilated to 35mm using a graded angioplasty balloons. This stent-graft combination was crimped onto a 30mm angioplasty catheter (William Cook Europe, Denmark), and the entire device backloaded into 21Fr Teflon sheath (William Cook Europe). The device was subsequently delivered through a 25 Fr sheath and commonly through a temporary iliac conduit. The ipsilateral internal iliac artery was ligated. In order to minimize distal ischaemia a 10mm Dacron graft was anastomosed end to side to the contralateral common femoral artery and tunneled subcutaneously to the ipsilateral femoral artery at this point. The stent-graft combination was then deployed just below the lowest renal artery. The extra-anatomic bypass was then completed by anastomosing the proximal portion of the femoro-femoral crossover graft to the ePTFE graft in the groin. The aortic balloon was then deflated allowing immediate restoration of blood flow to the contralateral limb. The distal ePTFE graft was then anastomosed to the ipsilateral common femoral artery (aortomonofemoral). The procedures were completed by occluding the contralateral common iliac vessel with a covered Gianturco stent (William Cook Europe)(Nasim et al. 1996a; Thompson et al. 1997b). In two patients with suitable common iliac vessels the ePTFE was sized exactly and the distal end stented in the ipsilateral common iliac artery with a further Palmaz stent (aortomoiliac). One of these patients had an isolated 6cm iliac artery aneurysm after previous conventional surgery for
Figure 2.3: The Palmaz stent; a balloon expandable stent made from annealed stainless steel.

Figure 2.4: Raw materials required to construct the Leicester endovascular device; a Palmaz stent, an 8mm PTFE graft, a 30mm angioplasty balloon, a 35mm achalasia balloon and two Teflon sheaths.
Figure 2.5: Dilated PTFE graft.

Figure 2.6: Palmaz stent being sutured to dilated PTFE graft with two 2/0 Prolene sutures.
ruptured AAA. The endovascular device in this patient was placed from the aorta down the contralateral iliac system to allow continuity of flow through the contralateral internal iliac vessel and the ipsilateral internal and external iliac vessels occluded using a combination of coils and a covered Gianturco stent (Thompson et al. 1997b).

The Endovascular Technologies' Endograft

The EndoVascular Grafting System (EGS) (Figure 2.7) is a manufactured transluminal prosthetic vascular graft delivery system that was invented and patented by Lazarus in 1988 (Lazarus 1992), and subsequently developed and manufactured by Endovascular Technologies (Menlo Park, California, USA). The EGS system comprises of two main components: an introducer system and the graft delivery apparatus. During manufacture a premeasured prosthetic vascular graft is incorporated into each graft delivery device. The endograft consists of a standard Dacron vascular graft with metal attachment systems affixed to both ends. These self-expanding cylindrical metal frames are equipped with a series of angled metal attachment hooks directed radially away from the centre of the graft that allow attachment to the aortic wall. The delivery apparatus also contains a balloon catheter that facilitates secure deployment of the endograft.

The deployment of the device occurs in the operating theatre under general anaesthesia with the patient prepared for conversion to conventional AAA repair if required. In addition a ‘marker board’ containing two movable radiopaque marker lines is positioned under the patient to mark the lowest renal artery and the aortic bifurcation for subsequent endograft deployment. A femoral arteriotomy is performed and a guidewire and angiographic catheter advanced into the aorta to obtain an aortogram. This image is then superimposed on the marker board to allow accurate positioning of the proximal and distal attachment sites. The 28 Fr introducer sheath is then inserted over the guidewire and advanced into the aneurysm and once fully expanded the obturator is removed and the delivery apparatus containing the endograft is advanced until the proximal fixation site is optimally positioned in the proximal neck. The jacket covering the graft is then retracted with real-time fluoroscopic visualisation and the proximal capsule advanced causing the proximal attachment system to spring open and engage the aortic wall. The balloon is then inflated twice in the proximal end of the graft to secure the device and the
Figure 2.7: Photograph of EVT tube endograft, demonstrating self expanding stent and attachment hooks.
Figure 2.8: Completion arteriogram after the deployment of a bifurcated EVT system.
procedure repeated to deploy the distal attachment site. A completion angiogram is then performed to document correct positioning of the graft and total aneurysm exclusion.

During the course of this thesis EVT developed a tapered aortomonoiliac and a bifurcated aortic endograft. Two patients in the subsequent experimental chapters had bifurcate prostheses inserted, which required a slightly modified technique as described below.

The deployment of a bifurcated graft required access to both femoral arteries. The bifurcated endograft delivery apparatus is designed with a guidewire, which is attached to the contralateral limb within the capsule. The free end of this wire is passed up the ipsilateral femoral artery and positioned just above the aortic bifurcation where it is captured by a snare catheter inserted through a sheath in the contralateral groin. The wire is then withdrawn through the contralateral iliac system leaving the contralateral graft limb guidewire in a transfemoral position. The endograft is then delivered and the proximal attachment system positioned as with the tube device. The distal attachment sites are then withdrawn into position in the common iliac arteries taking care to correct any twists in the graft before deployment. The proximal, contralateral iliac and ipsilateral iliac attachment systems are then deployed and secure by balloon catheters in this order and a completion arteriogram obtained (Figure 2.8).

The MinTec Stentor and Boston Scientific Vanguard Systems

The Stentor System (MinTec Ltd., Freeport, Bahamas) evolved into the Vanguard (Boston Scientific Corporation, New Jersey USA) during the period of this thesis. The devices consist of a self-expanding biocompatible Nitinol framework annealed into a tubular zigzag configuration by a 7-0 prolene thread. This Nitinol stent is covered with a 0.1mm woven polyester fabric graft. The devices are available in both tube and bifurcated designs. The bifurcated device is modular having two components, the aortic segment with one attached iliac limb and a separate iliac limb that are deployed separately and married together within the aneurysm sac.

The implantation of this device is commonly performed in the angiographic suite under general anaesthetic (Blum et al. 1997a), though our experience is within the standard operating theatre setting. The endograft is inserted through a three-stage delivery
Figure 2.9: Photograph of MinTec Stentor endograft.

Figure 2.10: Postoperative contrast enhanced CT image of the Bard Tube device successfully excluding the AAA.
system comprising of an 18 Fr introducer sheath, an aortic and iliac pusher, the stent-graft and a latex balloon. The system is delivered through a femoral arteriotomy over a stiff 0.035-inch guidewire and positioned at the level of the renal arteries.

The proximal tube graft is anchored at the desired position with the latex balloon and the outer sheath fully withdrawn to deploy a straight endograft. The sheath is only withdrawn just beyond the radiopaque marker of the iliac cuff in the case of the bifurcated device. The proximal segment is then fixed and then the sheath is withdrawn completely to deploy the ipsilateral iliac limb. The contralateral limb is then deployed percutaneously over a guidewire through a 10 Fr introducer sheath. Again a completion angiogram is performed.

**The Bard System**

One patient presented in the subsequent experimental chapters was treated with the prototype Bard tube graft as part of the Bard initial clinical trial. This device consisted of a fully supported self-expanding stent-graft. It was deployed via a single femoral arteriotomy under fluoroscopic control and had the added advantage of re-sheathing the endograft and subsequent redeployment if the initial positioning was not satisfactory. This endograft was limited in its size range and was not anatomically suitable for the majority of aneurysms.

**Other Devices**

In addition to the above devices numerous other endovascular stent-grafts have been described and deployed by various authors. Initially, in the absence of any commercially available endografts, a variety of ‘home-made’ devices were constructed using commercially available stents and graft materials. (Parodi et al. 1991; Chuter et al. 1993; White et al. 1994; Lawrence-Brown et al. 1996). Subsequently a number of commercially developed devices have become available with the greatest experience to date with the EVT and MinTec/Boston Scientific devices described above. The main categories of currently implanted devices are listed in table 2.1 below.
<table>
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<tr>
<th>Endograft configuration</th>
<th>Subgroups</th>
<th>Named Devices</th>
</tr>
</thead>
</table>
| Aortoacoartic                   | Graft with proximal and distal stent or other attachment device | EVT (Endovascular Technologies)  
Parodi device  
Passager (Boston Scientific)  
AneuRx (Medtronic)  
Excluder (Gore)  
Corvita (Corvita) |
|                                 | Externally covered stent                       |                                                   |
|                                 | Internally covered stent                       |                                                   |
|                                 | Modular graft fully supported                 | Sydney endograft (Baxter Healthcare)              |
| Bifurcated aortoiliac          | Graft with proximal and distal stent or other attachment device | EVT  
Chuter-Gianturco  
Vanguard (Boston Scientific)  
Talent (World Medical)  
Perth bifurcated system  
AneuRx (Medtronic)  
Excluder (Gore)  
Corvita  
Sydney endograft |
|                                 | Externally covered stent                       |                                                   |
|                                 | Internally covered stent                       |                                                   |
|                                 | Modular graft fully supported                 |                                                   |
| Aortomonoiliac (femoro-femoral crossover) | Graft with proximal and distal stent or other attachment device | EVT  
Ivancev-Malmo system  
Parodi device  
Leicester device  
Sydney endograft |
|                                 | Modular graft fully supported                 |                                                   |

Table 2.1: Endovascular grafts in current use for the treatment of abdominal aortic aneurysms (Modified from Woodburn et al 1998) (Woodburn et al. 1998).
2.12 Aneurysm morphology and patient selection

Accurate assessment of aneurysm morphology prior to endovascular AAA repair is vital to select suitable patients and identify the correct endograft dimensions. Precise preoperative measurements are obtained usually with a combination of contrast enhanced CT and biplanar angiography with a calibrated catheter. However in all, four imaging modalities are used to assist aortic endografting.

Ultrasound

External B-mode ultrasound can provide information on diameter and, less reliably, length of the abdominal aorta. The generally acceptable limit for diameter accuracy is ± 5mm (Lederle et al. 1995). This degree of resolution is not acceptable for sizing endografts.

Duplex ultrasound does have a role in monitoring patients after endovascular AAA repair and we have found it particularly useful in identifying endoleaks (Thompson et al. 1998). The most useful advance in ultrasound technology however is the development of intravascular ultrasound (IVUS). This enables the operator to gather information on luminal morphology, dimensions and branch arteries immediately after the first guidewire is manipulated into the aorta. After endovascular AAA repair, the IVUS can confirm complete endograft deployment and aneurysm exclusion. This reduces screening time and contrast volume used during the procedure, indeed it may soon be possible to deliver an endograft under complete IVUS control (White et al. 1997). The IVUS does have some limitations however, the catheter does not usually lie in the co-axial plane and therefore the vessel image is often elliptical requiring further interpretation and length measurements may be inaccurate as the catheter cuts across the radius of a tortuous aortic aneurysm (Beebe 1997). Despite this IVUS technology is rapidly evolving and, even in its present state of development, its measurements are comparable to CT (Verbin et al. 1995) and it appears to be the best method available for determining accuracy of stent deployment (Diethrich 1993).
Arteriography

Arteriography is the traditional mainstay of arterial luminal assessment. It however has a number of limitations that are important when assessing patients for endovascular aneurysm repair. Firstly magnification artifact occurs because of divergence of the X-ray beam (Elisevich et al. 1995). This magnification artifact may be determined in the individual patient by the use of arteriographic marker catheters (Beebe 1997). Secondly arteriography is very poor at identifying luminal thrombus. This is of great importance in endovascular grafting as only small amounts of thrombus have implications for long-term results. We have experienced this problem where a thin, initially unnoticed, layer of thrombus at the distal attachment site caused a late endoleak (Nasim et al. 1996c). CT best appreciates thrombus identification. Thirdly angiography tends to foreshorten the appearance of a tortuous vessel which may lead to undersizing of an endograft (Beebe 1997). During the deployment of an endograft parallax error is of major importance, but can be overcome by the use of marker boards, immobilising the X-ray tube and marking key anatomical landmarks on the fluoroscopy screen.

Computed tomography

Computed tomography has enabled more accurate assessment of aneurysm morphology and has been important in the development of endovascular AAA repair, however conventional CT still has limitations. Firstly the diameter of an arterial segment may be distorted if angled tangentially to the horizontal plane of the CT image (Ouriel et al. 1992) producing an elliptical section. In addition diameter measurement from CT scans suffer from methodology errors and observer variability (Jaakkola et al. 1996). Furthermore conventional CT has problems determining length, underestimating distance in the case of a tortuous lumen and missing anatomical landmarks that appear between slices. These problems can be overcome to a certain extent by using 3mm slices and combining the CT data with angiography. For reasons described above both of these imaging modalities need to be used for planning endovascular AAA repair, since neither alone provides sufficient data (Beebe et al. 1995).
Spiral CT and subsequent computerized processing has been the major advance in imaging prior to endovascular surgery of the last few years. The technique allows much greater appreciation of anatomical details such as location of small renal or lumbar arteries. Also the ability to construct a 3D model at an angle perpendicular to the flow axis increases significantly the ability to accurately measure diameters and length (Beebe 1997). Spiral CT still has some disadvantages and limitations, the proximal aorta moves with respiration, therefore for accurate imaging patients are required to hold their breath, the technique uses large amounts of contrast and there is ample opportunity for operator error during image reconstruction. Finally spiral CT is a powerful tool in assessing procedural success after endovascular AAA repair (Balm et al. 1996).

Magnetic Resonance Angiography

MRA is in its early stages of evolution in imaging vascular disease. There are however some encouraging reports of its use in assessing aortic morphology (Durham et al. 1993; Ecklund et al. 1994; Prince et al. 1995). Indeed in a recent study from our centre MRA was significantly better at visualizing AAA morphology when compared to conventional CT relevant to patient selection and graft sizing for endoluminal AAA repair (Nasim et al. 1998a). MRA also has limitations, it is poorly tolerated by some claustrophobic patients and is contraindicated in-patients with pacemakers or intracranial metallic clips, it is also considerably more expensive than conventional CT. However it avoids the use of contrast medium and ionizing radiation and may play an increasing role in patient selection and endograft sizing as the technology develops further.

At present we utilize conventional CT with 3mm slices as a preliminary imaging modality with those patients identified as potentially suitable for endovascular AAA repair going on to marker catheter arteriography. MRA although still available is too expensive at present.

Anatomical Suitability for endovascular AAA repair

The purpose of precise imaging is firstly to identify those patients who are suitable for endovascular AAA repair and subsequently to choose the most appropriate endograft. Anatomical factors that are important for endovascular repair vary depending
on the type of device considered. The general morphological features that do not favor endoluminal repair are listed below in Table 2.2.

<table>
<thead>
<tr>
<th>Morphological Feature</th>
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<tbody>
<tr>
<td>Involvement of renal artery origins</td>
</tr>
<tr>
<td>Infra-renal proximal neck length &lt; 15mm</td>
</tr>
<tr>
<td>Proximal aortic diameter &gt; 26mm</td>
</tr>
<tr>
<td>Common iliac artery diameter &lt; 9mm</td>
</tr>
<tr>
<td>Common iliac artery length &lt; 25mm</td>
</tr>
<tr>
<td>Common and external iliac diameter &gt; 14mm</td>
</tr>
<tr>
<td>Distal aortic cuff length &lt; 10 mm</td>
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<tr>
<td>Thrombus within lumen at endograft attachment sites</td>
</tr>
<tr>
<td>Angulation of the iliac arteries &gt; 90°</td>
</tr>
<tr>
<td>Angulation of proximal aortic neck &gt; 60°</td>
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</table>

Table 2.2: Contraindications to endovascular AAA surgery (Adapted from Woodburn et al 1998) (Woodburn et al. 1998).

The number of AAA patients actually suitable for endovascular repair depends on the intended type of device. We have recently shown that only 36% of patients can be treated with the commercially available tube and bifurcated devices. Other authors have reported similar results, Blum et al treated 154 patients (47%) of 331 patients with AAA endoluminally with 21 (6.3%) straight and 133 (40.2%) bifurcated Stentor/Vanguard devices (Blum et al. 1997b). Armon et al assessed 154 aneurysms with spiral CT and identified 6 patients (4%) suitable for straight endovascular grafting 15 (10%) for bifurcated devices and 85 (55%) for aortomonoiliac endografts (Armon et al. 1997). The difference in the suitability for bifurcated devices in these two series may be partly explained by the addition of further stent-grafts to the iliac limbs in 18 patients of the first series to treat distal endoleaks, thus technically these patients were not treated with a standard bifurcated device (Blum et al. 1997b). Secondly the patients in the first series had smaller aneurysms. The tapered aortomono-femoral device developed in our centre
has less rigid anatomical requirements and therefore is suitable for a larger percentage of aneurysms. The device requires a thrombus free proximal neck of 15mm length and < 28mm diameter. In addition one CIA < 20mm diameter is required to allow retrograde flow into the contralateral internal iliac artery after ligation or occlusion of the common iliac system (Thompson et al. 1997b). This technique is therefore suitable for patients with aneurysms involving the iliac arteries and potentially stable patients with rupture as anatomical requirements are limited (Thompson et al. 1997b). This technique does require extra-anatomic bypass and long-term results are awaited.

At present a significant proportion of patients remain unsuitable for endovascular repair, this percentage may fall with technological advantages but a significant minority still require conventional open repair.

2.13 Complications of endovascular AAA surgery

Complications after endovascular surgery can be divided into those specific to the procedure and those common to both conventional and endoluminal repair. The latter have been discussed in detail in the relevant parts of section 2.8 above. In addition, perhaps the most devastating complication of endovascular AAA surgery, 'microembolisation' is referred to in the 'Arterial embolisation and thrombosis' part of section 2.8. Other complications that are unique to endovascular repair and those rarely seen after conventional surgery are discussed below.

Postimplantation Syndrome

This syndrome has been observed by some authors after endovascular AAA repair and presents with a marked fever (38.0-39.7°C) and a mild or marked elevation in C-reactive protein lasting for 4-10 days after surgery, without any evidence of graft infection or bacteraemia (Blum et al. 1997b). Blum et al observed this syndrome in 87 of 154 patients (Blum et al. 1997b). Transient unexplained fever was also reported in 9 of 46 patients in North America and 4 of 31 patients in Europe in the initial EVT trials (Balm et al. 1996; Moore and Rutherford 1996). Interestingly recent work by Syk et al comparing 23 endovascular and 13 conventional AAA repairs demonstrated moderate
postoperative temperature rises in both groups, without any significant differences between the groups. The also reported rises in CRP in both groups and although these were slightly higher after conventional surgery there was again no statistical difference (Syk et al. 1998).

Local vascular complications

The passage of large diameter delivery systems through femoral and iliac vessels has often resulted in injury to these vessels requiring surgical repair. Moore et al reported iliofemoral injury in 17% of patients in the North American EVT phase 1 trial (Moore and Rutherford 1996). Most other authors have reported this complication less frequently (Balm et al. 1996; Blum et al. 1997b). May et al reported femoral artery damage in 4%, dissection of the iliac artery in 2%, perforation of the iliac artery in 2% and iliac artery occlusion in 4% with greater than one quarter of patients suffering a local or vascular complication after endoluminal AAA repair (May et al. 1998). In addition to direct vessel damage some authors have reported groin haematomas that were treated conservatively (Balm et al. 1996) or required surgical intervention (Blum et al. 1997b). Groin lymph leaks have been similarly reported by a number of authors (Balm et al. 1996; Moore and Rutherford 1996; Blum et al. 1997b).

Endoleak

Endoleak is a complication exclusive to endovascular aneurysm repair. White et al have constructed a precise definition: Endoleak is a condition associated with endoluminal vascular grafts, defined by the persistence of blood flow outside the lumen of the endoluminal graft but within an aneurysm sac or adjacent vascular segment being treated by the graft. Endoleak is due to incomplete sealing or exclusion of the aneurysm sac or vessel segment, as evidenced by imaging studies, such as contrast-enhanced CT scanning, ultrasonography, or angiography (White et al. 1997). They then went on to classify various types of endoleak. Endoleaks were divided into primary and secondary depending on time of development and further into graft-related endoleak and non-graft related endoleak thus distinguishing a persistent channel of blood flow due to inadequate endograft seal, from persistent flow into the aneurysm sac from patent lumbar or other
collateral vessels. A detailed classification is listed in Table 2.3 below. The authors have recently further modified their definition (White et al. 1998).

**Endoleak** by definition implies failure to exclude the aneurysm from the circulation and carries the risk of aneurysm growth (Thompson et al. 1998) and possible rupture (Lumsden et al. 1995; White et al. 1997). Put another way endoleak implies failure of treatment.

A variety of factors cause the development of an endoleak. Poor patient selection, accuracy of preoperative imaging and dimensional measurements have a major influence on the risk of developing primary endoleak. Specific anatomical features associated with endoleak include angulation of the proximal neck, non-circular attachment zones, the presence of mural thrombus at attachment sites and possibly calcification. Also a short aortic neck or low deployment of the proximal stent have been a major cause of primary endoleak in both our own and others experience (White et al. 1997; Thompson et al. 1997b). Secondary endoleak may result from any of the above factors and in addition, material fatigue, such as hook breakage observed with the EVT endograft (Balm et al. 1996) may lead to device migration. Device migration has also been observed to cause late endoleak by other authors and appears to be a particular problem with self-expanding proximal stents. Chuter et al reported four cases in 52 patients treated with bifurcated endografts, incorporating Gianturco stents and they attributed this to short, thrombus lined proximal necks in three cases (Chuter et al. 1997). More recently Resch et al observed proximal stent migration of greater than 5mm in 26 (45%) of patients in their series at mean follow-up of 13 months. They highlighted proximal neck dilatation and poor patient selection as the main causes of migration. Migration was complete in 8 cases leading to rupture in two cases, all requiring open repair (Resch et al. 1999).

The diagnosis of endoleak is made radiologically in virtually all cases. A primary endoleak is usually detected at completion angiography or by CT (Figure 2.11) or Duplex scans (Figure 2.12) in the early postoperative period. The potential development of a late endoleak has made rigorous follow-up after successful endovascular AAA repair vital. Periodic surveillance by contrast-enhanced CT scan must be carried out at 6 to 12 monthly intervals for all patients (Matsumura and Moore 1998). We have also found Duplex to be as good a tool for endoleak detection with the added advantage of
Figure 2.11: A contrast enhanced CT image showing a proximal endoleak after endovascular repair with an EVT tube device.

Figure 2.12: A colour duplex scan demonstrating the endoleak in the same patient.
identifying the site of the endoleak (Thompson et al. 1998). Other authors however routinely use angiography as part of a follow-up protocol (Malina et al. 1997b), although most would reserve more invasive investigations for definite endoleaks and their treatment (White et al. 1997).

The natural history of an endoleak may depend on its size and site. Primary endoleaks may well seal spontaneously, Matsumura and Moore reported this phenomenon in 50% of 28 leaks (Matsumura and Moore 1998). They also commented that none of 4 patients who were identified with secondary endoleaks underwent spontaneous closure and suggested that late endoleaks may have a worse prognosis. A number of other authors have also described spontaneous sealing of primary endoleak (Blum et al. 1997b)

(Balm et al. 1996; Parodi et al. 1997b).

The management strategies for an endoleak can be grouped into five categories. Most cases of minor endoleak detected at the time of surgery, that are perhaps due to graft porosity, and those detected in the early postoperative period are usually treated conservatively initially. Patients should undergo frequent imaging to determine aneurysm size and the site and extent of the endoleak. Endoleaks may seal spontaneously as alluded to above, however if a leak persists it must be considered a failure of treatment and further intervention should be contemplated. An endoleak may be closed by the deployment of a further endoluminal device or covered stent (White et al. 1997). These techniques may be of particular use for proximal and distal endoleaks and also for leaks around an iliac occluder in the case of an aortomonoiliac graft. If the aneurysm sac is undergoing persistent perfusion via collateral lumbar or mesenteric vessels the use of coils or other devices to embolize these vessels has been advocated (Ivancev et al. 1996).

If further endovascular intervention is unsuccessful then a conventional surgical approach should be considered. Some authors have described surgical band ligature of the aortic neck to reinforce the seal between the seal between the endograft and the aortic wall; this technique requires laparotomy and is not in widespread use (White et al. 1997). Most authors have described conversion to conventional surgical repair. May et al reported conversion in 18 (16%) of 113 patients after failed endoluminal repair. The removal of incorrectly deployed endografts required considerable modification of the
standard open technique and resulted in a higher morbidity and mortality rate than is generally accepted after conventional surgery (May et al. 1997b). They also reported a mortality rate of 43% in-patients who required conversion who had initially been turned down for conventional surgery because of severe co-morbidity (May et al. 1997b). In these cases it may be better to treat patients conservatively rather than resort to open repair (White et al. 1997).

The long-term outlook of a sealed endoleak, whether spontaneous or secondary to further endovascular intervention is not known. Cases of recurrent endoleak have been reported after both spontaneous closure (Rozenblit et al. 1995) and endovascular correction (White et al. 1997). This highlights the need for regular radiological follow-up after endovascular AAA repair and secondary intervention.

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<td>Proximal or distal graft attachment zones</td>
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<td></td>
<td>Perigraft Channel</td>
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<td>Graft-related endoleak</td>
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<td>Type II endoleak</td>
<td>Retrograde endoleak</td>
<td>Patent lumbar, inferior mesenteric or intercostal arteries</td>
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<td>Contralateral stump disconnection</td>
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<td>Type IV endoleak</td>
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<td>Suture holes</td>
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<td></td>
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<tr>
<td>Nonendoleak</td>
<td>Endopressure</td>
<td>High sac pressure, no endoleak shown</td>
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Table 2.4 Modified from White et al 1998. (White et al. 1998)
2.14 Early Results of Endovascular AAA repair

Mortality

Most early series report mortality rates following endovascular AAA repair to be similar to that reported for conventional surgery. May et al reported equal mortality rates of 5.6% in a concurrent comparison of 303 patients undergoing endovascular and conventional aneurysm repair (May et al. 1997a). Similarly Parodi and Chuter have reported mortality rates of 5-6% (Chuter et al. 1997; Parodi et al. 1997b). The are however some discrepancies with mortality rates reported as low as 1% and as high as 28% by some authors (Marin et al. 1995; Blum et al. 1997b). Marin et al reported death after 5 of 18 endoluminal AAA repairs, although all these deaths occurred in patients with severe co-morbidities and one with contained rupture (Marin et al. 1995). Indeed much of the early experience was with high-risk patients who had been refused conventional surgery (Parodi 1995; May et al. 1996; Yusuf et al. 1997). Blum et al reported a mortality of 1% in a large series of patients in whom 32% of patients were classed as American Society of Anesthesiologist grade IV (Blum et al. 1997b). The fact that many series contain high-risk patients and contained ruptures makes comparison with conventional surgery difficult to interpret.

Endoleak

The incidence of endoleak after endovascular AAA repair and its relationship to type of endoluminal device and configuration has recently been established. Schurink et al reported an overall endoleak rate of 24% in a meta-analysis of 1189 patients. They also identified that tube grafts were associated with a significantly higher endoleak rate (35%), than either bifurcated (18%) or aortounilateral devices (20%). In addition endoleak was significantly associated with the EVT (44%) and Corvita (52%) systems and with self-expandable (34%) rather than balloon expandable devices (17%). The most concerning data presented in this study was that the endoleak rate did not decrease with time as might have been expected with the evolution in technology and overcoming the learning curve (Schurink et al. 1999).
This data reflects our own practice to a certain extent, in that our endoleak rate has been greater with EVT tube devices than with the aortomonoiiliac or bifurcated endografts (Nasim et al. 1998b). However we have certainly observed a decrease in early endoleak incidence as our learning curve has been overcome (Thompson et al. 1997b).

At present we are still awaiting long-term follow-up data for endovascular AAA repair, the mean follow-up in the recent meta-analysis was only 11 months (Schurink et al. 1999). In contrast follow-up after conventional surgery extends beyond ten years (Ernst 1993). Therefore despite its appeal longer follow-up is required before endovascular AAA repair can be adopted as an effective alternative to conventional aortic reconstruction.

2.15 Scope and Design of the Thesis

The preceding two chapters have described in detail the pathophysiology and clinical aspects of abdominal aortic aneurysm. Two main problems have been identified in their current management. Firstly, how to manage those patients who present with small aneurysms (<5.5cm) in whom the risk of surgery is greater than that of rupture. Secondly, how to manage those patients who present with aneurysms of >5.5cm in whom co-morbid risk factors predict significant morbidity and mortality, or preclude conventional surgery altogether.

The work presented in chapters 3, 4 and 5 investigates possibility of pharmacological manipulation of the aneurysm process with the aim of inhibiting aneurysm growth. The experimental work uses a porcine aortic organ culture model of aneurysmal disease to investigate the effects of, both, an MMP inhibitor, doxycycline, and an MMP agonist, Amlodipine on aortic degradation.

Endovascular AAA surgery has a number of potential benefits over conventional repair. Firstly, its avoids the need for a laparotomy incision and secondly, it decreases the aortic occlusion time. It may therefore dramatically reduce the morbidity and mortality associated with conventional surgery and has potential to be used preferentially in those patients with severe co-existent medical disease. The experimental work presented in chapters 6, 7, 8 and 9 evaluates, respiratory, cardiovascular, renal and inflammatory
indices in comparative cohorts undergoing conventional and endovascular AAA repair with the aim of establishing whether endovascular surgery is indeed less invasive. Chapter 10 identifies problems associated with the development of an endovascular program and its implications for workload and patient co-morbidity.

Finally chapter 11 highlights the main findings arising from this thesis and suggests directions for future research.
# CHAPTER THREE

Materials and Methods

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3.1 Introduction

This chapter details the general methods used in the subsequent in vitro experimental chapters, and describes organ culture techniques in depth. The source and composition of tissue culture, extraction, zymography, and immunoblotting reagents are outlined in Appendix A. The histological staining method used for the stereological analysis is described in Appendix B.

3.2 Porcine Aortic Organ Culture

The in vitro organ culture techniques used throughout this thesis were based upon the methods originally described and subsequently modified by Koo and Gotlieb (Koo and Gotlieb 1989; Koo and Gotlieb 1991), who were able to maintain segments of porcine aorta in culture for up to four weeks. In contrast to the culture of isolated cell lines, this technique involves the culture of segments of full-thickness vessel wall. Using this in vitro model, the structural integrity of the aortic wall is retained and the different cell types are located within their native extracellular matrix, preserving important cell-cell and cell-matrix interactions.

Collection and Preparation of Porcine Aorta.

Intact abdominal aortas from 18-36 month old sows were obtained from the local slaughterhouse (Dawkins Intl., Congerstone) and transported to the laboratory in 500 mls of sterile MEM (Appendix A) on ice. All subsequent handling of the aortas was done under sterile conditions in a laminar flow hood (Gelaire, UK).

After removal of fat and loose adventitia, a 7 cm length of aorta was cut along its longitudinal axis adjacent to the ostia of the lumbar arteries and transferred to a sterile 140 mm Petri dish (Sterilin, UK) filled with MEM to be washed and cleaned. Here the remaining adventitia was dissected away and square pieces of aortic wall (1 cm²) were excised with a scalpel blade, at sites distant from branch vessels.

These aortic segments were then pinned intimal surface uppermost onto a polyester gauze support resting on set sylgard resin (Dow Corning, Seneffe, Belgium) in
the base of a 60 mm glass Petri dish (Fisons, Loughborough, UK). Endothelial denudation was carried out by mechanical injury on every specimen so as to remove the possible confounding influence endothelial cells may play in organ culture. A gentle surface scraping of the pinned segment with a sterile cotton bud removed all surface endothelial cells, leaving the rest of the intima intact. This was confirmed by light microscopy on tissue stained with haematoxylin and eosin (Appendix B), and on trypan blue staining of intact aortic segments. Previous work in our department had validated this technique by demonstrating significantly reduced neointimal formation in denuded specimens after 14 days of culture (Wills et al. 1996), thus confirming the work of Koo and Gotlieb (Koo and Gotlieb 1989).

Depending upon the type of experiment to be performed, aortic specimens were cultured in either 7 mls of standard culture media (Appendix A), or 7 mls of culture media supplemented with exogenous porcine pancreatic elastase (Calbiochem, UK), doxycycline or amlodipine (Pfizer, Sandwich, UK). The concentration of exogenous elastase depended upon the context of the experiment, with concentrations of 100 units/ml (doxycycline) and 50 units/ml (amlodipine) being derived by sequential serial dilution (Appendix A). Cultures were then incubated at 37°C in a humidified atmosphere with 5% (v/v) CO₂ in air (Queue cell culture incubator, West Virginia, USA) and refed with fresh culture media every two days.

3.3 Stereological Tissue Analysis

Stereology (Point Counting)

The relative proportions of elastin, collagen, and smooth muscle cells have been found to alter dramatically in human aneurysmal tissue (Koch et al. 1990; Baxter et al. 1992; Baxter et al. 1994). Therefore, in developing an in vitro model of aneurysm formation, it was necessary to examine quantitative changes in the concentrations of the major ECM components. Stereological analysis or "point-counting" provided an accurate and reproducible method to determine such changes in concentration, as several structural components may be studied simultaneously. This method was previously described in a
study by He and Roach (He and Roach 1994) which examined the medial composition of human abdominal aortic aneurysms.

Stereology, or "point counting", aims to determine the relative concentration of one or more identifiable components in a defined area (Weibel 1979). A light microscopic image of a section appropriately stained for the components of interest is superimposed onto a transparent lattice grid, with intersecting lines forming "test points" (Weibel 1979). Quantitative characterisation of each uniquely identifiable element can be calculated by counting the number of respective test points falling on that particular structure. The only requirements are that the various structural components be clearly separated and distinguishable on section, and that no point falls on more than one structure (Weibel 1979). If a 10x10 grid defining 100 points is used, each point then constitutes one percent of the total field. The number of test points on that particular structure hence represents the percentage concentration.

After the cultured aortic tissue was fixed in 10% formalin and embedded in paraffin blocks, sections were stained with Miller's elastin and Van Gieson stain (Appendix B). This stains elastin black/blue, smooth muscle cells yellow, and collagen pink/red thus visually delineating the major structural components of the aortic wall. Point counting was performed on an Olympus light microscope with x40 objective and x10 ocular to view the sections (i.e. x400 magnification). An eyepiece graticule with an etched grid (Graticules Ltd., Kent, UK) was inserted into the light path to superimpose the test points on the image in the microscope. A 10x10 sub-section of the grid was utilised giving one hundred points and allowing percentage fractions to be obtained. The test points hitting black were counted for elastin, pink for collagen, and yellow for smooth muscle cells, and the volume fraction of each component was calculated (Weibel 1979; He and Roach 1994).

To maintain a constant frame of reference throughout the entire course of experiments, only the adventitial aspect of the media was point counted. Eight distinct and randomly chosen viewing fields were analysed for each section of tissue. This allowed 800 points to be analysed for each aortic sample.
**Inter and Intra Observer Error.**

One of the technical difficulties encountered in point counting is that the test points are produced by intersections of "lines" which have a defined width. This may introduce uncertainty as to whether the intersecting "point" lands on one particular structure or another. A subjective decision is made with respect to this occurrence. Inter- and intra-observer ranges have been previously calculated and this technique validated for determination of ECM component concentrations in our department (Wills et al. 1996).

Inter-observer ranges of agreement were measured by plotting the difference in elastin concentration in 30 different samples as measured by two separate observers, against the corresponding mean for each sample (Brennan and Silman 1992). The 95% range of agreement was -5.1 to +6.3%. A measure of inter observer bias was calculated by determining the mean difference between the two observers, based on the sample studied. The 95% confidence interval for this value was -0.44 to 1.64. As zero lies within this interval, it may be assumed that there was no bias between the two observers.

Elastin concentration in 30 samples, as measured by the same observer at two time points 3 days apart were used to calculate intra-observer error. The 95% range of intra-observer agreement was from -3.1% to 3.6%. The 95% confidence interval for the presence of bias was -0.38% to 0.84%, which again indicated no bias in the measurement.

Therefore, the use of a single observer throughout the entire course of experiments contributes no bias to the component volume fraction calculations.

**3.4 Enzymography**

Accurate quantification of enzymatic activity within the organ cultures was achieved by substrate gel zymography. This technique identifies enzymes by their distinct molecular weights. In addition MMP identification, zymography may also serve to delineate between the active and latent forms of MMP's since activation involves the proteolytic cleavage of ~10-kDa (Woessner, Jr. 1991).
Enzyme Extraction and Purification

Metalloproteinases were extracted from aortic tissue by the method described by Vine and Powell (Vine and Powell 1991). Snap frozen tissue stored at -80°C was thawed over ice and weighed. The aortic tissue was then diced into 1mm² pieces with a scalpel and homogenised with a Polytron (Kinematica, Switzerland), using 10 ml of homogenising buffer for each gram wet weight (Appendix A), to yield soluble proteins, proteins entrapped in vesicles and proteins tightly bound to connective tissue (Campa et al. 1987; Vine and Powell 1991). The homogenate was centrifuged at 10,000g for 1 hour at 4°C. The supernatant was dialysed overnight at 4°C in 12,000 to 14,000 molecular weight cut-off tubing (Fisons, Loughborough, UK) against a dialysing buffer (Appendix A). The protein concentration of the sample was estimated by using the dye-binding technique (BioRad Laboratories) with bovine serum albumin as standard. The samples were stored at -80°C until needed.

Further attempts at enzyme purification using ammonium sulphate fractionation with 30% and 70% cut-offs (Vine and Powell 1991) had previously been abandoned (Wills) since both the 30% fractionated pellet and the 70% fractionated supernatant were found to contain MMP's upon substrate gel zymography.

Sodium Dodecyl Sulphate (SDS)-Substrate Gel Electrophoresis - (Zymography)

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is a long established and powerful technique for the separation of proteins by molecular weight. This technique has been used in many studies analysing the enzyme profile in human aneurysmal tissue (Newman et al. 1994a; Newman et al. 1994b). In simple terms, SDS included in both the sample buffer and the gel itself denatures the proteins. The SDS binds to the proteins, giving them a negative charge, which is proportional to their size, and allows them to be separated on an electrical gradient. For SDS-PAGE the proteins are reduced to prevent their re-activation, but for zymography re-naturation is essential for enzyme activity to be regained.

For the MMP's to be detected, a known substrate such as gelatin (denatured collagen) is included when preparing the gel (Matrisian 1992; Birkedal-Hansen et al.)
Following electrophoresis, the proteins are re-natured by removing the SDS from the gel by washing it in an ionic detergent. To allow the enzymes to become active, the gel is then incubated in buffer at 37°C overnight. The gels are stained with Coomassie Blue and evidence of enzymatic activity is demonstrated by the absence of staining in areas where the gelatin substrate has been degraded. Physiologically inactive zymogens or pro-forms of the MMP's are visualised using this technique as the denaturation-renaturation process activates them.

The ability to detect proteolytic activity based on MW separation is a powerful tool, since the active and latent forms of the enzymes have different MW's and the presence of both may be visualised using zymography. Furthermore, the electrophoretic process efficiently resolves these enzymes from their endogenous inhibitors, the TIMP's.

Type III collagen from calf skin (Sigma) was denatured by dissolving 10 mg in 4.0 ml double distilled water, and heating to 60°C in a water-bath (Grant) for an hour. The gelatin substrate was incorporated within the 10% polyacrylamide separating gel (Appendix A) during its casting, to a final concentration of 1.0 mg/ml. The 4% polyacrylamide stacking gel (Appendix A) was then cast with a 15-well comb (BioRad Laboratories).

Meanwhile, samples of the aortic extracts were thawed over ice and 200 µl aliquots of each were protein normalised (0.9 mg/ml) by diluting with appropriate volumes of PBS (pH 7.5). 15 µl of each sample was mixed at room temperature with an equal volume of 2 x non-reducing sample buffer (Appendix A) before being applied to the wells of the stacking gel. A positive control sample of conditioned media from HT-1080 cells, a human fibrosarcoma cell line known to constitutively express MMP's (obtained from the European Collection of Animal Cell Cultures, ECACC No. 85111505) was included and a MultiMark Multi-Coloured Standard ladder (15 µl) (Novex, San Diego, CA) consisting of myosin (250 kDa), phosphorylase B (148 kDa), glutamic dehydrogenase (60 kDa) and carbonic anhydrase (42 kDa) was also loaded. Both reservoirs of the Bio-Rad Mini-Protean II Gel System (BioRad Laboratories) were filled with electrode running buffer (pH 8.3) (Appendix A) and electrophoresis was carried out at a constant current of 100 mAmps at 4°C for approximately 4 hours.
On completion of the run, the fractionated proteins were renatured by removing the SDS through extensive washing (3 x 15 minutes) in a solution of 2.5% (v/v) Triton X-100 (Sigma) in double distilled water on a rotary shaker at ambient temperature. The gels were then incubated at 37°C in incubation buffer (Appendix A) for 18 hours. At the end of this period, the gels were stained for 1-2 hours at room temperature with a solution of 0.1% Coomassie Blue R250 dissolved in a mixture of 50% methanol, 20% acetic acid and 30% double distilled water.

Bands of proteolytic activity are visualised as regions of clear lysis against the blue background of the gel. By comparing the relative migration of these bands and the molecular weight markers an estimation of the size of the proteases may be made. As activity of the protease is reflected by the amount of lysis it produces, direct comparison of samples may be made.

3.5 Western Blotting

Western blotting allows positive identification of specific protein species, which have been fractionated using gel electrophoresis. The separated proteins are transferred to a nitrocellulose membrane, to which antibodies are applied in conjunction with a suitable detection complex to locate protein-antibody complexes. Correlation with molecular weight markers allows estimation of the size of detected species.

Immunoblot analysis was utilised chiefly to detect the presence of MMP-9. Monoclonal antibody raised in mice against human MMP-9 (Oncogene, Camb. MA, US) was used. This detects the inactive form only displaying no cross-reactivity (manufacturer's protocol).

SDS-Polyacrylamide Electrophoresis

The stored frozen samples were thawed on ice and 200 μl aliquots were normalised for protein concentration as before (0.9 mg/ml). Both the SDS 10% polyacrylamide separating gel and the SDS 4% polyacrylamide stacking gel were assembled as above. However, the separating gel was cast without the incorporation of the gelatin substrate. The chosen samples were loaded along with the multi-coloured
ladder and HT1080 positive control and the SDS-polyacrylamide gels were run under non-reducing conditions as described above.

**Immunoblotting**

The proteins separated in the gel were then transferred electrophoretically to nitrocellulose membrane (ECL, Amersham, Bucks. UK) at 0.11 amps for 14 hours. Non-specific binding sites were blocked by immersing the membrane in 5% blocking buffer (Appendix A) for one hour at room temperature on a rotary shaker. The filter was then rinsed using two changes of washing buffer (TBS-T - Appendix A) and then washed once for 15 min and twice for 5 min with fresh changes of the buffer at room temperature.

The membrane was then incubated in primary monoclonal antibody (diluted 1:1000 with blocking buffer - Appendix A) for one hour at room temperature. The membrane was then washed three times as before, and then incubated with a 1 in 2000 diluted horseradish peroxidase labeled anti-mouse secondary antibody (Amersham, UK) at room temperature. The membrane was then washed for fifteen minutes and then a further four times for five minutes each with fresh changes of washing buffer. Thorough washing of the membrane was essential to minimise the background staining.

The following steps were then undertaken in a dark room. The excess buffer was drained from the washed membranes and placed on a piece of SaranWrap (Dow Chemical), protein side up. Equal volumes of detection solution 1 (ECL, Amersham, Bucks. UK) with detection solution 2 (ECL, Amersham, Bucks., UK) were mixed to cover the membrane for precisely one minute at room temperature. The ECL (Enhanced Chemiluminescence) detection system employs the emission of visible light (428 nm) following the cleavage of a luminal substrate by the horseradish peroxidase enzyme conjugated to the secondary antibody. Luminel is oxidised to an excited state from which it decays to ground state via a light emitting pathway, which may be detected by exposure to blue light sensitive autoradiography film. The excess detection reagent was drained off and the membranes wrapped in SaranWrap, with air pockets gently smoothed out. The membrane was placed protein side up in a film cassette (Siemans) and with the lights switched onto red, a sheet of autoradiography film (Kodak X-OMAT-AR, Rochester, NY, US) was carefully placed on top of the membrane and exposed for varying lengths of
time. The film was then developed using a Curix 60 developer (AGFA-Gevaert, Middlesex, UK).

3.6 Preparation of Histological Samples

Light Microscopy

At the end of the culture period, tissue samples for paraffin waxed sections and subsequent light microscopy were stored in 10% formyl saline (BDH, Merck Ltd., Poole, UK) for at least 18 hours prior to processing. The samples were then dehydrated in 95% IMS (Sigma, Poole, UK) for 2 hours followed by immersion in 99% IMS for a further 7 hours. The specimens were transferred into xylene (Sigma, Poole, UK) for 3 hours prior to embedding in paraffin wax for at least 4 hours. The wax embedded tissue was serially sectioned to 4 μm thickness and sections dried at 37°C prior to staining. Staining techniques are described in Appendix B.

3.7 Statistical Analysis

Individual statistical methods are included in each chapter with the results. Statistical analysis was performed using the Minitab Release 8.1 statistical program (Minitab Inc., Pennsylvania, US) on a Macintosh LC personal computer (Apple Computer Inc., California, US).
APPENDIX 3.A
REAGENTS

Minimal Essential Medium

Minimal Essential Medium (MEM) was prepared by diluting a solution of concentrated (x10) MEM (GibcoBRL Life Technologies, UK) with sterile water.

Culture Medium

This standard 5% FCS culture medium was composed of RPMI 1640 (GibcoBRL Life Technologies, UK) with supplements added under sterile conditions to the final concentrations given below:

Fetal calf serum (FCS) 50ml/l (5%) (Sera lab, Lot, UK)
Penicillin/streptomycin 10ml/l of 5000IU/ml (GibcoBRL Life Technologies, UK)
L-glutamine 10ml/l of 200MM (GibcoBRL Life Technologies, UK)

Culture Medium Supplemented by Pancreatic Elastase

Culture media with concentrations of 100 units/ml and 50 units/ml were derived by successive serial dilutions. 5000 units of porcine pancreatic elastase (Calbiochem, UK) was obtained in powder form, dissolved in 50mls of culture medium to give a concentration of 100 units/ml which was further diluted with equal volumes of culture medium to obtain a concentration of 50 units/ml.

Culture Medium Supplemented by Doxycycline and Amlodipine

Doxycycline and Amlodipine were obtained in powder form (Pfizer, Sandwich, UK) and dissolved in culture medium with quoted concentrations achieved by serial dilution.
**Tissue Homogenising Buffer**

Tissue homogenising buffer was prepared by dissolving the following in sterile water:

- **Urea**: 2 mol/l (Sigma, Poole, UK)
- **Tris-HCL**: 50mmol/l (Sigma, Poole, UK)
- **NaCl**: 1g/l (Fisons, Loughborough, UK)
- **Ethylenediaminetetraacetic Acid (EDTA)**: 1g/l (Fisons, Loughborough, UK)
- **Brij 35**: 1ml/l (Sigma, Poole, UK)
- **Phenylmethanesulphonyl Fluoride (PMSF)**: 0.1mmol/l (Sigma, Poole, UK)
- **NaOH**: to a final pH of 7.6

**10% Polyacrylamide Separating Gel**

The polyacrylamide separating gel was prepared by sequentially mixing together the following agents:

- **Double Distilled water**: 1ml
- **Resolving buffer (pH 8.8)**: 2.5ml
- **30% Acrylamide/Bis solution**: 3.34ml (National Diagnostics, Georgia, US)
- **10% SDS solution**: 100μl
- **10% Ammonium persulphate**: 50μl
- **Tetramethylethlenediamine (TEMED)**: 5μl (Sigma, Poole, UK)

The ammonium persulphate was added just before the gels were ready to be poured otherwise the gels would have set. Gelatin substrate was incorporated when needed at a final concentration of 1mg/ml.

**4% Polyacrylamide Stacking Gel**

The stacking gel was prepared by sequentially mixing together the following reagents:

- **Double Distilled water**: 6.1ml
Resolving buffer (pH 8.8) 2.5ml
30% Acrylamide/Bis solution 1.3ml
10% SDS solution 100μl
10% Ammonium persulphate 50μl
Tetramethylethylenediamine (TEMED) 10μl

2 x Non-reducing Sample Buffer
8.0ml of “non-reducing sample buffer” was prepared by mixing together the following reagents:
   Double Distilled water 2.8ml
   0.5M Tris-HCl 1ml pH 6.8
   Glycerol 0.8ml (Sigma, Poole, UK)
   10% SDS solution 0.2ml

5 x Electrode Running Buffer (pH 8.3)
This solution was prepared by dissolving 15g Tris-Base, 72g glycine (chromatography grade) and 5.0g SDS in double distilled water with gentle stirring and adjusting final volume to 1 litre. This solution was stored at 4°C. Before use, if precipitation had occurred, this solution was warmed to 37°C and for each electrophoresis run it was diluted 5-fold with double distilled water.

Incubation Buffer
This buffer was prepared by dissolving the following in 800mls of double distilled water:
   Tris-Base 6.06g (50mM)
   CaCl₂.2H₂O 1.47g (10mM)
   NaCl 2.92g
   Brij 0.5ml (0.05%)  
The final volume was then made up to 1 litre with double distilled water and adjusting to pH 7.6 with 1M HCl. The buffer was stored at 4°C for 3 days.
**Blocking Buffer**

The blocking buffer used for Western blotting was prepared by dissolving the following in double distilled water:

- **Dry defatted milk**: 50g/l (Somerfield, UK)
- **Tris-HCl**: 0.05mol/l pH 7.5
- **NaCl**: 0.15mol/l

**Tris-Buffered Saline-Tween (TBS-T)**

The washing buffer (TBS-T) used for Western blotting was prepared by dissolving the following in 100ml of double distilled water:

- **Tris-Base**: 2.42g (20mM)
- **NaCl**: 8g (137mM)
- **1M HCl**: 3.8ml pH 7.6
- **Polyoxyethylene sorbitan monolaurate (Tween-20)**: 1ml (Fisons, UK)

**Trypan Blue**

Trypan blue was obtained in powdered form (Sigma, Poole, UK), and made up to a 0.2% solution using 9 parts distilled water to 1 part normal saline.

**Phosphate Buffered Saline (PBS)**

PBS was prepared by dissolving the following in 1000ml of double distilled water and altering the pH to 7.5:

- **Di-sodium hydrogen orthophosphate anhydrous**: 11.5g (80mM)
- **Sodium dihydrogen orthophosphate**: 2.96g (20mM)
- **NaCl**: 5.84g.
APPENDIX 3B
HISTOLOGICAL METHODS

This section details histological methods for staining paraffin embedded tissue prior to light microscopy. Staff of the Department of Pathology, University of Leicester, carried out the staining procedures.

Haematoxylin and Eosin (H and E)

Haematoxylin and eosin denotes staining of nuclei by oxidised haematoxylin through chelate bonds of metals such as aluminium, followed by counterstaining by the xanthine dye eosin, which colours in varying shades the different tissue fibres and cytoplasm (Bancroft 1984)

Reagents

Mayer's Haematoxylin Solution.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematoxylin</td>
<td>1g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>Potassium alum</td>
<td>50g</td>
</tr>
<tr>
<td>Sodium iodate</td>
<td>0.2g</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1g</td>
</tr>
<tr>
<td>Chloral Hydrate</td>
<td>50g</td>
</tr>
</tbody>
</table>

Acid Alcohol

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>99% IMS</td>
<td>700ml</td>
</tr>
<tr>
<td>Pure water</td>
<td>300ml</td>
</tr>
<tr>
<td>Hydrochloric acid (concentrated)</td>
<td>10ml</td>
</tr>
</tbody>
</table>

Bicarbonate Solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Bicarbonate</td>
<td>20g</td>
</tr>
<tr>
<td>Pure water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>
Eosin Solution

Aqueous eosin 10g
Pure water 1000ml

Method

Sections were dewaxed in xylene and then rehydrated in 99% IMS followed by 95% IMS for 2min each. Sections were rinsed in water and then covered with haematoxylin solution for 5 min. Sections were washed again in water and immersed briefly in bicarbonate solution prior to wet mounting and viewing. The sections were then differentiated by repeated application of acid alcohol, alternated with bicarbonate solution, until only the nuclei were stained blue. The tissue was covered in eosin for 2min. and washed in water. Following staining the section was dehydrated in 95% and 99% IMS prior to immersion in xylene and mounting.

Miller’s Elastin and Van Giesons Stain (EVG)

This technique combines Miller’s elastin stain with Van Giesons' technique for demonstrating collagen fibres. The resultant stain allows differentiation between elastin fibres (blue/black), collagen (red/pink), and muscle (yellow).

Reagents

Miller’s Elastin Stain

Victoria blue 1g
New fuschin 1g
Crystal violet 1g

These reagents were dissolved in 200ml of deionised water, to which the following were added:

Reorcinol 4g
Dextrin 1g
30% ferric chloride 50ml
The solution was boiled, the precipitate removed and dissolved in 200ml 95% IMS. The resulting solution was boiled for 20min, prior to filtration and the addition of 2 ml concentrated hydrochloric acid.

Van Giesons' Stain

Saturated aqueous picric acid 100ml
1% aqueous fushin 10ml

Method

Sections were dewaxed and washed. The loose sections were then oxidised in 0.25% potassium permanganate solution for 5 min prior to bleaching in 1% oxalic acid for a further 1min. Following this process, sections were was and rinsed in 95% alcohol, before being immersed in Miller's staining for 2 hours. After staining the sections were washed and counterstained with Van Giesons' stain for 2min. Completed sections were dehydrated in alcohol and mounted.
CHAPTER FOUR

The influence of Doxycycline on the porcine aortic organ culture model of aneurysmal disease.

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4.1 Introduction

The prevalence of aneurysmal disease appears to be increasing in contrast to that of other vascular disorders including heart disease, stroke and peripheral vascular disease (Marmot MG 1992; Coggon D. et al. 1996). The age standardised death rates from aortic aneurysm in England and Wales rose 20 fold in men and 11 fold in women between 1950 and 1984 (Fowkes et al. 1989). The scale of the rise suggests that it is probably not an artifact of improved diagnosis through better imaging and the introduction of aneurysm screening. Indeed a review of all post-mortem examinations in Malmo between 1958 and 1986 demonstrated a clear rise in prevalence (Bengtsson et al. 1992a).

Abdominal aneurysms and their management, therefore, remain a significant health problem, which is likely to assume greater importance with the expansion of the elderly population. At present, there is a general surgical consensus that patients with abdominal aneurysms in excess of 5.5 cm should be recommended for elective surgical repair which has a mortality approaching 5% (Johnston and Scobie 1988b; Johnston 1994). Evidence to further support this policy has been provided by the recent publication of the UK Small Aneurysm Trial, which reported no long-term survival advantage after early surgery in patients with aneurysms of 4.0-5.5 cm in diameter (Anonymous, UK Small Aneurysm Trial 1998).

Current management strategies for small (< 5.5 cm) aneurysms involve serial ultrasound examination with elective surgery when growth exceeds 1 cm per year, or the absolute aneurysm size reaches 5.5 cm (Thompson and Bell 1996). Small aortic aneurysms expand exponentially by approximately 10% of their diameter per year (Cronenwett et al. 1990; Wolf and Bernstein 1994), although this rate may be significantly higher in patients who are hypertensive or who smoke (Cronenwett 1996). Collin (Collin 1996) monitored 145 patients with screen detected aneurysms < 4 cm, and demonstrated that 14% required elective surgery due to rapid expansion or growth to a diameter exceeding 5.5 cm. In addition to the complications of elective surgical repair, patients with small aneurysms also have a finite risk of rupture. Cronenwett et al. (Cronenwett et al. 1985) demonstrated that 9% of patients with small AAA's required
emergency surgery during a 3 year study, whilst Katz et al (Katz et al. 1992) demonstrated an annual rupture rate of 3.3% for patients with aneurysms < 5 cm. The UK Small Aneurysm Trial reported the mean risk of rupture of aneurysms of 4.0-5.5 cm in diameter was 1.0% per year (Anonymous, UK Small Aneurysm Trial 1998). With the advent of community or hospital based screening programs, the number of small aneurysms presenting to vascular surgeons is likely to increase. Although adhering to a number of Wilson and Junger's criteria of screening for disease (Wilson and Junger 1968), the lack of an adequate treatment strategy for the significant number of small aneurysms that are identified at present limits its efficacy. It is obviously not desirable to expose patients to the risk of elective repair of small AAAs that may not rupture during their lifetime. Clearly, a therapeutic strategy is required to limit both the expansion and rupture rate of small abdominal aneurysms, and to offer an effective treatment to patients with this condition.

Consequent to this requirement, the biochemical and molecular mechanisms of aneurysm formation have begun to attract more attention, and these studies have suggested novel therapeutic avenues. The characteristic feature of early abdominal aneurysms is the segmental depletion of elastin. The loss of elastin from the arterial wall has been related to a generalized enhancement of systemic and localised proteolytic capacity, with increased levels of neutrophil elastase (Cohen et al. 1992) and tissue matrix metalloproteinases (MMP's) (Newman et al. 1994c) being reported in patients with AAA's. The MMP's are a family of zinc dependent enzymes that have the capability to degrade all components of the extracellular matrix. Mesenchymal cells, of the arterial wall, and infiltrating white cells secrete these enzymes. The enzymes are divided on the basis of their substrate specificity into the collagenases (MMP-1), the gelatinases (MMP-2 and -9) and the stromelysins (MMP-3) (Matrisian 1992). Recent evidence has suggested that elevated levels of MMP's may be the crucial determinant of aneurysm formation and growth as all members of the elastolytic and collagenolytic MMP's are demonstrable in aneurysm tissue at elevated levels (Okada et al. 1986; Vine and Powell 1991; Woessner, Jr. 1991; Senior et al. 1991; Brophy et al. 1991c; Irizarry et al. 1993; Newman et al. 1994c; Newman et al. 1994d).
These enzymes therefore provide a potential target for pharmacological therapy aimed at preventing aortic wall matrix degradation and aneurysm growth. The aim of this study was to investigate the use of doxycycline, a non-specific MMP inhibitor, in a previously described organ culture model of aneurysmal disease (Wills et al. 1996). The model utilised porcine aortic segments, which were cultured in sterile conditions. The segments were exposed to a brief pulse of exogenous elastase, to initiate matrix degradation and endogenous MMP production. The arterial organ culture model facilitated the study of isolated cellular interactions and allowed the investigation of various doses of doxycycline within the system.

4.2 Methods

Organ Culture

Dawkins International Ltd. (Nuneaton, UK) kindly provided porcine thoracic aortas. Thoracic as opposed to abdominal aorta was used, as numerous sections were obtained from each aorta and this proved easier using thoracic tissue. Porcine thoracic aorta has a predictable structure containing numerous elastic lamellae (Koo and Gotlieb 1991). Under sterile conditions, fat and loose adventitial tissues were removed. One cm² segments of aorta were excised and pinned, intimal surface uppermost, onto a polyester gauze support resting on sylgard resin (Dow Corning, Seneffe, Belgium), in the base of a 6 cm Petri dish (Fisons Loughborough, UK). Samples were denuded of endothelium, in order to remove any confounding effects of endothelial mediators, and cultured for 14 days in standard medium (7mls) containing 5% fetal calf serum (Sera Lab, Crawley, UK), which was changed after 24 hours and then every 48 hours (Wills et al. 1996).
Experimental Design

Porcine thoracic aortas were divided into six sections. One sample was cultured fresh. One sample was cultured for 14 days without exposure to elastase or doxycycline. Four samples were pre-incubated in culture medium supplemented with porcine pancreatic elastase (100 units/ml) (Calbiochem, Nottingham, UK) for 24 hours. After this period, one sample was harvested and the three remaining aortic sections were washed thoroughly to remove all traces of exogenous elastase and cultured for a further 13 days in standard culture medium or culture medium supplemented by two concentrations of doxycycline (1mg/l and 10 mg/l). The experiment was replicated in eight separate aortas.

Exposure to a brief pulse of elastase was necessary in this experimental design because previous studies had demonstrated that incubation with elastase for 24 hours initiated a time-dependant elastin degradation and MMP production in aortic organ cultures, even though exogenous elastase was not detectable by casein zymography at 3 days. Interestingly, culture of aortic tissue in standard conditions was associated with MMP induction, but did not result in elastin degradation in the absence of exogenous elastase (Wills et al. 1996).

Histology

Following fixation in formalin, samples were dehydrated in 99% industrial methylated spirit (Sigma, Poole, UK), transferred into xylene (Sigma, Poole, UK) for 4 hours, and embedded in paraffin wax. Sections (4 m) were stained with both haematoxylin and eosin (H&E) which stains nuclei, and Miller's elastin and van Gieson's stain (Bancroft and Cook 1984) (EVG) which allows easy identification of elastin fibres (blue/black), collagen (red/pink), and smooth muscle cells (yellow), thus visually delineating the major structural components of the aortic wall.

Stereological Tissue Analysis.

The volume fractions of elastin, collagen and smooth muscle cells in the extracellular matrix were determined by stereological analysis as described previously (Weibel 1979; He and Roach 1994). Aortic sections, stained with EVG, were viewed at 400x magnification using an Olympus microscope incorporating an eyepiece
graticule with a 100-point test grid (Graticules Ltd., Kent, UK). One hundred test points were then analysed with test points hitting black indicating elastin, points hitting yellow indicating smooth muscle cells, and points hitting pink indicating collagen. Following analysis, the relative volume fraction of each component was calculated. To maintain a constant frame of reference throughout all experiments, the adventitial aspect of the media was point counted, with eight randomly chosen fields quantified for each sample. Stereologic tissue analysis reflects the ability of the tissue to take up histological stain, and thus measures of collagen, elastin and smooth muscle cells, reflect changes in the relative concentrations of each of these elements, rather than changes in protein content.

Previous experiments have determined the inter-observer limits of agreement for this technique in our institution (Wills et al. 1996).

**Gel Enzymography**

Metalloproteinases were extracted from frozen tissue using the method of Vine and Powell (Vine and Powell 1991) as previously described (Wills et al. 1996). Tissue was thawed over ice, diced into 1mm pieces and homogenised in buffer. The homogenate was centrifuged, dialysed and the protein concentration was standardised for each sample to 0.9mg/ml with phosphate-buffered saline.

Substrate gels were prepared by incorporating gelatin (1mg/ml - Sigma, Poole, UK) into a 10 % SDS-polyacrylamide gel. Fifteen microlitres of standardised tissue extract plus an equal volume of non-reducing sample buffer were loaded onto the gel. Electrophoresis was performed at 100mA for 4 hours at 4°C with the Mini-Protean II system (Bio-Rad, Hemel Hempsted, UK), after which the gel was washed three times with 2.5% Triton X-100 (Sigma, Poole, UK), incubated in buffer for 18 hours and finally stained with Coomassie Blue R250. The molecular weight of each band was estimated by comparison with the positions of known molecular weight standards (Bio-Rad, Hemel Hempstead, UK). The relative density of each lytic band was determined from negative photographic images of gels with a Pharmacia LKB Imagemaster scanning densitometer (Pharmacia LKB, St Albans, Herts.) and expressed as a product of the optical density and area of the band. The protein concentration utilised in this analysis had been previously determined to be within the
linear range for densitometric quantification (data not shown). To allow for variation between zymographic gels, all paired samples were run on the same gel, and paired statistics used in analysis. No comparison was made between different gels.

**Immunoblotting**

Tissue extracts were fractionated on a 10% SDS-polyacrylamide and transferred to a nitrocellulose membrane (Hybond ECL, Amersham, UK) in the Mini-transblott apparatus (Bio-Rad, Hemel Hempsted, UK) as previously described (Wills et al. 1996). Mouse monoclonal antibodies specific for MMP-2, MMP-9, TIMP-1 and TIMP-2 (Oncogene Science, Paris, France) were used to identify MMP's and TIMP's within the samples. These anti-human antibodies had been demonstrated to cross react with porcine proteins in a prior study (Wills et al. 1996).

**Statistical analysis**

Median values and inter quartile ranges for the volume fractions of elastin, collagen and smooth muscle cells were calculated for all sections. These were then compared using non-paired, non-parametric analysis (Mann-Whitney U test).

The densitometric analyses of MMP’s were compared using the non-parametric one tailed Wilcoxon test.

**4.3 Results**

**Stereological Analysis**

The histological appearances of 4 paired aortic segments are illustrated in Fig 4.1. There was no reduction in the elastin concentration in the sections of aorta cultured in standard conditions for 14 days. Exposure to a 24-h pulse of elastase (100u/ml) induced matrix degradation in a time dependant manner as described previously (Wills et al. 1996), resulting in complete elastin depletion at 14 days.

Stereological analysis confirmed that there was a significant preservation of elastin in the elastase exposed aortic sections cultured in standard medium supplemented
by doxycycline (10mg/l) when compared to the sections not treated with doxycycline (p<0.001, W=28.0, 95% CI 36.00 to 9.9). There was also a trend to elastin preservation in the sections treated with a dose of 1mg/l, although this was not significant (p=0.09, W=39.0, 95% CI 12.5 to -0.62). The percentage elastin, collagen and smooth muscle cell concentrations for all aortic segments is illustrated in Figure 4.2. The reduction in elastin concentration after exposure to elastase was accompanied by a parallel increase in collagen concentration, which obviously reflects the type of stereological volume fraction analysis used. The median concentrations of elastin, collagen and smooth muscle cells and the inter quartile ranges are tabulated in Table 4.1 below.

<table>
<thead>
<tr>
<th></th>
<th>Elastin</th>
<th>Collagen</th>
<th>SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh aorta</td>
<td>53.81(52.62-57.0)</td>
<td>14.31(10.75-17.88)</td>
<td>31.87(25.12-38.62)</td>
</tr>
<tr>
<td>Cultured 14 days</td>
<td>54.25(45.38-62.25)</td>
<td>10.88(10.12-14.38)</td>
<td>31.38(26.87-42.0)</td>
</tr>
<tr>
<td>Elastase 24 hours</td>
<td>47.44(45.0-49.88)</td>
<td>25.0(24.5-25.5)</td>
<td>26.56(24.63-28.5)</td>
</tr>
<tr>
<td>Elastase 14 days</td>
<td>1.0(0-7.5)</td>
<td>71.62(67.75-83.87)</td>
<td>25.38(16.72-39.41)</td>
</tr>
<tr>
<td>Doxycycline 1mg/l</td>
<td>8.75(0.87-13.5)</td>
<td>63.13(57.25-84.88)</td>
<td>31.38(14.38-33.12)</td>
</tr>
<tr>
<td>Doxycycline 10mg/l</td>
<td>21.94(13.66-33.34)</td>
<td>42.62(29.09-59.41)</td>
<td>33.56(20.63-37.66)</td>
</tr>
</tbody>
</table>

Table 4.1: Median values and inter quartile ranges for elastin, collagen and smooth muscle cell concentrations for all the aortic segments of the eight aortas.

Gelatinolytic Activity

Gelatin enzymography confirmed a time dependant increase in MMP activity within elastase treated cultures compared to the control samples. Fresh aortic tissue demonstrated lytic bands at 70 kDa, whilst elastase treated samples demonstrated a progressive increase in gelatinolytic activity at 70 kDa (doublet), and appearance of proteolytic bands at 90 kDa and 250 kDa.
Figure 4.1: Four histological sections from the same aorta stained with Miller's elastin and van Gieson's stain (x400). The cultured control (4.1a).
Figure 4.1b: Section of aorta exposed to a pulse of elastase and subsequently cultured in standard medium.
Figure 4.1c: Section of aorta exposed to a pulse of elastase and subsequently cultured in standard medium supplemented by doxycycline 1mg/l.
Figure 4.1d: Section of aorta exposed to a pulse of elastase and subsequently cultured in standard medium supplemented by doxycycline 10mg/l.
Immunoblotting with specific monoclonal antibodies demonstrated immunoreactivity of a 70-kDa doublet with MMP-2 antibody and a 90 and 250-kDa protein reacting with an antibody to MMP-9 (data not shown).

A representative zymogram of four paired aortic sections is depicted in Fig 4.3. Densitometric analysis of the aortic segments treated with doxycycline demonstrated that these samples had significantly less MMP-9, activity when compared to those cultured in standard medium after elastase exposure (p = 0.04, W = 33.0 and p = 0.02, W= 35.0 for doxycycline concentrations of 1mg/l and 10mg/l respectively). MMP-2 and MMP-9
Figure 4.3: A photograph of a gelatin zymogram demonstrating metalloproteinase activity for all sections of one aorta and demonstrating reduction in MMP-9 activity in the doxycycline treated segments. Positive C, HT 1080 fibrosarcoma cell line, which produces large quantities of MMP-2 and MMP-9. Fresh, freshly harvested, noncultured aortic sample. Cultured C, aorta cultured without exposure to elastase for 14 days. Elastase, aorta exposed to elastase for 24 hours and then cultured in standard conditions for a further 13 days. Doxy 1mg/l and Doxy 10mg/l, aorta exposed to elastase for 24 hours and then cultured with doxycycline for 13 days.
Figure 4.4: Immunoblot demonstrates TIMP-1 immunoreactivity. Cultured C, aorta cultured without exposure to elastase for 14 days. Elastase, aorta exposed to elastase for 24 hours and then cultured in standard conditions for a further 13 days. Doxy 1mg/l and Doxy 10mg/l, aortas exposed to elastase for 24 hours and then cultured with doxycycline for 13 days. Positive C, HT 1080 fibrosarcoma cell line, which produces large quantities of MMP-2, MMP-9 and TIMP-1.
activities are plotted as percentages of the cultured control are illustrated in Figure 4.5. The reduction in MMP-9 activity was accompanied by a lesser reduction in MMP-2 activity, although this was not significant (p = 0.45, W=9.0, and p=0.08, W=25.0 for doxycycline concentrations of 1 and 10 mg/l respectively).

Figure 4.5: Graph plotting MMP-2 and MMP-9 activity (median values and Q3) as a fraction of the cultured control (CC), confirming that elastase exposure induced MMP-9 Activity (E14), that was progressively inhibited by an increasing dose of doxycycline at 1 and 10 mg/l (ED1 and ED10).
Table 4.2. Median values and inter quartile ranges in arbitrary densitometric units in the elastase exposed and doxycycline treated segments after 14 days culture. MMP-2 data is presented as the total of the pro and active forms.

Immunoblotting for the endogenous MMP inhibitors, the tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) demonstrated no obvious difference in activity in the doxycycline treated segments. A representative immunoblot is shown in Figure 4.4.

4.4 Discussion

The management of small aneurysms is currently one of the critical problems in vascular surgery, and it is clearly desirable that pharmacological treatments are developed to retard the expansion rate of such aneurysms. In previous studies a number of pharmacological agents have been proposed to inhibit aneurysm growth. Simpson et al. suggested that the Beta 2 adrenergic antagonist, dl-propranolol and reserpine both reversed the B-aminoproprionitrile mediated reduction in aortic strength and the risk of aortic dissection in a turkey model, whereas the Beta 1 antagonists sotalol and prolactol had very little or no effect on tensile strength(Simpson et al. 1976; Simpson and Boucek 1983). More recent experimental studies have suggested that -blockers may inhibit aneurysm growth by lowering blood pressure and promoting collagen and elastin cross linkage(Leach et al. 1988; Brophy et al. 1988; Slaiby et al. 1994). Interest in these agents was further stimulated by a report from Gadowski et al.(Gadowski et al. 1994) who demonstrated that the expansion rate of large aneurysms was lower in a cohort of patients taking -blockers. Unfortunately, this was an uncontrolled, unrandomised study,
and recent data from the MRC small aneurysm study has suggested that \(\beta\)-blockade has no effect of aneurysm growth (Brown 1996). Whilst the role of \(\beta\)-blockers in aneurysms is defined by a randomised trial (Ricci et al. 1996a), the necessity for alternative strategies has suggested that potential therapeutic agents should be specifically targeted to pathophysiological processes within the aneurysm wall.

Many of the features observed during aneurysm development and expansion may be related to an overproduction of MMP's within the arterial wall. Particularly important in this respect are the gelatinolytic enzymes MMP-2 and MMP-9. Recently Freestone et al. (Freestone et al. 1995) investigated the enzyme profile in aneurysms of differing diameters and demonstrated that the elastolytic MMP-2 was the dominant MMP in small aneurysms, whereas MMP-9 was preferentially elevated in larger vessels. This investigation suggested that MMP-2 might play a role in aneurysm genesis whereas MMP-9 may be more important in expansion of larger aneurysms. Sakalihasan et al. (Sakalihasan et al. 1996) confirmed these observations by describing high levels of activated MMP-9 in aneurysmal tissue whilst Newman et al. (Newman et al. 1994b) have previously localised MMP-9 activity to the mononuclear infiltrate in the aneurysm wall.

An aetiological role in aneurysm genesis for the elastolytic MMP's and MMP-2 in particular, has been suggested by two contemporary studies which demonstrated that smooth muscle cells cultured from the abdominal aneurysm wall expressed higher levels of MMP-2 than cells from control tissue culture (Crowther et al. 1996; Patel et al. 1996). These results suggested that a primary elastolytic insult from high levels of MMP-2 might initiate aneurysm formation and growth. Clearly, these findings require extensive further investigation into the control of MMP-2 production, as traditionally MMP-2 is considered a relatively nonresponsive gene with different promoters to the majority of the MMP family (Matrisian 1992).

Theoretically, elastin degradation will release elastin derived peptides (EDP's), which further stimulate MMP production and induce leukocyte infiltration into the tissue (Cohen et al. 1987; Cohen et al. 1992). These changes may then initiate a cascade leading to elastin degradation, leukocyte infiltration and aneurysm formation. Recent in vitro investigations from our department have confirmed that an initial wave of elastin degradation may induce aneurysmal type changes in arterial tissue (Wills et al. 1996).
and these sequelae have also been observed in the elastase infusion animal model of aneurysmal disease (Dobrin et al. 1984; Anidjar et al. 1990; Anidjar et al. 1992). Evidence from the above studies has suggested that the elastolytic MMPs-2 and -9 may be intimately involved in aneurysm pathogenesis and growth, and these enzymes provide an attractive target for pharmacological agents aimed at reducing small aneurysm expansion and rupture.

One agent, which may fulfill the criteria for use in small aneurysms, is doxycycline. Doxycycline belongs to the family of tetracyclines and chemically modified tetracyclines. These groups of antibiotics have proven long-term safety and efficacy in the treatment of acne vulgaris. The drugs are frequently used in relatively low doses for many months or years and have good side effect profiles (Sauer 1976; Harrison 1988; Layton and Cunliffe 1993). Doxycycline non selectively inhibits MMP's by binding to the active zinc sites (Sorsa et al. 1994) and also by binding to a non active calcium site which causes conformational change (Lovejoy et al. 1994) and loss of enzymatic activity. Secondary mechanisms of inhibition have also been proposed which include a reduction in activation (Ramamurthy et al. 1993), decreased gene expression (Petrinec et al. 1996) and stabilization of specific and non-specific inhibition (Golub et al. 1994).

The present study has shown that doxycycline significantly reduced elastin degradation and MMP-9 activity in an in vitro model of aneurysmal disease, and also had a non-significant effect to reduce the production of MMP-2. The findings from our in vitro study were similar to those from Petrinec et al. (Petrinec et al. 1996) who investigated the therapeutic potential of doxycycline in the elastase infusion rodent model. The authors observed preservation of aortic medial elastin with doxycycline administration and suggested that this was as a result of reduced MMP-9 production by the infiltrating inflammatory cells. Similarly Holmes et al. (Holmes et al. 1996) have recently demonstrated that indomethacin, a non-steroidal anti-inflammatory drug, reduces aortic aneurysm formation in the same model and suggested that this results from decreased MMP-9 expression by infiltrating macrophages. These findings corroborate those of Ricci et al. who showed that aneurysm growth, in a similar rat model, could be slowed by inhibiting the inflammatory response using the anti-CD 18 monoclonal
antibody (Ricci et al. 1996b). Furthermore recent studies utilising the rat infusion model have demonstrated that both methylprednisolone and cyclosporine attenuate aortic dilatation after elastase infusion by reducing the inflammatory response (Dobrin et al. 1996).

The tetracycline group of antibiotics is frequently used in relatively low doses for many months or years and has a good side effect profile. The incidence of serious side effects is reported to be rare (Sauer 1976; Harrison 1988; Layton and Cunliffe 1993). Doxycycline therefore provides a potentially attractive pharmacotherapeutic agent for the long-term treatment of patients with small AAAs, with the aim of inhibiting or reducing aneurysm growth rate. The doses of doxycycline utilised in the present study are achievable with oral administration. A loading dose of 200mg followed by 100mg/day gives a serum level of 3.5mg/l, with a tissue/serum concentration of greater than one (Steigbigel et al. 1968). A further recent human study has demonstrated serum concentrations of doxycycline of 5.7mg/l two hours after the administration of a 200mg oral dose (Thompson and Baxter 1999). This study also showed that a 7 day course of 100mg doxycycline bd produced a three-fold reduction in aortic wall expression of MMP-2 and a four-fold reduction in MMP-9 (Thompson and Baxter 1999). These data suggest that a doxycycline dose of 100mg bd would provide a good starting point for a larger clinical trial. There is little doubt that the therapeutic potential of this family of drugs in aneurysmal disease deserves further consideration.

### 4.5 Summary

The results presented in this chapter are extremely encouraging when considering possible therapeutic avenues for the treatment of small abdominal aortic aneurysms. The non-specific MMP inhibition of the tetracycline family of drugs has already been demonstrated to have clinical benefits in the treatment of both reactive and rheumatoid arthritis and adult periodontitis in humans. The benefits were mediated by a reduction in interstitial collagenase activity (MMP-8). These studies however did not demonstrate a reduction in MMP-9 activity in salivary samples.
The role-played by MMPs in tumour invasion and metastasis (Parsons et al. 1997a) has led to the development of more specific MMP inhibitors (MMPIs). Early clinical studies with the first generation MMPI, balimastat, have demonstrated clinical benefit in-patients with malignant ascites (Parsons et al. 1997b). Marimastat is a synthetic low molecular weight second generation MMPI with a collagen mimicking hydroxamate structure, which facilitates chelation of the zinc ion of the MMP active site. It is a potent and specific MMPI and is at present undergoing clinical trials in-patients with advanced metastatic disease. The use of these drugs in aneurysmal disease warrants further investigation and the organ culture model described herein provides an ideal model for this purpose.

The results of this and previous animal studies may provide the basis for a randomized placebo controlled trial of doxycycline in the treatment of small abdominal aortic aneurysms.
CHAPTER FIVE

The influence of Amlodipine on the porcine aortic organ culture model of aneurysmal disease.

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5.1 Introduction

The findings of the previous chapter have demonstrated that the non-specific metalloproteinase inhibitor, doxycycline, reduces elastin destruction in the porcine organ culture model of aneurysmal disease and has corroborated the evidence of Petrinec et al. who have reported similar findings in a rat model (Petrinec et al. 1996). The logical implication is that a metalloproteinase agonist will potentiate MMP activity and thus accelerate elastin destruction in the same model. As such, no specific MMP agonists have been described. However a number of authors have recently suggested that calcium antagonists, a widely used family of agents in the treatment of angina pectoris and hypertension, exert a number of late onset effects that cause structural remodeling of the arterial wall. In particular amlodipine, a dihydropyridine calcium channel blocker has been demonstrated to act at three different levels on the composition of the extracellular matrix. Firstly, it inhibits the expression of procollagens I, III, and IV in both fibroblasts and vascular smooth muscle cells, secondly it increases the proteolytic activity of MMP-2 and thirdly it inhibits the transcription of TIMP-2 in both fibroblasts and VSMC (Roth et al. 1996; Eickelberg et al. 1997). These mechanisms result in regression or retardation of atherosclerotic plaque development within arteries. If these findings are extrapolated it may be hypothesised that calcium antagonists may increase MMP and reduce TIMP activity in aneurysmal disease and potentially hasten elastin degradation with the further potential to accelerate aneurysm growth.

As already alluded to the incidence of abdominal aortic aneurysms is increasing (Fowkes et al. 1989). The ageing population and improved diagnosis may account for part of this increase, however a clear rise in the prevalence of AAA at autopsy in Sweden has been demonstrated (Bengtsson et al. 1992a). The rise in incidence is in contrast to that of peripheral vascular disease, despite similar risk factor profiles (Coggon D. et al. 1996). The reasons for this apparent disparity remain undetermined. Despite the greater understanding of the underlying pathophysiology of aneurysmal disease, the variation of growth rates between individuals remains poorly understood. It is likely however that both genetic and environmental influences play a role. Calcium antagonists have been widely prescribed for the treatment of hypertension
and angina pectoris over the past 25 years. It may be hypothesised that this family of
drugs might influence aneurysm development by the mechanisms described above.

We set out to test this hypothesis investigating the effects of Amlodipine (a
dihydropyridine calcium antagonist) in an organ culture model of aneurysmal
disease (Wills et al. 1996). The model utilised porcine aortic segments, which were
cultured in sterile conditions. The segments were exposed to a brief pulse of exogenous
elastase, to initiate matrix degradation and endogenous MMP production. The arterial
organ culture model facilitated the study of isolated cellular interactions and allowed the
investigation of various doses of amlodipine within the system.

5.2 Methods

Organ Culture

Porcine thoracic aortas were kindly provided by Dawkins International Ltd
(Nuneaton, UK). Aortas were retrieved fresh and transported in minimal essential
medium on ice and then set up as described in the previous chapter.

Experimental Design

The experimental design described for doxycycline was modified slightly. In
order to demonstrate enhanced elastin degradation the duration of culture and the
concentration of exogenous elastase were reduced so that control segments contained
significant concentrations of elastin at the termination of culture. Porcine aortic segments
were pre-incubated in culture medium supplemented with porcine pancreatic elastase (50
units/ml) (Calbiochem, Nottingham, UK) for 24 hours. After this period aortic samples
were washed thoroughly to remove all traces of exogenous elastase and cultured for a
further 6 days in standard culture medium or culture medium supplemented by two
concentrations of amlodipine (10μg/l and 100μg/l). The experiment was replicated in 8
separate aortas. One aortic segment was harvested fresh, another was cultured in standard
conditions for 7 days, without prior exposure to elastase, and four segments were exposed
to porcine elastase for 24 hours. One of these four segments was harvested after 24 hours
and the remaining three were cultured for 6 days in standard medium and medium
supplemented by amlodipine (Pfizer, Sandwich, UK) at a concentration of 10μg/l and 100μg/l. At the termination of organ culture the tissue was divided into two equal segments which were prepared for histological evaluation and metalloproteinase quantification.

Histology

Following fixation in formalin, samples were dehydrated in 99% industrial methylated spirit (Sigma, Poole, UK), transferred into xylene (Sigma, Poole, UK) for 4 hours, and embedded in paraffin wax. Sections (4mm) were stained with both haematoxylin and eosin (H&E) and Miller's elastin and van Gieson's stain (EVG) (Bancroft and Cook 1984).

Stereological Tissue Analysis

The volume fractions of elastin, collagen and smooth muscle cells in the extracellular matrix were determined by stereological analysis as described in the previous chapter.

Gel Enzymography

Metalloproteinases were extracted from frozen tissue using the method of Vine and Powell (Vine and Powell 1991) as previously described in Chapter 4.

Immunoblotting

Immunoblotting was performed as described in Chapter 4.

Statistical analysis

Median values and inter quartile ranges for the volume fractions of elastin, collagen and smooth muscle cells were calculated for all sections. These were then compared using non-paired, non-parametric analysis (Mann-Whitney U test).

The densitometric analyses of MMPs were also compared using the non-parametric paired, one-tailed Wilcoxon test.
5.3 Results

Stereological Analysis.

The histological appearances of 4 paired aortic segments are illustrated in Figure 5.1. There was no reduction in the elastin concentration in the sections of aorta cultured in standard conditions for 7 days. Exposure to a 24 hr pulse of elastase (50u/ml) induced matrix degradation in a time dependant manner as described previously (Wills et al. 1996) resulting in significant elastin depletion at 7 days.

Stereological analysis confirmed that there was a significant acceleration of elastin degradation in the elastase exposed aortic sections cultured in standard medium supplemented by amlodipine (100µg/l), when compared to the sections not treated with amlodipine (p< 0.05, W=87.5, 95% CI 0.00, 24.00). There was also a trend to elastin destruction in the sections treated with a dose of 10µg/l, although this was not significant (p=0.345, W=77.5, 95% CI -4.99,16.5). The percentage elastin concentration for all aortic segments is illustrated in Fig 5.2. The median concentrations of elastin, collagen and smooth muscle cells and their inter quartile ranges are tabulated in Table 5.1.

<table>
<thead>
<tr>
<th></th>
<th>Elastin</th>
<th>Collagen</th>
<th>SMC</th>
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<tr>
<td>Fresh aorta</td>
<td>54.67 (52.55-58.2)</td>
<td>12.72(9.85-16.38)</td>
<td>29.8 (25.95-38.27)</td>
</tr>
<tr>
<td>Cultured 7 days</td>
<td>52.58 (48.6-58.74)</td>
<td>13.24(10.86-15.33)</td>
<td>31.58(26.35-41.12)</td>
</tr>
<tr>
<td>Elastase 24 hours</td>
<td>50.21(46.2-52.86)</td>
<td>18.32 (14.5-20.28)</td>
<td>27.8 (24.28-38.1)</td>
</tr>
<tr>
<td>Elastase 7 days</td>
<td>40.25(31.06-48.75)</td>
<td>17.52(15.62-26.69)</td>
<td>31.12(29.01-37.0)</td>
</tr>
<tr>
<td>Amlodipine 10 g/l</td>
<td>33.62(27.94-40.75)</td>
<td>25.27(21.25-28.05)</td>
<td>30.25(27.06-36.56)</td>
</tr>
<tr>
<td>Amlodipine 100 g/l</td>
<td>27.0(18.5-38.25)</td>
<td>30.12(28.56-35.69)</td>
<td>32.12(28.71-34.25)</td>
</tr>
</tbody>
</table>

Table 5.1: Median elastin collagen and smooth muscle cell concentrations with inter quartile ranges in parentheses for all aortic segments.
Figure 5.1: *Four histological sections from the same aorta stained with Miller's elastin and Van Gieson's stain (x 400).* 5.1a The cultured control.

Figure 5.1b: *A section exposed to a pulse of elastase and subsequently cultured in standard medium.*
**Figure 5.1c:** A section exposed to a pulse of elastase and subsequently cultured in standard medium supplemented by amlodipine 10µg/l.

**Figure 5.1d:** A section exposed to a pulse of elastase and subsequently cultured in standard medium supplemented by amlodipine 100µg/l.
Figure 5.2: Graph plotting percentage elastin, collagen and smooth muscle cell concentrations (median values and Q3) for fresh aorta, cultured control (CC), elastase exposed after 24 hours (E24) elastase exposed after 7 days (E), amlodipine treated 10μg/l (EA10) and amlodipine treated 100μg/l (EA100).

Gelatinolytic Activity

Gelatin enzymography confirmed a time dependant increase in MMP activity within elastase treated cultures compared to the control samples. Fresh aortic tissue demonstrated lytic bands at 70 kDa, whilst elastase treated samples demonstrated a progressive increase in gelatinolytic activity at 70 kDa (doublet), and appearance of proteolytic bands at 90 kDa and 250 kDa.
Figure 5.3: Photograph of a gelatin zymogram showing metalloproteinase activity for all sections of one aorta and demonstrating enhanced MMP-9 activity in the amlodipine treated segments.
Immunoblotting with specific monoclonal antibodies demonstrated immunoreactivity of a 70 kDa doublet with MMP-2 antibody and a 90 and 250 kDa protein reacting with an antibody to MMP-9 (data not shown).

A representative zymogram of four paired aortic sections is depicted in Fig 5.3. Densitometric analysis of the aortic segments treated with amlodipine demonstrated that these samples had significantly enhanced MMP-9 activity, when compared to those cultured in standard medium after elastase exposure (p = 0.007, W = 42.0, CI-2.082, -0.419, and p = 0.013, W= 44.0, CI-2.153, -0.231, for amlodipine concentrations of 10μg/l and 100μg/l respectively). MMP-9 activity in arbitrary densitometric units is illustrated in Fig 4. The enhanced MMP-9 activity was accompanied by lesser increases in both proMMP-2 (p= 0.128, W=53.0 and p=0.318, W=58.0) and active MMP-2 (p=0.227, W=56.0 and p=0.083, W=51.0) activity, although this was not significant for amlodipine concentrations of 10 and 100 μg/l respectively).

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<tr>
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<th>MMP-9 activity</th>
<th>MMP-2 activity</th>
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<tr>
<td>Cultured control</td>
<td>2.408 (2.011-2.99)</td>
<td>2.832 (2.081-3.023)</td>
</tr>
<tr>
<td>Elastase</td>
<td>2.658 (1.73-3.628)</td>
<td>2.809 (2.146-3.16)</td>
</tr>
<tr>
<td>Amlodipine 10 g/l</td>
<td>4.075 (3.281-4.546)</td>
<td>3.071 (2.131-3.581)</td>
</tr>
<tr>
<td>Amlodipine 100 g/l</td>
<td>4.109 (2.617-4.740)</td>
<td>3.127 (2.076-3.357)</td>
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</table>

Table 5.2: Median MMP-9 and MMP-2 activity in arbitrary densitometric units with interquartile ranges in parentheses, for aortic segments after 7 days culture. MMP-2 data is presented as the total of the pro and active forms.

Immunoblotting for the endogenous MMP inhibitors, the tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) did not demonstrate any difference in TIMP activity in the amlodipine treated segments (data not shown).
Figure 5.4: Graph plotting the MMP-2 and MMP-9 activity (median values and Q3) in arbitrary densitometric units for the cultured control (CC) and elastase exposed segments (E7) and demonstrating that MMP-9 activity was progressively potentiated by an increasing dose of amlodipine at 10 and 100μg/l (EA10 and EA100).
5.4 Discussion

The scale of the rise in incidence of abdominal aortic aneurysm in contrast to that of peripheral vascular disease suggests that it is probably not simply an artifact of an aging population or improved diagnosis (Coggon D. et al. 1996). Despite this, similar risk factors are associated with both pathologies (Reed et al. 1992), suggesting that other undetermined influences are important during the development of aneurysmal disease.

Animal research has identified agents that accelerate aneurysm growth or precipitate rupture. Reilly et al demonstrated that hydrocortisone precipitated aortic rupture in genetically susceptible mice and caused aneurysm formation in normal mice (Reilly et al. 1990).

Similarly B-aminopropionitrile (BAPN) fed broad breasted white turkeys succumb from aortic dissection, BAPN causing a marked disruption of elastin and collagen fibres (Simpson et al. 1976; Simpson and Boucek 1983).

A greater understanding of the pathophysiology of aneurysmal disease has been recently established, with an overproduction of MMP-2 and MMP-9 within the aortic wall integral to the process. Freestone et al. (Freestone et al. 1995) suggested that MMP-2 might play a role in aneurysm genesis whereas MMP-9 may be more important in expansion of larger aneurysms. As already alluded to in the previous Chapters, the evidence suggests that MMP’s-2 and -9 are intimately involved in aneurysm pathogenesis and growth. The potentiation of their action may therefore accelerate aneurysm growth.

Roth et al have recently demonstrated that a number of calcium antagonists, including amlodipine, enhanced human VSMC MMP-2 activity and inhibited TIMP-2 transcription in vitro (Roth et al. 1996). They suggested that this ability to modulate ECM metabolism was protective in atherosclerosis (Roth et al. 1996). The increase in MMP-2 activity occurred in the presence of lower intracellular calcium levels, a finding that was consistent with the work of Lohi and Keski-Oja, who showed that the pericellular gelatinolytic activity of MMP-2 and MMP-9 was inhibited by increasing intracellular calcium levels with calcium ionophores (Lohi and Keski-Oja 1995). The results of this study are in keeping with both these previous investigations. We have shown that the
calcium antagonist, amlodipine, not only potentiates the activity of MMP-9, but this in turn accelerates elastin breakdown in this model of aneurysmal disease. This corroborates the finding of Cohen et al who described verapamil, as a poor drug to use to manipulate the protease system in abdominal aortic aneurysms (Cohen et al. 1990). In short, these studies suggest that calcium antagonists enhance the proteolytic activity of the MMPs within the arterial wall, a phenomenon that has a protective effect in atherosclerosis by influencing plaque morphology and detrimental consequences in aneurysmal disease by accelerating elastin destruction.

One must not however, consider the calcium antagonist's effect on MMPs in isolation of its other pharmacological actions. Amlodipine is a potent antihypertensive agent, and hypertension is a well-recognised risk factor for aneurysm development, with aneurysms expanding significantly faster in patients who are hypertensive (Cronenwett 1996). Previous experimental studies have suggested that β-blockers may inhibit aneurysm growth by lowering blood pressure and promoting collagen and elastin cross linkage (Leach et al. 1988; Brophy et al. 1988; Slaiby et al. 1994). Interest in these agents was further stimulated by a report from Gadowski et al. (Gadowski et al. 1994) who demonstrated that the expansion rate of large aneurysms was lower in a cohort of patients taking β-blockers. Unfortunately, this was an uncontrolled, unrandomised study, and recent data from the MRC small aneurysm study has suggested that β-blockade has no effect of aneurysm growth (Brown 1996). However, the antihypertensive actions of calcium antagonists may be beneficial in reducing aneurysm expansion in vivo.

Recent reports have identified a possible link between calcium channel blockade and an excess risk of cancer (Pahor et al. 1996; Olsen et al. 1997). Pahor et al quoting a relative risk of 1.72 (Pahor et al. 1996). They suggested that the possible mechanism was via inhibition of apoptosis or programmed cell death of neoplastic cells, thereby promoting cancer growth. In addition to this there is now strong evidence that MMPs play a major role in tumour invasion and metastasis (Parsons et al. 1997a). When considering this evidence together with the findings of this study and the work of Roth et al. (Roth et al. 1996) it may be hypothesised that calcium antagonists may also potentiate MMP activity in cancer and thus, tumour invasion and metastasis.
The concentrations of amlodipine used in this study are in keeping with those achieved in elderly patients taking a standard oral dose for hypertension (Abernethy et al. 1990). Further studies are required to establish whether the detrimental effect of calcium antagonists on elastin destruction and enhanced MMP activity described in this study are observed in an animal model or man.

5.5 Summary

The results described in this chapter demonstrate that metalloproteinase activity is potentiated by amlodipine and this increased activity translates to accelerated elastin destruction in this model of aneurysmal disease. To some extent this work generates more questions than it answers. The pharmacological mechanisms of amlodipine in this model require further delineation. Whether these in vitro results will translate to the in vivo situation requires future investigation. Further work in the previously described rat model may be the next step (Anidjar et al. 1990). Also information on aneurysm expansion rate and concomitant pharmacotherapy may become available from the UK small aneurysm trial, although this was not the primary aim of this study (Powell et al. 1996). There is however, little doubt that the incidence of aneurysmal disease is increasing and the likelihood is that this is related to environmental influences. Whether one of these influences is calcium antagonist therapy remains to be seen.
# CHAPTER SIX

Patients and Methods

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6.1 Introduction

This chapter describes in detail the methods used in the subsequent clinical chapters. In particular it details patient selection and pre-operative assessment, anaesthetic and surgical techniques, tests of respiratory and cardiovascular function and the laboratory assays of metabolic parameters.

Data has been collected prospectively on all patients admitted to Leicester Royal Infirmary with asymptomatic abdominal aortic aneurysms since January 1994. The majority of the subsequent chapters concentrate on two comparative cohorts of patients who underwent endovascular or conventional aneurysm repair between September 1995 and December 1996. The local Ethics Research Committee approved the study and all patients gave informed consent.

6.2 Patient selection and pre-operative assessment

Patients with asymptomatic AAAs of 5cm diameter or greater were admitted for two days and underwent a number of investigations to assess fitness for surgery and aneurysm morphology. The patient’s age and aneurysm diameter, as assessed by ultrasound, were recorded. A standard history was taken and a full external examination was performed and recorded on a pre-designed proforma for all patients. Particular attention was paid to respiratory and cardiac co-morbidities and current drug therapies. All patients had standard haematological and biochemical blood assays, an electrocardiogram (ECG), spirometry and radioisotope (MUGA) scanning to assess cardiac function. In addition plain chest radiography and arterial blood gas sampling were performed if clinically indicated. Patients identified as, ‘high risk’ after these investigations, were assessed by an anaesthetist prior to admission for surgery.

Suitability for endovascular abdominal aortic aneurysm repair was determined initially by a contrast enhanced CT scan. Those patients demonstrating favourable anatomy then underwent further assessment with intra-arterial digital subtraction angiography (IA-DSA).
Computed tomography

CT was performed using a Siemens HiQ scanner (Erlangen, Munich, Germany). Continuous slices of 10mm thickness were obtained from the diaphragm inferiorly. On reaching the renal arteries 3mm slices were used. Once distal to the renal vessels scanning continued at 10mm intervals until reaching the aortic bifurcation where 3mm cuts were again utilised to obtain greater definition. Images were enhanced by delivering 100mls of intravenous contrast, (Omnipaque 320, Nycomed, Birmingham, UK) which was hand-injected at approximately 2mls/second when scanning from the level of the renal vessels. This was performed dynamically in quiet respiration and commenced after a 40-second delay.

Intra-arterial digital subtraction angiography

IA-DSA was performed by percutaneous catheterisation of the femoral artery using the Seldinger technique. A marker pigtail catheter with eight side holes (6Fr, 65cm long, Cordis, Miami, Florida, USA) was used. Approximately 80mls of contrast (Omnipaque 300, Nycomed, Birmingham, UK) was injected at 20mls/second. Anterioposterior and lateral views of the abdominal aorta, and anterior posterior and oblique views of the iliac arteries, were obtained with a Siemens Multiskop equipped with a Digitron computer system (Erlangen, Munich, Germany).

Suitability for endovascular repair depended on specific aneurysm morphology. Exact anatomical requirements varied depending on the type of device to be implanted. The minimum requirements were a proximal neck of normal aorta, below the lowest renal artery, of at least 15mm in length and less than 30mm diameter, and in addition one non-aneurysmal iliac bifurcation. Patients whose aortic anatomy was suitable for endovascular repair made an informed choice on whether to proceed with surgery by this or a conventional technique. Patients whose morphology precluded endovascular intervention were offered standard open surgery.
6.3 Anaesthetic

All patients received a standard general anaesthetic given by one of two anaesthetists. Premedication comprised diazepam 10mg and ranitidine 150mg orally. Anaesthesia was induced with a sleep dose of thiopental approximately 4-5mg/kg, alfentanil 15-μg kg⁻¹, and maintained with isoflurane and nitrous oxide 70% in oxygen and muscle relaxation with vecuronium 0.1mg/kg. Morphine 0.15-0.25mg kg⁻¹ and infusions of dopamine 3-μg kg⁻¹ min⁻¹ and glyceryl trinitrate 0-120-μg kg⁻¹ min⁻¹ were administered during surgery. Intravenous fluids were administered as a mixture of crystalloids and colloids, with 5mls/kg crystalloid given at induction and subsequent infusion guided by a pulmonary artery flotation catheter.

At the end of surgery, patients were transferred to the Intensive Care Unit for weaning from artificial ventilation and further management. Sedation was continued with intravenous propofol and morphine infusions before weaning. The intensive care management, timing of weaning and discharge was at the discretion of intensivists. Within 2 hours of tracheal extubation, all patients commenced intravenous patient controlled analgesia (PCA) with morphine (1mg bolus, 5-min lockout).

6.4 Operative Details

The study group of 43 patients underwent aneurysm repair by a variety of techniques. Twenty three patients were treated with endovascular devices (17 aortomonofemoral, 2 aortomoiliac(Thompson et al. 1997b), 2 EVT bifurcates (Menlo Park, CA, USA), 1 Mintec bifurcate (Freeport, Bahamas) and 1 Bard straight (New Jersey, USA)) and twenty conventionally with inlay grafts (12 tube grafts and 8 bifurcates). No conventional cases required supra-renal clamping. Patients were only offered aortomoiliac repair, if their aortic anatomy precluded the use of a commercially available device. All the commercially available products were deployed in accordance with the manufacturer’s protocols and in the presence of a manufacturer’s representative. The basic design of the aortomoiliac endograft, a long, tapered graft sutured to
Figure 6.1: Photograph illustrating the 5cm long Palmaz stent secured to PTFE graft by two diametrically opposed 2/0 polypropylene sutures.
Figure 6.2: Photograph illustrating the stent-graft combination mounted on a 30mm angioplasty balloon and backloaded into a 21Fr Teflon sheath.
a balloon expandable stent, was adapted from the endovascular prostheses described by Parodi et al (Parodi et al. 1991) and Richter et al (Richter et al. 1994) and is similar to that deployed by Marin et al (Marin et al. 1995). The devices were constructed as previously described, using a thinwalled 8mm pre-expanded ePTFE tube graft (Impra UK) which was serially dilated to 35mm using a graded angioplasty balloons followed by a 35mm achalasia balloon (Boston Scientific Ltd., St Albans, UK). The graft was then tapered from 35mm proximally to 15 mm at its distal terminus. The graft was deliberately oversized to allow for retraction of the ePTFE, and the same size graft was used for all patients.

The proximal end of this graft was then sutured to a 4 or 5cm long partially predilated Palmaz stent (Johnson and Johnson, Berskshire UK) using two diametrically opposed 2-0 Prolene sutures (Ethicon Ltd., Edinburgh, UK)(Figure 6.1). This stent-graft combination was then crimped onto a 30mm angioplasty catheter (William Cook Europe, Denmark), and the entire device backloaded into a 21Fr teflon sheath (William Cook Europe)(Figure 6.2).

All endovascular procedures were performed in a standard operating theatre under balanced general anaesthesia with fluoroscopic imaging provided by a portable C-arm with roadmapping capabilities. The patients were prepared for conventional surgery and both common femoral arteries were exposed and controlled. The endograft was preferentially inserted through the most aneurysmal iliac segment. Commonly a temporary iliac conduit was constructed via a retroperitoneal approach, with division of the internal iliac artery to allow full mobilisation of the CIA. Following iliac mobilisation a 10mm Dacron graft was anastomosed end-to-side to the external iliac artery. This graft was then tunneled beneath the inguinal ligament into the groin wound to provide straight-line access to the iliac system. During preparation of the iliac conduit and in order to minimize distal ischaemia a 10 mm dacron graft was anastomosed end to side to the contralateral common femoral artery and tunneled subcutaneously to the ipsilateral femoral artery at this point. This graft formed the distal part of the femorofemoral crossover graft. A guidewire was then inserted through contralateral femoral artery into the aorta, and a pigtail catheter (Cordis) was positioned just above the renal arteries.
A standard 0.89mm guidewire (Meadox, Bedfordshire, UK) was then manipulated via the iliac conduit into the suprarenal aorta under fluoroscopic control. A tapered Van Andel dilation catheter was passed over the guide wire into the aorta, and the standard wire exchanged for a 0.89mm J-curved Lunderquist guidewire (Meadox). Following removal of the dilation catheter, the Dacron conduit was incised longitudinally, and a 25 Fr sheath with mandrill was passed over the Lunderquist wire into the suprarenal aorta.

An aortogram was then performed via the pigtail catheter in the contralateral femoral artery to identify the position of the renal arteries (Figure 6.3). The mandrill of the sheath was then removed, and the endograft, packaged in a 21 Fr sheath, was inserted into the aorta through the introducer sheath. The 25 Fr sheath was then removed and 21 Fr sheath withdrawn to expose the stent-graft combination. The stent was fluoroscopically positioned immediately below the lowest renal artery. The devise was deployed, by inflating the angioplasty balloon to 2-atm pressure, using a saline filled manometer syringe (Boston Scientific Corp., MA, USA). During balloon inflation, the position of the stent was continually adjusted to compensate for stent shortening during expansion.

After deployment, the packaging sheath was removed and the extra-anatomic bypass was then completed by anastomosing the proximal portion of the femoro-femoral crossover graft to the ePTFE graft in the groin. At this point, the aortic balloon was deflated allowing immediate restoration of blood flow to the contralateral limb. Finally the distal ePTFE graft was anastomosed to the ipsilateral common femoral artery (aortomonofemoral). The procedures were completed by occluding the contralateral common iliac vessel with a covered Gianturco stent (William Cook Europe) deployed through a 16 Fr sheath(Thompson et al. 1997b) or by direct ligation. A completion arteriogram was then performed (Figure 6.4). In two patients with suitable common iliac vessels the ePTFE was sized exactly and the distal end stented in the ipsilateral common iliac artery with a further Palmaz stent (aortomonoiliac). One of these patients had an isolated 6cm iliac artery aneurysm after previous conventional surgery for ruptured AAA. The endovascular device in this patient was placed from the aorta down the contralateral iliac system to allow continuity of flow through the contralateral internal iliac vessel and
Figure 6.3: *Intraoperative arteriogram demonstrating both renal arteries, the aneurysm sac and neck and the 25 Fr Teflon introducer sheath within the aorta.*
Figure 6.4: Completion intra operative arteriogram after aortomonofemoral endovascular repair.
the ipsilateral internal and external iliac vessels occluded using a combination of coils and a covered Gianturco stent (Thompson et al. 1997b).

Data on each procedure was collected prospectively. The exact repair performed and incisions used for each patient were documented. In addition the total operative time, aortic occlusion time, limb ischaemia time and intra-operative blood loss and fluid replacement with crystalloids, colloids and blood were recorded. In the postoperative period in addition to routine observations, total IPPV ventilation time and ITU and Hospital stays were determined. Further cardiovascular and respiratory investigations performed in the perioperative period are described below.

6.5 Respiratory and cardiovascular assessments

Respiratory Tests

Pain scores at rest and on movement (defined as deep inspiration and coughing) were assessed using a 4-point scale, (0=no pain at rest or on movement, 1= pain on movement only, 2= pain at rest, worse on movement, 3= continuous pain at rest, worse on movement), by the responsible nurse at hourly intervals for the first 4h, at 2h intervals up to 24h, and at 4h intervals thereafter. PCA morphine was discontinued by the Acute Pain nurses according to standard clinical criteria.

Forced vital capacity (FVC) and forced expired volume in 1s (FEV₁) were measured using a Vitalograph® spirometer on the day before surgery and repeated on the third and fifth postoperative days. The best of 3 attempts was recorded on each occasion. Overnight arterial oxygen saturation (SpO₂) was recorded using an Edentrace® digital recorder model 3711 (EdenTec, MN, USA) and analysed automatically using the manufacturer’s software. In addition, recordings were checked visually for any obvious artifact, caused for example by patient disconnection. Accuracy is quoted at ± 2% at SpO₂>70% and ± 3% at SpO₂ 61-70%. Recordings were made between approximately 2200h and 0700h within 2 nights before surgery and on the 3rd and 5th postoperative nights. Values for average and minimum overnight SpO₂, number of episodes of desaturation per hour of recording (desaturation index, DI) and saturation-time curves were produced. A desaturation was defined as a reduction in SpO₂ of >4% for >10s,
according to the default settings of the analysis software, and in accordance with previous data (Reeder et al. 1992a). The average SpO2 was calculated as the mean SpO2 during the analysis time, after exclusion of artifact.

Oxygen therapy (4l min\(^{-1}\) by mask or 2l min\(^{-1}\) by nasal cannulae) was administered to all patients for 24h postoperatively. After conventional repair, patients received oxygen for 48-72h postoperatively, according to our current hospital practice. In addition, oxygen was administered to patients at the clinical discretion of the ward medical and nursing staff as appropriate. In all cases, the nursing staff were unaware of the purpose of the Edentrace recordings, and blinded to the values, so that the administration of oxygen was independent of overnight oximetry.

**Cardiovascular tests**

After induction of anaesthesia a thermodilution pulmonary artery flotation catheter was placed via the internal jugular route for continuous cardiac output (CCO) monitoring using the Vigilance\(^{\circledR}\) monitor (Baxter Healthcare Corporation, Irvine, CA, USA). The monitor was operated in ‘stat’ mode; such that measurements were updated every 54s. Measurements were made at 15-minute intervals throughout surgery but recorded specifically at standard times. These were: before commencement of surgery (time 1), within 5 minutes before (time 2), and 5 min after (time 3) aortic clamping (conventional group) or occlusion (endovascular group), before, 5 and 30 min after aortic unclamping or release (times 4, 5 and 6). Arterial blood samples for blood gas analysis and mixed venous blood samples for measurement of plasma catecholamine concentrations were also obtained at these times. The sample times were taken in relation to unclamping of the first limb with bifurcated endovascular or conventionally placed aortic grafts. Plasma was separated immediately by centrifugation, and stored at -70°C pending assay by reverse phase high-pressure liquid chromatography with electrochemical detection (Thompson et al. 1997). Inter and intra-assay coefficients of variation were 3.16% and 2.50% for noradrenaline, and 4.18% and 2.14% for adrenaline respectively with a lower limit of sensitivity of 0.15 pmol ml\(^{-1}\). Body temperature was measured from the tip of the PA catheter.
Intravenous fluids (crystalloid [Hartmanns solution and 0.9% saline] and colloid [3.5% gelatin]), were administered according to arterial and pulmonary capillary wedge pressures (PCWP). PCWP was maintained above 10mmHg throughout surgery. Blood (concentrated red cells, each ‘unit’ having a nominal volume of 240-270mls) was transfused when estimated blood loss (according to swab weight and suction losses) had exceeded 20% of calculated blood volume. Dopamine 3μg kg⁻¹ min⁻¹ was administered throughout surgery; glyceryl trinitrate 0.2 - 2.4 μg kg⁻¹ min⁻¹ was infused before aortic occlusion or clamping until just before release as guided by measured haemodynamic variables. No other inotropic or vasodilator drugs were used. At the end of surgery, patients were transferred to the Intensive Care Unit for weaning from artificial ventilation and further management. The intensive care management, time of weaning and discharge from ICU was at the discretion of intensivists who were not involved in the study.

6.6 Blood and plasma analyses

Sample Collection

Serial blood and urine specimens were collected on all patients at distinct time points during the peri-operative period. A peripheral venous blood sample was collected the day before surgery (1). At five further time points during surgery peripheral venous blood was collected directly from iliac or femoral veins and mixed venous blood obtained via a pulmonary artery catheter. The five time points were defined as (2) Start of procedure, (3) Just prior to clamping (conventional) or balloon inflation (endovascular), (4) Maximum ischaemia (just prior to clamp release or balloon deflation), (5) 5 minutes after reperfusion, (6) 30 minutes after reperfusion. In addition further venous samples were collected during the post operative period at (7) 6 hours and (8) 24 hours and on the (9) 2nd, (10) 3rd, (11) 4th and (12) 5th days. Blood samples were collected in sterile bottles containing ethylene diamine tetra-acetic acid (EDTA), as the anticoagulant, and immediately centrifuged at 6000rpm for ten minutes. Plasma was then snap frozen in liquid nitrogen and stored at -80°C for subsequent analysis. Further EDTA and lithium heparin samples were sent for immediate full blood count and clotting analysis respectively. Urine samples were obtained at all specified time points and were also snap
frozen in liquid nitrogen for subsequent analysis. The first urine sample was taken on bladder catheterisation in theatre, and therefore all urine results contain one less time point, with time point (1) corresponding to the start of the procedure (or time point (2) of the plasma assays). Thus leading to all further urine sample numbers corresponding to one number higher on the plasma assays.

Assays

ELISAs

The cytokines, interleukin-6 (IL-6), interleukin 1β (IL-1β) and tumour necrosis factor alpha (TNFα) were quantified by commercially available ELISAs (Genzyme Diagnostics, MA, USA). Similarly 11-dehydro Thromboxane B2 (Caymen Chemical Company, MI, USA) and sL-selectin (Immunotech, Marseille, France) concentrations were calculated by ELISAs on urine and plasma respectively. The techniques used are described in greater detail below.

The IL-6 assays were performed in concordance with the manufacturer’s protocols. Briefly, the Interleukin-6 enzyme immunoassay kit contains a 96-well microtiter pipette plate pre-coated with monoclonal antibody to IL-6. A measured volume of sample, standard or control was added to each test well and incubated to allow any IL-6 present to be bound by antibodies on the microtiter plate. The wells were then washed and a biotin labeled polyclonal antibody to IL-6 was added which binds to the captured IL-6. The wells were then washed and a peroxidase labeled avidin reagent was added which attached to biotin in the immune complex on the plate. The wells were washed and a substrate (peroxide) and chromagen (tetramethyl benzidine) were added producing a blue colour in the presence of peroxidase enzyme. The colour reaction was then stopped by the addition of acid, which changed the blue colour to yellow. The intensity of the yellow colour was in direct proportion to the amount of IL-6 present in the sample, standard or control. The absorbance of each well was read at 450 nm and a standard curve constructed to quantitate IL-6 concentrations in the controls and samples. All samples and controls were tested in duplicate. The interleukin 1β and tumour necrosis factor alpha ELISAs followed very similar protocols and are not described in further detail.
The ELISA for 11-dehydro-thromboxane B2 operated on the basis of competition between the enzyme conjugate and the 11-dehydro-thromboxane B2 in the sample for a limited number of binding sites on the antibody-coated plate. Briefly the sample or standard solution was added to the microplate. The diluted enzyme conjugate was then added and the mixture shaken and incubated at room temperature for one hour, during which time competition for binding sites is taking place. The plate was then washed to remove all unbound material. The bound enzyme conjugate was detected by the addition of K-Blue substrate, which generates an optimal colour after 30 minutes. Quantitative test results were obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards with a microplate spectrophotometer at 650 nm. The extent of the colour development was inversely proportional to the amount of 11-dehydro-thromboxane B2 in the sample or the standard. All samples and controls were tested in duplicate.

The plasma sL-selectin was measured by sandwich ELISA. The assay used monoclonal antibodies directed at two different epitopes of human sL-selectin. Samples or standards were added to the wells, which were coated with anti-human sL-selectin monoclonal antibody, MHL-1, and the incubated in the presence of biotin conjugated monoclonal antibody, MHL-2. The wells were then washed and streptavidin-peroxydase was added. After further washing the microwells were developed with O-phenylenediamine as a substrate. The optical density of each well was read at 492 nm using a microplate reader. The concentration of human sL-selectin was calculated from the dose response curve based on reference standards. All samples and controls were tested in duplicate.

N-acetyl glucosamidase assay

N-acetyl glucosamidase (NAG) was assayed using a fluoroscopic method based on that of Whiting (Whiting et al. 1979), which was adapted from that of Dance (Dance et al. 1969). The assay is based on the cleavage of 4-methylumbrelliferyone-2-acetamido-2-deoxy-b-D-glucosaminide (4-MU-NAG), a non-fluorescent compound to release 4-methylumbrelliferyone (4-MU), a fluorescent compound. 4-MU fluoresces and NAG is
inhibited at a pH of greater than 10 so the reaction is inhibited by the addition of 0.5M glycine buffer, pH 10.4. The absorbence is read spectrophotometrically at 360nm.

Initially a series of standards (0-145 nmol/ml) were prepared in triplicate by diluting aliquots (0-1ml) of the standard stock solution to 1 ml with water. To these 2 mls of 0.5 M glycine (pH 10.4, adjusted with 5M sodium hydroxide) were added. The 3 ml samples were then read spec spectrophotometrically at 360nm using a zero standard as the blank. The absorbencies were averaged, corrected for the blank and the standard curve drawn. The standard curve showed a good linear relationship to concentration ($R^2=0.9997$).

Samples were then analysed in duplicate and a blank prepared for each sample. Firstly 0.65ml of 20mmol trisodium citrate (pH 4.3, adjusted with phosphoric acid) was pipetted into a glass tube and 0.1ml of urine added. At the same time 2mls of 0.5 M glycine (pH 10.4, adjusted with 5M sodium hydroxide) were added to the blank tubes. Both tests and blanks were incubated for 10 minutes at 37 C and then 0.25ml of 4-methylumbrelliferyone-2-acetamido-2-deoxy-b-D-glucosaminide (30mg/l) was added. The tubes were then incubated for a further 30 minutes before the reaction was halted by the addition of 0.5M glycine (pH 10.4, adjusted with 5M sodium hydroxide). All tubes had a final volume of 3ml and the absorbence was then read at 360nm.

The concentration of the 4-MU released is proportional to the enzymes activity and therefore its concentration. The absorbencies of the two test tubes were averaged and the blank subtracted. The amount of 4-MU being subsequently determined from the standard curve. This gives a concentration of 4-MU released in 30 minutes of incubation by 0.1ml urine. The concentration was therefore multiplied by 20 to determine the amount of 4-MU released by 1ml of urine in 1 hour. To adjust for differing concentrations of urine this figure was then divided by the urinary creatinine concentration. The results are therefore expressed as mMol of NAG activity per mMol of creatinine per hour. The normal value was less than 40mmol/mmol(Whiting et al. 1979). Quality control was assessed by the use of control urine.
Catecholamine Assays

The following assays were performed in conjunction with the University Department of Anaesthesia, who had considerable experience with the technique. Stored plasma was thawed on ice with 0.75 ml required for each assay. Catecholamines were extracted as follows. Firstly the internal standard was added, 6 pmol -methyl-dopamine(Merck, Sharp & Dohme) in 10 l 0.1 M perchloric acid (Merck). The sample was then diluted with 0.75ml of distilled water before adding the antioxidant; 40 l of freshly prepared 5mM sodium bisulfite. The pH of the solution was then adjusted to at least 8.0 by the addition of 200 l 1M Tris (Sigma) buffer pH 8.6 with 2g Na₂EDTA per 100mls. 15 mg of acid washed alumina was then added and the mixture vigorously shaken for 15 minutes. The alumina was then washed three times with distilled water and the supernatant removed as completely as possible afterwards. Desorption of the catecholamines was then achieved with 50 l 0.1 M perchloric acid.

After swirling the tubes to ensure complete elution of catecholamines from the alumina, 20 l of the supernatant were injected into a 50 cm, 2mm i.d. glass column packed with strong cation exchange resin. The eluent was acetate-citrate buffer (pH 5.2) and a flow rate of 40-50ml/hour was used with the pressure kept at approximately 450 psi with the help of a resistance coil. The effluent was passed over a graphite detector electrode housed in a lucite block and +0.6 V was applied between this and the reference electrode. The potential was kept constant and the current flow was measured using an electronic controller (Bioanalytical Systems, IN, USA). The chromatogram was recorded on a Y-t-recorder.

The water used during all the above steps and preparing solutions was deionised and the double distilled. Before the last distillation 1g KMnO₄ was added and the pH raised to approximately 12 by addition of sodium hydroxide. The perchloric acid contained 0.4moles of sodium bisulfite. Conical 2.2ml tubes (Eppendorf) were used in the extraction procedure.

A full blood count and clotting analysis (fibrinogen and fibrinogen degradation products (FDPs) were determined using standard automated techniques on each sample by the Haematology Department of the Leicester Royal Infirmary. Urinary albumin/creatinine
Figure 6.5: Follow-up duplex scan 18 months after endovascular AAA repair with an EVT tube graft demonstrating that the aneurysm sac has almost disappeared.
ratios were determined using a standard automated technique by the Biochemistry Department of the Leicester Royal Infirmary

6.7 Postoperative imaging and follow-up

All patients who had successful endovascular AAA repair were entered into a surveillance program. They underwent physical examination in combination with duplex scanning, CT and plain radiography at 7 days, 3 months, 6 months, one year and then at yearly intervals. Patients who underwent successful conventional surgery were followed up once 6 weeks after discharge.

6.8 Summary

The patients and techniques described above form the basis for the investigations carried out in the subsequent experimental chapters.
CHAPTER SEVEN

Respiratory function after endovascular and conventional abdominal aortic aneurysm repair.

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7.1 Introduction

In order for any new surgical technique to be adopted proof of its superiority over the currently practiced procedure must be established. Conventional transperitoneal abdominal aortic aneurysm repair is associated with high morbidity and mortality rates; endovascular repair has been proposed a minimally invasive technique. In this and the subsequent two chapters we compare respiratory, cardiovascular and metabolic parameters in the comparative cohorts undergoing conventional and endovascular aneurysm repair described in chapter 6 to test the hypothesis that endovascular repair is a less invasive procedure.

Conventional abdominal aortic aneurysm (AAA) repair is a high-risk procedure, with substantial morbidity and mortality rates of up to 15% in some series(Campbell 1991). Cardiovascular and respiratory complications are common, because of the haemodynamic insults of aortic cross clamping and unclamping, large fluid losses and shifts, and the effects of a large abdominal incision in a patient group with a high prevalence of cardiovascular and respiratory disease(Hertzer 1987). Perioperative nocturnal hypoxaemia is common in vascular surgical patients(Reeder et al. 1992a), persists for several nights after surgery(Reeder et al. 1992b; Rosenberg et al. 1994a) and may be associated with myocardial ischaemia(Reeder et al. 1991). This is related to impairment of control of ventilation, upper airway patency and gas exchange caused by the effects of opioids, sleep disturbance, atelectasis and changes in FRC after surgery(Craig 1981; Catley et al. 1985; Knill et al. 1990; Rosenberg et al. 1994b). Endovascular repair of abdominal aortic aneurysms is a new technique, with several potential advantages over open repair(Parodi et al. 1991). It has been suggested that the avoidance of a laparotomy incision might have less effect on lung volumes and cause less postoperative hypoxaemia, with lower analgesic requirements compared with conventional surgery(Thompson et al. 1995); this might be advantageous in medically unfit patients. However, there are no data to support these suggestions. We have therefore measured respiratory function, nocturnal oxygen saturation and analgesic
requirements in comparative cohorts undergoing conventional and endovascular AAA repair.

7.2 Methods

Patients
The local Ethics Research Committee approved the study design. After obtaining informed consent we studied 43 patients undergoing infrarenal aortic aneurysm repair. Suitability for endovascular repair was assessed by a combination of 3-dimensional spiral computed tomography, magnetic resonance imaging and conventional arteriography according to standard criteria (Thompson et al. 1995). Those with suitable aneurysms were offered endovascular repair; consecutive patients with aneurysm morphology unsuitable for endovascular repair were offered conventional surgery.

Conventional aneurysm repair was performed using a transperitoneal approach and midline incision by the same two surgeons (MT and PB). Endovascular repair was performed using an aorto-mono-iliac (Thompson et al. 1997a) or aorto-femoral tapered endovascular graft with contralateral iliac occlusion and femoro-femoral crossover graft or commercially available devices (EVT, Menlo Park, CA, USA; Bard, New Jersey, USA; Mintec, Freeport, Bahamas).

Anaesthetic
All patients received a standard general anaesthetic administered by the same anaesthetist (JT or GS). Details of the anaesthetic technique are described in chapter 6.

Analgesia
Pain scores at rest and on movement (defined as deep inspiration and coughing) were assessed using a 4-point scale (0 = no pain at rest or on movement, 1 = pain on movement only, 2 = pain at rest, worse on movement, 3 = continuous pain at rest, worse on movement) by the responsible nurse at hourly intervals for the first 4h, at 2h intervals up to 24h, and at 4h intervals thereafter. PCA was discontinued by the Acute Pain nurses according to standard clinical criteria.
**Pulmonary Function**

Forced vital capacity (FVC) and forced expired volume in 1s (FEV₁) were measured using a Vitalograph® spirometer on the day before surgery and repeated on the third and fifth postoperative days. The same investigator (JB) made all measurements and the best of 3 attempts was recorded. Overnight arterial oxygen saturation (SpO₂) was recorded using an Edentrace® digital recorder model 3711 (EdenTec, MN, USA) and analysed automatically using the manufacturer's software. In addition, recordings were checked visually for any obvious artifact, caused for example by patient disconnection. Accuracy is quoted at ± 2% at SpO₂>70% and ± 3% at SpO₂ 61-70%. Recordings were made between approximately 2200h and 0700h within 2 nights before surgery and on the 3rd and 5th postoperative nights. Values for average and minimum overnight SpO₂, number of episodes of desaturation per hour of recording (desaturation index, DI) and saturation-time curves were produced. A desaturation was defined as a reduction in SpO₂ of >4% for >10s, according to the default settings of the analysis software, and in accordance with previous data (Reeder et al. 1992a). The average SpO₂ was calculated as the mean SpO₂ during the analysis time, after exclusion of artifact.

Oxygen therapy (4l min⁻¹ by mask or 2l min⁻¹ by nasal cannulae) was administered to all patients for 24h postoperatively. After conventional repair, patients received oxygen for 48-72h postoperatively, according to our current hospital practice. In addition, oxygen was administered to patients at the clinical discretion of the ward medical and nursing staff as appropriate. In all cases, the nursing staff were unaware of the purpose of the Edentrace recordings, and blinded to the values, so that the administration of oxygen was independent of overnight oximetry.

**Statistical Analysis**

Data were analyzed by the Kolmogorov-Smirnov goodness of fit test. Spirometry results were found to be normally distributed and are presented as mean (SEM). These data were analysed by multivariate analysis of variance (manova) for repeated measures, and paired and unpaired t-tests for within and between group comparisons as appropriate. Overnight pulse oximetry data (minimum SpO₂ and DI), pain scores and morphine
requirements were not-normally distributed and are presented as median (range). These data were analyzed by Friedman analysis of variance for repeated measures and Wilcoxon signed rank matched pairs tests for within-group comparisons, and Mann-Whitney tests for comparisons between groups. Values of $S_{50}$ for overnight SpO$_2$ curves (i.e. the value of SpO$_2$ which was exceeded for 50% of the analysis time) were computed by non-linear regression using a sigmoidal dose response curve with variable slope. A convergent fit was found for all data sets. Dose response curves and $S_{50}$ values were compared by Friedman two way analysis of variance and Wilcoxon signed rank matched pairs tests. Spearman correlation coefficients were calculated for comparison of spirometric, morphine consumption and oximetry data.

7.3 Results

Demographics

Spirometric values, morphine doses and pain scores were calculated for the entire cohort of 43 patients. Complete pulse oximetry data was only available on a sub-group of 20 patients. Despite this the demographics of the entire group and the sub-group only differed slightly. The co-morbidities for the entire group are shown in Table 7.1, which demonstrates that the endovascular and conventional patients were well matched.

The conventional patients were younger, median 67 years, than those undergoing endovascular surgery, median 72 years, although this difference was not significant, (Mann-Whitney, $W=547.0$, CI (-2.0,6.0), $p = 0.3226$). The endovascular procedures took significantly longer than the conventional operations, median times 175 minutes versus 124 minutes respectively ($W= 282.0$, CI (-78.01,-27.99) $p< 0.0001$). Aortic occlusion time (clamp time for conventional operations, balloon inflation time for endovascular procedures) was significantly lower in the endovascular group, median 15 versus 48 minutes ($W=609.0$, CI (21.0,39.0) $p< 0.0001$). The blood loss was significantly greater in the endovascular group, median 2500 mls versus 1500mls for the conventional patients ($W=286$, CI (-2000.2,-499.9) $p< 0.002$). The median hospital stays were also significantly lower after endovascular surgery, median 8 versus 14 days ($W=598.5$, CI (3.0,7.999) $p< 0.0001$).
### Table 7.1: Patient Co-morbidity, absolute values and percentages in parentheses.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Endovascular Repair (N=23)</th>
<th>Conventional Repair (N=20)</th>
<th>Chi-square value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>10 (43%)</td>
<td>12 (60%)</td>
<td>1.169</td>
</tr>
<tr>
<td>Ischaemic Heart Disease</td>
<td>15 (65%)</td>
<td>10 (50%)</td>
<td>1.018</td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td>11 (48%)</td>
<td>7 (35%)</td>
<td>0.723</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>0</td>
<td>3 (15%)</td>
<td>3.709</td>
</tr>
<tr>
<td>Cerebral Vascular Disease</td>
<td>3 (13%)</td>
<td>7 (35%)</td>
<td>2.89</td>
</tr>
<tr>
<td>Renal Failure (Dialysis)</td>
<td>1 (4%)</td>
<td>0</td>
<td>0.89</td>
</tr>
<tr>
<td>COPD</td>
<td>4 (17%)</td>
<td>2 (10%)</td>
<td>0.487</td>
</tr>
<tr>
<td>Current/Ex smokers</td>
<td>21 (91%)</td>
<td>20 (100%)</td>
<td>1.824</td>
</tr>
</tbody>
</table>

**Spirometry**

Pre-operative spirometry values were similar in each group. There was a reduction in FEV₁ and FVC at day 3 postoperatively in both groups (p<0.005), which was significantly greater in the conventional group (p<0.05 for FEV₁ and FVC). In the endovascular group, FEV₁ and FVC had returned to approximately 87% and 85% of
Table 7.2: Operative details presented as medians and complete ranges and *medians and inter quartile ranges.

preoperative baseline by the 5th postoperative day respectively, although these remained significantly lower than preoperative values (p<0.05). However, FEV₁ and FVC in the conventional group were less than 66% of preoperative values on day 5 and were decreased in comparison to the endovascular group (p<0.005).

Table 7.3: Mean (SEM) spirometry data. † p < 0.005 compared with preoperative values. p < 0.05 compared with endovascular group.
Figure 7.1 Mean (SEM) forced expiratory volume in one second for both groups preoperatively and on the third and fifth postoperative days.
Figure 7.2: Mean (SEM) forced vital capacity for both groups preoperatively and on the third and fifth postoperative days.
**Morphine consumption and pain scores**

Pain scores at rest and upon movement were compared using calculated AUC for the first 24h after initiation of PCA and found to be similar in both groups (Table 7.4).

<table>
<thead>
<tr>
<th></th>
<th>PCA Morphine (mg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endovascular</td>
<td>Conventional</td>
</tr>
<tr>
<td>12 hours</td>
<td>11 (0-39)</td>
<td>30* (18-47)</td>
</tr>
<tr>
<td>24 hours</td>
<td>22 (0-63)</td>
<td>48* (32-120)</td>
</tr>
<tr>
<td>48 hours</td>
<td>35 (0-118)</td>
<td>92* (38-218)</td>
</tr>
<tr>
<td>72 hours</td>
<td>35 (0-189)</td>
<td>112* (38-286)</td>
</tr>
<tr>
<td>Total</td>
<td>41 (0-215)</td>
<td>128* (37-323)</td>
</tr>
<tr>
<td>PCA time (hours)</td>
<td>41 (0-86)</td>
<td>93** (36-110)</td>
</tr>
<tr>
<td>AUC pain scores after 24h</td>
<td>13 (0-25)</td>
<td>13 (0-38)</td>
</tr>
</tbody>
</table>

* Table 7.4: Median (range) cumulative morphine consumption, duration of PCA and 24 hour AUC pain scores. * p < 0.005, ** p < 0.001 Mann Whitney test.

The endovascular group used significantly less morphine via the PCA device both overall and in all the defined time periods demonstrated in Table 7.3, (p<0.005, as a result of multiple comparisons within this group significance was deemed to be achieved at the 1 in 200 level) and required the device for a significantly shorter duration (p<0.005).

**Pulse oximetry**

Pulse oximetry data were incomplete in 10 of the 90 recording nights, because of technical difficulties (probe displacement during the recording period), prolonged artificial ventilation or administration of oxygen after the 1st night following endovascular repair or the 3rd night after conventional repair. One patient was given an epidural infusion in the intensive care unit and was therefore excluded. In order to maximize within-subject comparisons, complete data sets only (data from 11 endovascular and 9 conventional patients) were included for analysis.
<table>
<thead>
<tr>
<th></th>
<th>Preop</th>
<th>3rd night</th>
<th>5th night</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean overnight SpO₂ (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>94 (90-98)</td>
<td>97 (91-98)</td>
<td>92 (87-94)</td>
<td>ns</td>
</tr>
<tr>
<td>Endovascular</td>
<td>94 (93-97)</td>
<td>93 (89-98)</td>
<td>94 (89-98)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Desaturation index (no. h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>3 (1-36)</td>
<td>3.5 (0-33)</td>
<td>17* (8-35)</td>
<td>*p&lt; 0.05 within-group</td>
</tr>
<tr>
<td>Endovascular</td>
<td>5 (0-27)</td>
<td>4 (1-31)</td>
<td>9* (1-25)</td>
<td>within-group ns *p&lt;0.05 between groups night 5</td>
</tr>
<tr>
<td><strong>Minimum SpO₂ (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>78 (64-87)</td>
<td>69.5* (60-87)</td>
<td>70 (60-79)</td>
<td>*p&lt;0.01 within-group</td>
</tr>
<tr>
<td>Endovascular</td>
<td>81 (68-85)</td>
<td>78 (60-80)</td>
<td>79* (68-85)</td>
<td>ns within-group *p&lt;0.005 between groups night 5</td>
</tr>
</tbody>
</table>

Table 7.5. Median (range) pulse oximetry results. Conventional group was receiving oxygen on the 3rd postoperative night. Within-group comparisons made by Friedman two-way analysis of variance, between group comparisons by Mann Whitney tests.

Average SpO₂, minimum overnight SpO₂, and desaturation index are presented in Table 7.5. Oxygen saturation-time curves are displayed in Figures 7.3 and 7.4 for the endovascular and conventional groups respectively. The conventional group were receiving oxygen on the third night and therefore no between-group comparisons have been made for night 3. Preoperative values were similar in both groups (p=0.29, Mann Whitney u test). Movement of the curve to the left represents a deterioration in
oxygenation. In the endovascular group there was a leftward shift on nights 3 and 5 compared with preoperative values, (p<0.05). In the conventional group, there was a significant shift to the right on the third postoperative night, when all these patients were receiving oxygen (p<0.005). On the 5th postoperative night, the curve was shifted significantly to the left compared with preoperative values (p<0.01). Absolute within-group differences were greater for the conventional group, but between-group comparisons at night 5 showed no significant differences (p=0.36).

Desaturation index (DI) and minimum SpO$_2$ were similar in both groups preoperatively. DI increased significantly at night 5 (p<0.05) in the conventional group and was significantly greater at night 5 compared with the endovascular group. Minimum SpO$_2$ was significantly lower at night 5 in the conventional group compared with preoperatively (p<0.01) and compared with the endovascular group on night 5 (p<0.005). There were no correlation between morphine consumption, DI, minimum or average overnight SpO$_2$, or spirometry in either group. There was a correlation between preoperative DI and BMI ($r=0.63$, p<0.05).
Figure 7.3: Overnight Saturation-time curves: Endovascular group

Graph of mean (±SEM) overnight oxygen saturation on the preoperative (■), 3rd (▲) and 5th (▼) postoperative nights vs % of analysis time. Movement of curve to the left represents an increased % analysis time at lower mean SpO₂ i.e. deterioration in oxygen saturation.
Figure 7.4: Overnight Saturation-time curves: Conventional group
Graph of mean (±SEM) overnight oxygen saturation on the preoperative (■), 3rd (▲) and 5th (▼) postoperative nights vs % of analysis time. Movement of curve to the left represents an increased % analysis time at lower mean SpO₂ i.e. deterioration in oxygen saturation.
7.4 Discussion

In this chapter it has been demonstrated that respiratory function as assessed by spirometry was impaired less after endovascular repair of AAA than conventional surgery, and function returned to near normal by the 5th postoperative day. There was significantly less morphine consumption by PCA, and a shorter duration of PCA usage. Pain scores were similar in both groups for the first 24h of PCA usage, suggesting patients were titrating morphine consumption to pain. Pain scores were not formally assessed after discontinuation of PCA but all patients had registered pain scores of 0 consistently for period of 4-8h before discontinuation. No comparisons of AUC pain scores were made after the first 24h as they are related to duration of usage.

The study has also documented the occurrence of episodic nocturnal hypoxaemia in both groups of patients, pre- and postoperatively. Mean SpO₂, minimum recorded SpO₂, and frequency of episodes of desaturation did not change significantly in the endovascular group, but the frequency and magnitude of episodic desaturation was greater in patients undergoing conventional AAA repair at the fifth postoperative night (Table 7.4). Analysis of oximetry-time curves showed a significant left shift in overnight SpO₂ (i.e. each individual patient had a decreased overnight SpO₂ compared with the preoperative night) in both groups, persisting on the 5th postoperative night. The absolute shift was less for the endovascular than for the conventional group.

Upper abdominal surgery is associated with a characteristic reduction in lung volumes, with a decrease in FEV₁, FVC, VC and FRC, which may lead to postoperative atelectasis and hypoxaemia(Craig 1981; Wahba 1991). These changes are greater following upper rather than lower abdominal surgery, and persist for several days(Craig 1981; Dureuil et al. 1987). They have been related to the site of incision, pain, and diaphragmatic dysfunction(Dureuil et al. 1987; Putensen-Himmer et al. 1992; Wahba et al. 1995). Our findings of a postoperative decrease of FEV₁ and FVC after conventional AAA repair to approximately 45% of preoperative values at day 3 and 66% at day 5 are consistent with previous findings related to upper abdominal surgery(Craig 1981; Dureuil et al. 1987). FEV₁ and FVC after endovascular repair decreased to approximately 65%
and 85% of pre-operative values at days 3 and 5 respectively. This reduction is greater than that reported after inguinal hernia repair [18] and suprapubic prostatectomy or abdominal hysterectomy (Dureuil et al. 1987). However, in addition to one or two ipsilateral or bilateral groin incisions, endovascular AAA repair using a tapered aorto-mono-iliac device involves retroperitoneal dissection; the duration of surgery may be longer, and the previous studies used a different patient population and anaesthetic techniques.

Pain scores were similar in the two groups, and it seems likely that the difference in postoperative FEV₁ and FVC between endovascular and conventional groups was related to the site and extent of surgical incision and trauma.

Reeder and colleagues (Reeder et al. 1992a) documented the occurrence of late postoperative hypoxaemia in patients undergoing aortic reconstruction, and demonstrated that hypoxaemia can occur in patients receiving oxygen therapy. They used a fall in SpO₂ of greater than 4% with a prompt recovery toward baseline as the definition of a significant abnormality of respiration (SAR). All patients who experienced more than 5 SARs per hour preoperatively had significant episodes of hypoxaemia up to the 5th night postoperatively. However, the majority of episodes of postoperative hypoxaemia occurred in patients without preoperative abnormalities. Other workers have related hypoxaemia to opioid usage (Catley et al. 1985), and a rebound increase in REM sleep on the 3rd or 4th postoperative nights (Knill et al. 1990; Rosenberg et al. 1994b). Rosenberg and colleagues found a peak incidence of episodic hypoxaemia on the third night after abdominal surgery, and were able to relate preoperative overnight mean oxygen saturation with postoperative constant and episodic hypoxaemia in patients not given oxygen (Rosenberg et al. 1994a). The degree of constant hypoxaemia was lowest on the 2nd postoperative night.

We found no relationship between preoperative mean or minimum overnight SpO₂ and postoperative values, or between preoperative spirometry and postoperative hypoxaemia. Preoperative desaturation index was however related significantly to postoperative values in the conventional group. This apparent discrepancy may be due to the multifactorial etiology of postoperative hypoxaemia where constant hypoxaemia may be related to increased intrapulmonary shunt as a consequence of a reduction in FRC and
atelectasis and episodic hypoxaemia related to opioids, obesity or REM sleep (Jones et al. 1990; Rosenberg et al. 1994a). All patients in our study undergoing conventional surgery received oxygen for 3 nights postoperatively. Although this was associated with an increase in mean \( \text{SpO}_2 \), episodic hypoxaemia still occurred. We believe that the overnight mean and minimum \( \text{SpO}_2 \) would have been lower had oxygen not been administered, but it would be unacceptable to withhold oxygen in such circumstances. Rosenberg & colleagues found a similar increase in mean \( \text{SpO}_2 \) but no change in episodic hypoxaemia in patients given oxygen following hip replacement (Rosenberg et al. 1992). Episodic hypoxaemia may be related temporally to myocardial ischaemia (Rosenberg et al. 1990; Reeder et al. 1991; Gill et al. 1992) but both are commonly asymptomatic and undetected by observers in the perioperative period (Muir et al. 1991), and prevention or early detection is desirable. In contrast to Rosenberg (Rosenberg et al. 1994) who related opioid dose on the 2\textsuperscript{nd} postoperative day to episodes of hypoxaemia on the 2\textsuperscript{nd} postoperative night after abdominal surgery, we found no relation between morphine consumption by PCA and postoperative hypoxaemia, possibly because of the wide variation in PCA morphine consumption. Wheatley & colleagues (Wheatley et al. 1992) found no difference in the incidence of postoperative hypoxaemia in patients using PCA or intermittent i.m. morphine following upper abdominal surgery. The peak consumption of morphine was during the first 48-72h after surgery, during which all conventional patients were receiving oxygen, which will have alleviated constant hypoxaemia. Morphine consumption declined thereafter, and by the 5\textsuperscript{th} postoperative night, was minimal. Episodic hypoxaemia is related to factors other than opioid consumption and it is of note that there was a correlation between body mass index and preoperative DI.

In this study there were no differences in duration of artificial ventilation, and ICU stay between groups. This probably reflected ICU practice in our hospital, where weaning from artificial ventilation and discharge to the ward are less common out of 'daylight hours', and are therefore not dependent solely on patients' physiological status. These data are based on relatively early experience (surgical, anesthetic and ICU) of endovascular AAA repair in our unit, and may reflect this. Similarly the factors influencing hospital discharge include individual home circumstances in addition to the patients physical condition, although duration of stay was shorter in the endovascular
group. Duration of surgery, ICU and in-hospital stay in the endovascular group were all similar to previous reports (Baker 1997). The incidence of mortality and major morbidity after endovascular AAA repair in a high risk population was 8% and 37% respectively, predominantly from cardiovascular or respiratory complications (Baker 1997). Postoperative cardiovascular and respiratory morbidity occurred in both groups in our study, although in a study of this size it is not possible to draw conclusions regarding incidence or make comparisons between techniques.

We recognise some weaknesses in this study. Patients were allocated to group according to individual aneurysm morphology, and therefore not randomised; patients unsuitable for endovascular repair were offered conventional surgery. At the start of the study, the technique of endovascular repair was in an early stage of development with unknown morbidity and mortality, and it was not felt justified to allocate patients to a surgical treatment in a randomised manner. However, there were no preoperative differences in patient characteristics, spirometry or oximetry between the two groups, and we have no reason to suspect the two groups were physiologically different. In the UK the EVAR (EndoVascular Aneurysm Repair) randomised study has recently started recruiting and may provide data on pulmonary morbidity in due course.

During the course of the study, a change in clinical practice resulted in the administration of oxygen for several days to some patients by the ward staff, predominantly after conventional AAA repair. This was outside our control and was based on separate, intermittent pulse oximetry readings not related to the Edentrace recordings. Routine practice had been to administer oxygen for the first 48-72h after conventional surgery and 24h after endovascular repair, but prolongation of this period by the ward medical and nursing staff introduced a large confounding variable, and it was clearly not justifiable to withhold oxygen on this basis. We therefore analysed data from patients who had received oxygen for three nights after conventional repair, and for up to two nights after endovascular repair. We excluded patients in whom data were incomplete, to maximize the use of within-subject comparisons. With large between-subject variations in preoperative values (e.g. DI range 0-36 episodes per hour), and small groups, this was felt to be reasonable. We have not drawn any conclusions based on oximetry data from night 3 (as the conventional group received oxygen and the
endovascular group did not). One patient in the endovascular group was receiving artificial ventilation on night 3, and one patient was given epidural analgesia because of pulmonary atelectasis and poor expectoration; these patients were also excluded.

Despite a decrease in the severity of pulmonary dysfunction postoperatively, there were still significant deteriorations in spirometric measurements, and episodic nocturnal hypoxaemia in individual endovascular patients (Table 7.4). The minimum FEV₁ and FVC in the endovascular group were 50% and 54% of preoperative values at day 3 and 67% and 70% at day 5 respectively. Individual overnight mean and minimum SpO₂ were as low as 89% and 68% respectively in the endovascular group on the 5th postoperative night. In contrast to previous data relating episodic nocturnal hypoxaemia to opioid use, no clear correlation with morphine consumption has been demonstrated.

Endovascular repair was associated with less pain than conventional AAA surgery, as demonstrated by decreased morphine consumption to produce equivalent subjective pain scores. Duration of artificial ventilation and ICU stay did not differ significantly, and although hospital stay was shorter in the endovascular group, the longest individual ICU and hospital stay were for an endovascular patient. Individual overnight mean and minimum SpO₂ were as low as 89% and 68% respectively in the endovascular group on the 5th postoperative night. It is clear that endovascular AAA repair is not a benign procedure in this high risk patient population and should not be regarded as such. Further studies are warranted to define those patients at most risk of episodic hypoxaemia, and to establish the place of endovascular repair in the management of AAA in both low and high risk patients.

7.5 Summary

The data described in this chapter have demonstrated that endovascular AAA repair attenuates the respiratory dysfunction associated with the conventional transperitoneal approach. However endovascular surgery was still associated with significant reductions in postoperative spirometric values and overnight oxygen saturation. This in part may reflect the high percentage of aorto-uni-iliac procedures in this group, as this technique requires a femoro-femoral crossover graft and possibly a
retroperitoneal iliac fossa approach to the common iliac artery on one side. This operation is therefore more invasive than those involved in deploying the commercially available devices.

Although the patients in this study were unrandomised the groups were well matched and there was nothing to suggest that they were physiologically different. The rapid advances in endovascular technology has inevitably lead to smaller more user friendly devices that can be deployed in radiology suites rather than operating theatres and that may soon be deployed under local anaesthetic. It is therefore unlikely that further investigation comparing respiratory function in two such tightly controlled groups of patients will become available without a randomised controlled trial.

These data have demonstrated that endovascular AAA repair is associated with significantly less pulmonary dysfunction than conventional surgery. The improved spirometric values should translate to clinically improved gas exchange and functional residual capacity. This superior pulmonary function may reduce the incidence of respiratory morbidity and mortality associated with the transperitoneal approach.
CHAPTER EIGHT

Cardiovascular and catecholamine responses during endovascular and conventional abdominal aortic aneurysm repair.

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8.1 Introduction

The data presented in chapter 7 have demonstrated that endovascular AAA repair has significant benefits in terms of respiratory function when compared to conventional surgery. Although respiratory complications are common after transperitoneal repair, the incidence cardiac morbidity and mortality remains the greatest concern. Endovascular repair of abdominal aortic aneurysms (Parodi et al. 1991) has several potential advantages compared with conventional surgery. In particular from a cardiac perspective, aortic cross clamping is avoided and the duration of aortic occlusion is shorter. In addition, endovascular AAA repair is performed via one or two unilateral or bilateral groin incisions, so that surgical and tissue trauma should be less compared with conventional surgical approach.

Aortic cross-clamping may be associated with profound haemodynamic and metabolic changes (Gelman 1995), which may cause myocardial ischaemia in susceptible patients (Attia et al. 1976; Gooding et al. 1980). These changes include a decrease in cardiac index (CI), increases in afterload and systemic vascular resistance (SVR), and variable effects on preload, central venous (CVP) and pulmonary capillary wedge pressures (PCWP) (Gelman 1995). An increase in PCWP during cross clamping has been shown to be associated with myocardial ischaemia (Attia et al. 1976). Aortic clamp release is associated with a decrease in cardiac index and SVR, and a metabolic response, which includes lactic acidemia, and release of oxygen free radicals and cytokines (Andersson et al. 1979; Gelman 1995; Baxendale et al. 1996; Thompson et al. 1996a). Plasma catecholamine concentrations increase during conventional surgery (Normann et al. 1983; Derbyshire and Smith 1984; Gold et al. 1994) and may contribute to the cardiovascular changes (Derbyshire and Smith 1984; Gelman 1995). There are no data at present describing the changes in plasma catecholamine concentrations during endovascular AAA repair.

One previous investigation has suggested that changes in cardiac output in patients undergoing endovascular AAA repair with a bifurcated graft are less in comparison with those when conventional surgery is performed (Baxendale et al. 1996).
However, patients in Baxendale's study were predominantly healthy, 50% having no other evidence of cardiovascular disease. The overall incidence of risk factors such as ischaemic heart disease, hypertension, or peripheral vascular disease in patients presenting for AAA repair is approximately 65% (Hertzer 1987) which is consistent with our own experience (Sayers et al. 1997). The data presented in this chapter compares prospectively the changes in cardiovascular dynamics, acid-base status and plasma catecholamine concentrations in a comparative cohorts undergoing endovascular or conventional AAA repair.

### 8.2 Methods

**Patients**

After obtaining informed consent and local Ethics Research Committee approval we studied 30 patients presenting for infrarenal aortic aneurysm repair. These patients were a subgroup of the 43 presented in the previous and subsequent chapter. Aneurysm size, morphology and suitability for endovascular repair was assessed by a combination of computed axial tomography, magnetic resonance imaging and conventional arteriography using a marker catheter (Cordis, UK) according to standard criteria (Thompson et al. 1997b). Those with suitable aneurysms underwent endovascular repair; consecutive patients with aneurysm morphology unsuitable for endovascular repair underwent conventional surgery. Preoperative cardiac function was evaluated by clinical history, physical examination and radioisotope (MUGA) scanning. The diagnosis of ischaemic heart disease was based on a history of angina, previous MI, or signs of cardiac failure, and peripheral vascular disease on a history of intermittent claudication. Chronic respiratory disease was defined as a clinical history of chronic obstructive pulmonary disease or asthma.

Conventional aneurysm repair was performed using a transperitoneal approach and midline incision. Endovascular repair was performed using an aorto-mono-iliac or aorto-femoral tapered endovascular graft with contralateral iliac occlusion and femoro-femoral crossover graft (Thompson et al. 1997b), or commercially available devices (EVT, Menlo Park, CA, USA; Mintec, Freeport, Bahamas). In the aorto-mono-iliac
system, intra-aortic balloon inflation was achieved using an automated manometric syringe to a pressure of 2 atmospheres for 1 minute. The balloon was then left inflated at 1 atmosphere until all distal anastomoses were completed.

Anaesthetic, Sample collection and Assays

All patients received a standard general anaesthetic as described in chapter 6. A radial artery catheter for arterial pressure monitoring was inserted under local anaesthesia prior to induction of general anaesthesia.

After induction of anaesthesia a thermodilution pulmonary artery flotation catheter was placed via the internal jugular route for continuous cardiac output (CCO) monitoring using the Vigilance® monitor (Baxter Healthcare Corporation, Irvine, CA, USA). The monitor was operated in ‘stat’ mode; such that measurements were updated every 54s. Measurements were made at 15-minute intervals throughout surgery but recorded specifically at standard times. These were, before commencement of surgery (time 1), within 5 minutes before (time 2), and 5 min after (time 3) aortic clamping (conventional group) or occlusion (endovascular group), before, 5 and 30 min after aortic unclamping or release (times 4, 5 and 6). Arterial blood samples for blood gas analysis and mixed venous blood samples for measurement of plasma catecholamine concentrations were also obtained at these times. The sample times were taken in relation to unclamping of the first limb with bifurcated endovascular or conventionally placed aortic grafts. Plasma was separated immediately by centrifugation, and stored at -70°C pending assay by reverse phase high-pressure liquid chromatography with electrochemical detection (Thompson et al. 1997). Inter and intra-assay coefficients of variation were 3.16% and 2.50% for noradrenaline, and 4.18% and 2.14% for epinephrine respectively with a lower limit of sensitivity of 0.15 pmol ml⁻¹. Body temperature was measured from the tip of the PA catheter.

Intravenous fluids (crystalloid [Hartmanns solution and 0.9% saline] and colloid [3.5% gelatin]), were administered according to arterial and pulmonary capillary wedge pressures (PCWP). PCWP was maintained above 10mmHg throughout surgery. Blood (concentrated red cells, each ‘unit’ having a nominal volume of 240-270mls) was transfused when estimated blood loss (according to swab weight and suction losses) had
exceeded 20% of calculated blood volume. Dopamine $3 \mu g \text{ kg}^{-1} \text{ min}^{-1}$ was administered throughout surgery; glyceryl trinitrate $0.2 - 2.4 \mu g \text{ kg}^{-1} \text{ min}^{-1}$ was infused before aortic occlusion or clamping until just before release as guided by measured haemodynamic variables. No other inotropic or vasodilator drugs were used.

**Statistical Analysis**

Data were analyzed using the Kolmogorov-Smirnov goodness of fit test and found to be distributed normally. Unpaired t tests were used to compare patient characteristics, intraoperative and ICU data. Cardiovascular and catecholamine data were analyzed by general linear model multivariate analysis of variance for repeated measures using polynomial contrast, with time and type of surgery as within- and between-group factors, using the computer software SPSS for Windows 95 (version 7.0. 1995). Mauchly’s test of sphericity was applied and when significant, F values were adjusted by Greenhouse-Geisser epsilon values. Post hoc paired and unpaired t tests were performed where appropriate.

**8.3 Results**

**Demographics**

All patients in the endovascular group and 14 patients in the conventional group were male. There were no differences in age, weight, body mass index, pre-operative ASA status, incidence of co-existing disease or aneurysm diameter (Tables 8.1 and 8.2). Mean (SD) preoperative left ventricular ejection fraction as estimated by MUGA scanning was 61% (11.4) in the endovascular group and 58% (16.9) in the conventional group. 10 conventional tube and 5 bifurcated grafts were performed. In the endovascular group 12 aorto-femoral or aorto-iliac and 3 bifurcated grafts (2 Mintec and 1 EVT) were deployed.
<table>
<thead>
<tr>
<th></th>
<th>Endovascular</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>67.9 (5.9)</td>
<td>67.6 (7.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.2 (12.5)</td>
<td>84.1 (12.4)</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>26.0 (2.6)</td>
<td>27.5 (4.5)</td>
</tr>
<tr>
<td>ASA grade 2/3/4 (no.)</td>
<td>8/6/1</td>
<td>7/7/1</td>
</tr>
<tr>
<td>Aneurysm diameter (cm)</td>
<td>5.6 (0.7)</td>
<td>6.2 (1.3)</td>
</tr>
<tr>
<td>Intraoperative morphine (mg)</td>
<td>16.5 (4.7)</td>
<td>17.7 (3.3)</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>182 (59)**</td>
<td>126 (35)</td>
</tr>
<tr>
<td>Estimated blood loss (ml)</td>
<td>2513 (1268)*</td>
<td>1630 (819)</td>
</tr>
<tr>
<td>Aortic occlusion time (min)</td>
<td>17.4 (16.8)**</td>
<td>48.5 (16.9)</td>
</tr>
<tr>
<td>Crystalloids infused (ml)</td>
<td>2701 (1027)</td>
<td>2566 (530)</td>
</tr>
<tr>
<td>Colloids infused (ml)</td>
<td>1334 (858)</td>
<td>1766 (623)</td>
</tr>
<tr>
<td>Blood transfused (units)</td>
<td>4.6 (2.7)*</td>
<td>2.9 (1.5)</td>
</tr>
<tr>
<td>Duration of ICU ventilation (h)</td>
<td>12.2 (8.7)</td>
<td>19.3 (16.8)</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>8.8 (5.1)**</td>
<td>15.2 (4.9)</td>
</tr>
</tbody>
</table>

Table 8.1: Mean (SD) Patient characteristics. *p<0.05 compared with conventional group, **p<0.005 compared with conventional group.

The dose of morphine administered during surgery was similar in both groups. Duration of surgery was longer for endovascular repair (p<0.005). Intraoperative blood loss and blood transfused were greater in the endovascular group (both p<0.05), but there were no differences in crystalloid or colloid transfused. Haematocrit (Table 8.3) was slightly higher at the end of surgery in the conventional group but this was not statistically significant (p=0.26). Duration of postoperative artificial ventilation and ICU stay were similar but total hospital stay was significantly shorter for the endovascular group (p<0.005, Table 8.1).
Table 8.2: Co-existing Disease, absolute values with percentages in parentheses.

### Haemodynamic parameters

There were no differences in preoperative values for mean arterial pressure (MAP), heart rate (HR), cardiac index (CI), central venous pressure (CVP), pulmonary capillary wedge pressure (PCWP) or systemic vascular resistance index (SVRI) (Table 8.4). CI increased and MAP decreased during conventional surgery (both \( p<0.05 \)), but between-group differences were not statistically significant. Changes in MAP and CI in the endovascular group and changes in HR were not significant. SVRI decreased after the start of surgery in the conventional group and remained low throughout compared with pre-operative values (\( p<0.05 \)). A similar pattern was observed in the endovascular group although the within-group differences were not statistically significantly different. PCWP was higher during surgery in the conventional group (\( p<0.05 \)), but there were no within-group differences in either group. There was a decrease in CVP during surgery in the endovascular group to significantly lower values than preoperatively, and lower than in the conventional group (\( p<0.05 \)). There were no changes in cardiovascular variables.
associated with aortic cross clamping (conventional group) or occlusion (endovascular group) (Table 8.4, time points 2-3 and 4-5).

There were no significant changes in arterial pH or pCO₂ in the endovascular group throughout the procedure, but base deficit increased during aortic occlusion (p<0.05) (Table 8.3). pH decreased during aortic clamping (p<0.05) and decreased further after clamp release (p<0.005) in the conventional group (p<0.005 between groups). pCO₂ increased significantly after cross clamp release (p<0.005), but values for pH and pCO₂ were similar in the two groups 25 min later. There was a progressive decrease in plasma bicarbonate during the procedure in the conventional group (p<0.005 within-group comparison, p<0.05 compared with endovascular group), but corresponding changes in the endovascular group were not statistically significant (p=0.08).

Base deficit increased progressively during aortic cross-clamping (p<0.05) and after clamp release (p<0.001), and had returned towards neutral 30 min after clamp release. Changes in base deficit were significantly greater in comparison with the endovascular group (p<0.005). There were no significant changes in SpO₂, arterial pO₂ or calculated alveolar-arterial pO₂ within or between groups. Body temperature decreased during surgery in the conventional group (p<0.05). There were no significant within- or between-group changes in pulmonary artery pressures or other derived variables (stroke volume, LVSWI, RVSWI, PVRI).

Catecholamines

Plasma concentrations of adrenaline increased after the start of conventional surgery (p<0.05) and were greater than in the endovascular group throughout surgery (p<0.05). Plasma concentrations of noradrenaline increased in both groups after the start of surgery but within- and between group comparisons were not statistically significant (Figures 8.1, 8.2 and Table 8.5).

There was similar postoperative cardiovascular and respiratory morbidity in both groups (Table 8.6), although in a study of this size no conclusions are made regarding the incidence of complications, or comparisons between technique.
<table>
<thead>
<tr>
<th>Time point</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
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<tbody>
<tr>
<td><strong>pH (units)</strong></td>
<td><strong>Conv</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>7.39 (0.03)</td>
<td>7.38* (0.04)</td>
<td>7.38 (0.04)</td>
<td>7.33** (0.04)</td>
<td>7.27**# (0.05)</td>
<td>7.37(0.05)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>7.41 (0.05)</td>
<td>7.41 (0.04)</td>
<td>7.39 (0.07)</td>
<td>7.36 (0.06)</td>
<td>7.36†† (0.06)</td>
<td>7.39(0.07)</td>
</tr>
<tr>
<td><strong>pCO2 (kPa)</strong></td>
<td><strong>Conv</strong></td>
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<tr>
<td></td>
<td>5.24 (6.7)</td>
<td>5.08 (0.8)</td>
<td>4.99 (0.7)</td>
<td>5.23 (0.5)</td>
<td>6.15**# (0.9)</td>
<td>4.63 (0.5)</td>
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<td>5.11 (0.8)</td>
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<td>4.94 (0.7)</td>
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<tr>
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<td><strong>HCO3 (mmol l⁻¹)</strong></td>
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<td>23.4 (2.0)</td>
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<td>22.7** (2.4)</td>
<td>21.5** (1.9)</td>
<td>21.1** (1.7)</td>
<td>20.7* †(2.1)</td>
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<td>(mmol l⁻¹)</td>
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<td>1.1** (2.5)</td>
<td>1.5** (2.2)</td>
<td>3.3** (2.1)</td>
<td>5.2***# (2.0)</td>
<td>3.4* (2.6)</td>
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<td>35.4 (0.6)</td>
<td>35.3 (0.7)</td>
<td>35.0* (0.7)</td>
<td>34.9* (0.7)</td>
<td>35.2 (0.6)</td>
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<tr>
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<tr>
<td></td>
<td>35.8 (0.7)</td>
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<td>35.5 (0.8)</td>
<td>35.5 (0.8)</td>
<td>35.5 (1.0)</td>
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<td><strong>Haematocrit (%)</strong></td>
<td><strong>Conv</strong></td>
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<td></td>
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<td></td>
<td>36.2 (5.0)</td>
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<td>32.4* (4.8)</td>
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</tr>
<tr>
<td></td>
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<td>35.1 (2.5)</td>
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<td>32.0* (4.2)</td>
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Table 8.3: Mean (SD) arterial blood gas measurements.
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<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>Conv</td>
<td>92.3 (13.9)</td>
<td>87.7* (11.9)</td>
<td>81.3* (15.1)</td>
<td>83.2* (13.0)</td>
<td>78.8** (11.9)</td>
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<td>Endo</td>
<td>86.4 (13.2)</td>
<td>80.3 (12.6)</td>
<td>75.3 (10.7)</td>
<td>81.0 (12.7)</td>
<td>75.4 (7.8)</td>
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<td>Systolic pressure (mm Hg)</td>
<td>Conv</td>
<td>128 (16.1)</td>
<td>127 (14.4)</td>
<td>123 (24.3)</td>
<td>125 (21.3)</td>
<td>121 (21.3)</td>
</tr>
<tr>
<td></td>
<td>Endo</td>
<td>123 (24.3)</td>
<td>117 (17.2)</td>
<td>116 (19.7)</td>
<td>125 (20.5)</td>
<td>114 (11.4)</td>
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<td>HR (min⁻¹)</td>
<td>Conv</td>
<td>66 (21.4)</td>
<td>73 (12.1)</td>
<td>68 (20.4)</td>
<td>72 (9.1)</td>
<td>78 (18.3)</td>
</tr>
<tr>
<td></td>
<td>Endo</td>
<td>64 (13.8)</td>
<td>61 (8.5)</td>
<td>64 (12.1)</td>
<td>69 (9.3)</td>
<td>70 (15.3)</td>
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<tr>
<td>CVP (mm Hg)</td>
<td>Conv</td>
<td>11 (4.0)</td>
<td>11 (3.5)</td>
<td>11 (4.4)</td>
<td>12 (3.0)</td>
<td>11 (4.2)</td>
</tr>
<tr>
<td></td>
<td>Endo</td>
<td>10 (2.9)</td>
<td>10 (3.3)</td>
<td>8† * (3.7)</td>
<td>8† * (3.2)</td>
<td>9 (4.1)</td>
</tr>
<tr>
<td>PCWP (mm Hg)</td>
<td>Conv</td>
<td>17 (5.6)</td>
<td>16 (5.4)</td>
<td>14 (5.7)</td>
<td>15 (5.2)</td>
<td>16 (5.3)</td>
</tr>
<tr>
<td></td>
<td>Endo</td>
<td>15 (5.5)</td>
<td>14 (3.8)</td>
<td>12 (4.1)</td>
<td>12 (3.4)</td>
<td>13 (4.9)</td>
</tr>
<tr>
<td>CI (l min⁻¹ m⁻²)</td>
<td>Conv</td>
<td>2.9 (0.7)</td>
<td>3.4* (0.9)</td>
<td>3.4* (0.9)</td>
<td>3.4* (6.7)</td>
<td>3.5** (1.0)</td>
</tr>
<tr>
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<td>3.3 (1.0)</td>
<td>2.9 (0.8)</td>
<td>3.2 (0.9)</td>
<td>3.0 (0.7)</td>
<td>2.9 (0.7)</td>
</tr>
<tr>
<td>SVRI (dyne s. cm⁻⁵ m⁻²)</td>
<td>Conv</td>
<td>2420 (1015)</td>
<td>1799 (591)</td>
<td>1654 (673)</td>
<td>1710 (469)</td>
<td>1718* (616)</td>
</tr>
<tr>
<td></td>
<td>Endo</td>
<td>2467 (499)</td>
<td>2060 (844)</td>
<td>1761 (485)</td>
<td>1980 (548)</td>
<td>1906 (1503)</td>
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Table 8.4: Mean (SD) Cardiovascular measurements
<table>
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<tr>
<th>Time point</th>
<th>Plasma epinephrine (n mol ml⁻¹)</th>
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<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Endovascular</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Plasma epinephrine (n mol ml⁻¹)</td>
<td>Conventional</td>
<td>1.59 (0.78)</td>
<td>2.93*† (1.50)</td>
<td>2.73*† (1.86)</td>
<td>2.54 (2.21)</td>
<td>2.40 (2.51)</td>
</tr>
<tr>
<td>Plasma noradrenaline (n mol ml⁻¹)</td>
<td>Conventional</td>
<td>4.46 (1.09)</td>
<td>7.19 (3.40)</td>
<td>6.88 (2.95)</td>
<td>8.14 (4.25)</td>
<td>9.24 (3.98)</td>
</tr>
<tr>
<td>Plasma noradrenaline (n mol ml⁻¹)</td>
<td>Endovascular</td>
<td>4.29 (1.19)</td>
<td>4.93 (2.66)</td>
<td>6.11 (2.98)</td>
<td>6.33 (2.99)</td>
<td>7.45 (3.65)</td>
</tr>
</tbody>
</table>

Table 8.5: Mean (SD) plasma catecholamine concentrations.

Legends to tables 8.3-8.5.

Time point 1 = after induction of anaesthesia and before surgery. Time point 2 = 5 min before aortic clamping (conventional) or occlusion (endovascular). Time point 3 = 5 min after aortic clamping or occlusion. Time point 4 = 5 min before clamp release or balloon deflation. Time point 5 = 5 min after aortic clamp release or balloon deflation. Time point 6 = 30 min after aortic clamp release or balloon deflation. * Significant within-group difference compared with time 1 (p<0.05). ** Significant within-group difference compared with time 1 (p<0.005). # Significant within-group difference compared with time 4 (p<0.005). † Significant between-group difference (p<0.05). †† Significant between-group difference (p<0.005).
**Figure 8.1**: Mean adrenaline concentrations over time with y error bars representing the SEM.
Figure 8.2: Mean noradrenaline concentrations over time with y error bars representing the SEM.
## Table 8.6: Postoperative morbidity

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<th>Conventional</th>
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<tr>
<td>Pulmonary oedema</td>
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<tr>
<td>Pneumonia</td>
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<td>3</td>
</tr>
<tr>
<td>Microembolisation/Trash Foot</td>
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<td>0</td>
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<tr>
<td>Other</td>
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<tr>
<td>No complications</td>
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</table>

Other = wound infection, atrial fibrillation, (endovascular group), haematemesis (conventional group)

### 8.4 Discussion

In this study we have found differences in haemodynamic and metabolic parameters, and plasma catecholamines in patients undergoing during conventional AAA surgery compared with those undergoing endovascular repair. Cardiac index and heart rate were higher during conventional surgery, and SVRI lower, but there were no significant changes associated with aortic cross clamping or release. Aortic cross clamping was associated with the development of a metabolic acidosis, with a decrease in pH, HCO₃ and an increase in base deficit. Arterial pCO₂ increased after aortic unclamping with a further decrease in pH and HCO₃ and a further increase in base deficit, but these changes had largely resolved 30 minutes after clamp release. Within-group changes in CI, SVRI, pH, and pCO₂ during endovascular AAA repair were not statistically significant. Body temperature decreased during conventional repair, despite a smaller blood loss and a shorter duration of surgery.

The effects of aortic cross clamping on cardiac output are variable, depending on the level and duration of clamping, collateral circulation, vascular tone, pre-existing cardiac function, anaesthetic technique and intravascular fluid status(Gelman 1995). Clamping of the supracoeliac or thoracic aorta causes increases in arterial pressure and SVR with a decrease in cardiac index and no significant change in heart rate(Gelman
Base deficit increases during the period of clamp application (Andersson et al. 1979; Whalley et al. 1993). Release of the cross clamp is followed by a decrease in arterial pressure, venous return, cardiac index and a worsening of the lactic acidaemia (Whalley et al. 1993; Baxendale et al. 1996). Plasma catecholamines increase following cross clamping with a further increase after clamp release (Normann et al. 1983). The changes which follow infrarenal cross clamping are similar but less marked (Falk et al. 1981; Zaidan et al. 1982; Quintin et al. 1990; Gold et al. 1994). The lack of change in haemodynamic variables associated with cross clamp application or release in this study is consistent with these findings and partly reflects the anaesthetic technique and maintenance of intravascular volume. Baxendale and colleagues found significant changes in mean arterial pressure, SVR, cardiac output and plasma lactate in 10 patients undergoing infrarenal AAA repair; there were few changes in 10 patients undergoing endovascular surgery (Baxendale et al. 1996). However, 6 of the 10 patients undergoing conventional repair had no history of cardiovascular disease, and no vasodilator or inotropic drugs were used. In contrast, only 6 of 30 patients in our own study had no evidence of associated cardiovascular disease. We did not consider it appropriate to perform aortic cross clamping or release without attenuating the potentially harmful haemodynamic effects which occur in this patient group (Attia et al. 1976; Gooding et al. 1980). The decrease in pH and increase in pCO₂, HCO₃ and base deficit after aortic cross clamp release indicated that a significant metabolic acidaemia occurred. This had resolved 30 minutes after cross clamp release. Without active intervention using vasodilators or adjustments of minute ventilation these could have been associated with adverse cardiovascular effects. Thus the lack of cardiovascular changes documented at cross clamping and release is at least partly a consequence of the anaesthetic technique we used.

The increase in plasma catecholamines during conventional AAA repair is consistent with previous work (Derbyshire and Smith 1984) although there are no data relating to endovascular surgery. McCoy and colleagues (McCoy et al. 1993) studied plasma catecholamine and cortisol concentrations in patients undergoing elective AAA repair under high dose opioid-oxygen isoflurane anaesthesia (fentanyl 50 mcg kg⁻¹ bolus and infusion of 30 mcg kg⁻¹ h⁻¹). Plasma concentrations of cortisol, epinephrine and
noradrenaline were increased preoperatively and remained elevated throughout surgery with no significant changes at cross clamp application or release. The catecholamine concentrations were similar to those found in our study. In patients undergoing infrarenal AAA repair under high dose fentanyl (50-100 mcg kg⁻¹)/midazolam/nitrous oxide anaesthesia, Gold and colleagues found an increase in plasma concentrations of epinephrine and noradrenaline after the start of surgery with no further changes 1, 5 and 10 minutes after aortic cross clamping (Gold et al. 1994). Epinephrine and noradrenaline concentrations throughout were 30-70% of those in our study, probably because of differences in anaesthetic technique or patient characteristics (Derbyshire and Smith 1984).

It has been shown that the generation of oxygen free radical and inflammatory markers (cytokines and C-reactive protein) is less during endovascular compared with conventional AAA repair (Swartbol et al. 1996; Thompson et al. 1996b) but there are no data on acid-base changes during surgery. Although not statistically significant, we found that base deficit increased during surgery in the endovascular group (p<0.08). This may reflect tissue hypoperfusion caused by blood loss and the decrease in cardiac index during endovascular repair. Changes were less in comparison with the conventional group but this highlights the fact that endovascular repair in this patient population should not be regarded as a benign procedure.

We recognise some potential criticisms of this study. Patients were allocated to group according to individual aneurysm morphology; patients unsuitable for endovascular repair were offered conventional surgery. Endovascular repair was in an early stage of development with unknown morbidity and mortality when this study commenced, and it was not felt justified to allocate patients to a surgical treatment in a random manner. However, there were no preoperative differences in patient characteristics or cardiac function between groups. We studied patients undergoing all forms of AAA repair (aortic tube graft, bifurcated grafts and aorto-mono-iliac reconstructions). The type of aortic reconstruction (tube or bifurcated graft) was not controlled between groups, but our patients represent a typical cohort with AAA requiring a range of operative approaches. In the absence of a distal aortic neck but with relatively narrow calibre iliac arteries, patients would be suitable to receive either a
bifurcated endovascular prosthesis or a conventional tube graft. If iliac aneurysms were present, a bifurcated conventional or mono-iliac endovascular graft would be indicated, as a bifurcated endovascular repair would be inappropriate (Chuter et al. 1996). Comparison of the consequences of bifurcated endovascular or conventional repair may not therefore be applicable for an individual patient.

Cardiovascular measurements were made at specified times related to aortic occlusion or cross clamping as we had anticipated that cardiovascular changes would be related to these events. As used the Vigilance® monitor provides rapid trend estimates of cardiac output, although the response to rapid changes in haemodynamic conditions may be delayed. In an animal model, the time for an 80% response to a change in cardiac output of 40-50% caused by inferior vena Cava occlusion or arterial haemorrhage was 9.1 and 9.7 minutes respectively. Times to 50% response to these interventions were 6.7 and 7.8 minutes (Siegel et al. 1996). The minimum intervals between measurements in our study were 6-10 minutes (before and after aortic clamping or occlusion). Arterial, central venous and pulmonary arterial pressures were monitored continuously, but the method of measurement of cardiac output was the most rapid available to us. The timing of the recordings at a minimum of 5 minutes after each intervention is consistent with previous studies of changes in haemodynamic variables (Thompson et al. 1997) and plasma catecholamine concentrations (Normann et al. 1983; McCoy et al. 1993) after aortic cross clamping or occlusion and release. However it is likely that the maximum changes in cardiac output and derived variables occurred between our measurement times, and that the recorded values underestimated this variability.

The present study was undertaken early in our endovascular AAA program. The anaesthetic technique and ICU management reflected our practice at the time of inception of the study. However, the duration of surgery, ICU stay, incidence and type of complications were similar to those reported elsewhere (White et al. 1996; Baker 1997) but in contrast the duration of hospital stay in our study was shorter in the endovascular group whilst blood loss was greater. A consequence of the greater unanticipated blood loss was that intravascular volume was lower in the endovascular group, reflected in the lower CVP and PCWP. This renders strict comparison of the groups difficult, but there were significant differences that might have been magnified.
had the same volaemic status been maintained. In particular, there was a greater degree of metabolic acidosis and higher concentrations of plasma catecholamines, reflecting a higher degree of metabolic stress (Baranowski and Adiseshiah 1996). With increasing experience, we have changed some aspects of anaesthetic and surgical management, with a reduction in blood loss and alternative techniques have proved more appropriate for some patients, e.g. the use of regional anaesthesia. It has been recommended that specific attempts be made to decrease MAP during placement of the endovascular prosthesis (Clutter et al. 1980; Baker 1997), but this has rarely been necessary in our experience.

8.5 Summary

In summary, these data have demonstrated that endovascular AAA repair caused fewer intraoperative changes in cardiac index, plasma concentrations of adrenaline and acid-base status than during conventional surgery. The lack of changes in cardiovascular variables associated with aortic occlusion and lower limb reperfusion during conventional surgery may be attributed to our anaesthetic technique and management. However, the diminished peroperative metabolic and cardiovascular effects of endovascular AAA repair that we observed may be advantageous in patients with cardiovascular disease.
CHAPTER NINE

Metabolic and renal responses to endovascular and conventional aneurysm repair

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<td>9.2 Methods</td>
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9.1 Introduction

The data presented in the previous two chapters have demonstrated that endovascular aneurysm repair lessens the respiratory and cardiovascular insult associated with conventional surgery. The following experimental data aimed to quantify any difference in renal, cytokine and ischaemia reperfusion response between the two techniques. As already stated endoluminal surgery has a number of potential advantages, over conventional inlay AAA repair, in that it avoids the need for aortic cross-clamping, an abdominal incision, mobilization of the abdominal viscera and retroperitoneal dissection.

Aortic clamping and subsequent reperfusion initiate a systemic inflammatory response, producing endothelial damage and an increase in vascular permeability. Oxygen derived free radicals are thought to play an important role in this increased vascular permeability, which may result in increased renal albumin excretion, possible renal failure, pulmonary oedema and acute lung injury (Smith et al. 1994; Khaira et al. 1996). This systemic inflammatory reaction is also associated with increased concentrations of cytokines such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNFalpha) after complicated cases, and (Froon et al. 1996) interleukin 1beta (IL-1beta) (Swartbol et al. 1996). Conventional aneurysm surgery causes considerable perioperative disturbance of platelet function, sequestration and blood coagulation (Bradbury et al. 1997). The absence of aortic cross-clamping and extensive surgical dissection, during endovascular AAA repair, may potentially reduce these metabolic manifestations that are associated with conventional surgery. The aim of this study was to prospectively compare inflammatory and renal parameters in comparative cohorts undergoing conventional and endovascular aneurysm repair.

9.2 Methods

Patients

The study group consisted of 43 consecutive patients who underwent elective abdominal aortic aneurysm repair. All patients consented to inclusion within the trial, which was approved by our local ethical committee. Patients were allocated to
endovascular or conventional aneurysm repair, according to aneurysm morphology, as determined by pre-operative imaging with a combination of computed tomography (CT) and angiography. In addition to standard preoperative anaesthetic evaluation, all patients underwent radioisotope (MUGA) scanning to assess cardiac function. Twenty three patients were treated with endovascular devices (17 aortomonofermal, 2 aortomonoiliac (Thompson et al. 1997b), 2 EVT bifurcates (Menlo Park, CA, USA), 1 Mintec bifurcate (Freeport, Bahamas) and 1 Bard straight (New Jersey, USA)) and twenty conventionally with inlay grafts (12 tube grafts and 8 bifurcates). No conventional cases required super-renal clamping. Patients were only offered aortomonoiliac repair, if their aortic anatomy precluded the use of a commercially available device. All the commercially available products were deployed in accordance with the manufacturer’s protocols and in the presence of a manufacturer’s representative. The basic design of the aortomonoiliac endograft, a long, tapered graft sutured to a balloon expandable stent, was adapted from the endovascular prostheses described by Parodi et al (Parodi et al. 1991) and has been described in previous chapters.

**Anaesthetic**

All patients received a standard general anaesthetic as previously described.

**Sample Collection**

Serial blood and urine specimens were collected on all patients at distinct time points during the peri-operative period. A peripheral venous blood sample was collected the day before surgery (1). At five further time points during surgery peripheral venous blood was collected directly from iliac or femoral veins and mixed venous blood obtained via a pulmonary artery catheter. The five time points were defined as (2) Start of procedure, (3) Just prior to clamping (conventional) or balloon inflation (endovascular), (4) Maximum ischaemia (just prior to clamp release or balloon deflation), (5) 5 minutes after reperfusion, (6) 30 minutes after reperfusion. In addition further venous samples were collected during the post operative period at (7) 6 hours and (8) 24 hours and on the (9) 2nd, (10) 3rd, (11) 4th and (12) 5th days. Blood samples were collected in sterile bottles containing ethylene diamine tetra-acetic acid (EDTA), as the anticoagulant, and
immediately centrifuged at 6000rpm for ten minutes. Plasma was then snap frozen in liquid nitrogen and stored at -80°C for subsequent analysis. Further EDTA and lithium heparin samples were sent for immediate full blood count and clotting analysis respectively. Urine samples were obtained at all specified time points and were also snap frozen in liquid nitrogen. The first urine sample was taken on bladder catheterisation in the operating room, and therefore all urine assays contain one less time point.

Assays

The cytokines, tumour necrosis factor alpha (TNFα), interleukin 1β (IL-1β) and interleukin-6 (IL-6) were quantified by commercially available ELISAs (Genzyme Diagnostics, MA, USA). Similarly 11-dehydro Thromboxane B2 (Caymen Chemical Company, MI, USA) and sL-selectin (Immunotech, Marseille, France) concentrations were calculated by immunoassays on urine and plasma respectively.

Urinary albumin/creatinine ratios were determined by a standard automated technique. The N-acetyl glucosamidase (NAG) was assayed using a fluoroscopic method based on that of Whiting (Whiting et al. 1979), which was adapted from that of Dance (Dance et al. 1969).

A full blood count and clotting analysis (fibrinogen and fibrinogen degradation products (FDPs) were determined by standard automated techniques on each sample.

Statistical analysis

Demographic and operative data are presented as medians and interquartile ranges and compared using the Mann-Whitney U test. Plasma assays are presented as medians and interquartile ranges. Medians at each time point for each group were compared using Mann-Whitney U test. To allow for repeated group to group comparison, significance was deemed to be achieved below the 1% level. In addition to this analysis, the data was also compared using by the area under the curve and point by point with Student t-tests, both methodologies produced similar results, the data for the latter is included in Appendix 9A. All calculations were performed in Minitab for Apple Macintosh and SPSS for PC.
### 9.3 Results

<table>
<thead>
<tr>
<th></th>
<th><strong>Endovascular Repair</strong> (N=23)</th>
<th><strong>Conventional Repair</strong> (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>10 (43%)</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Ischaemic Heart Disease</td>
<td>15 (65%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td>11 (48%)</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>0</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Cerebral Vascular Disease</td>
<td>3 (13%)</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>Renal Failure (Dialysis)</td>
<td>1 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>Current/Ex smokers</td>
<td>21 (91%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>16 (70%)</td>
<td>12 (60%)</td>
</tr>
</tbody>
</table>

**Table 9.1: Patient co-morbidity.**

#### Demographics

The two groups of patients were well matched for age and aneurysm size. Twenty three patients (23 male) underwent endovascular AAA repair (1 isolated iliac aneurysm), median age 72 years (range 60-81 years) (CI (2,6), W=547, p=3.2) and aneurysm diameter 5.5cm (range 4.8-7.3cm) and 20 (18 male, 2 Female) had conventional surgery, median age 67 years (58-81 years) and size 5.7 cm (5.0-9.4cm) (CI (0.0001,0.7), W=439, p=0.1). The patients from each group were also well matched for co-morbid factors (Table 9.1). There was no significant difference in left ventricular ejection fractions, as assessed by radioisotope (MUGA) scanning, between the two groups (endovascular 62% (IQR 45-70%) versus conventional 62% (50-69%) CI (-16.0,7.99) W=232.0, p=0.767).

#### Operative Details

There were 3 peri operative deaths, two endovascular (8.7%) and one conventional (5%). The endovascular deaths occurred, firstly in a 72 year old man, who was converted to a conventional repair, after balloon malfunction caused stent deformation(Thompson et al. 1997b); the patient subsequently dying of multiple organ failure on the second post-
operative day. Secondly in a 61 year old man, who died after iatrogenic small bowel perforation on the sixth post operative day. This patient had a retroperitoneal approach to the left common iliac artery to facilitate delivery of the endovascular device through a dacron conduit, unfortunately damage occurred to the small bowel during closure of this incision. The conventional death occurred in a 74 year old man, who died of multiple organ failure two days after surgery. Major morbidity included two conventional patients who developed respiratory failure and three endovascular patients whose recoveries were complicated by pulmonary embolism, disabling CVA and trash foot respectively (Figure 9.12). In one further endovascular patient, technical difficulties required conversion to conventional surgery, however all results for this individual were analysed in the endovascular group.

The endovascular procedures took significantly longer than the conventional operations, median times 175 minutes versus 124 minutes respectively (Mann-Whitney W= 282.0; p< 0.0001). Aortic occlusion time (clamp time for conventional operations, balloon inflation time for endovascular procedures) was significantly lower in the endovascular group, median 15 versus 48 minutes (W=609.0, p< 0.0001). If however, the total ischaemia time was calculated to include the time that at least one common femoral artery was clamped during endovascular repair, there was no difference between the two groups, median 48 versus 48 minutes (W=472.0, p=0.4429). The blood loss was significantly greater in the endovascular group, median 2500 mls versus 1500mls for the conventional patients (W=659.0, p< 0.002) and as a result blood transfused was significantly higher in this group, median 4 units versus 3 units (W=586.0, p< 0.05). There were however no differences in infused volumes colloid or crystalloid during the intraoperative period (Table 9.2) or any statistical differences in the haematocrit (Figure 9.11) at anytime point between the groups. The median hospital stays were significantly lower after endovascular surgery, median 8 versus 14 days (W=598.5, p< 0.0001), (Table 9.2).
<table>
<thead>
<tr>
<th></th>
<th><strong>Endovascular Repair</strong> (N=23)</th>
<th><strong>Conventional Repair</strong> (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>72 years (60-81)</td>
<td>67 years (58-81)</td>
</tr>
<tr>
<td><strong>Aneurysm diameter</strong></td>
<td>5.5cm (4.8-7.3)</td>
<td>5.7cm (5.0-9.4)</td>
</tr>
<tr>
<td><strong>Operation time</strong></td>
<td>175 minutes (153-215)</td>
<td>124 minutes (99-149)</td>
</tr>
<tr>
<td><strong>Aortic Occlusion time</strong></td>
<td>15 minutes (7-23)</td>
<td>48 minutes (35.75-58.5)</td>
</tr>
<tr>
<td><strong>Total Ischaemia time</strong></td>
<td>48 minutes (20-57)</td>
<td>48 minutes (35.75-58.5)</td>
</tr>
<tr>
<td><strong>Blood loss</strong></td>
<td>2500 mls (2000-3500)</td>
<td>1500 mls (1000-2000)</td>
</tr>
<tr>
<td><strong>Crystalloids infused</strong></td>
<td>2700 mls (1500-3500)</td>
<td>2500 mls (1800-3300)</td>
</tr>
<tr>
<td><strong>Colloids infused</strong></td>
<td>1300 mls (500-2000)</td>
<td>1700 (1000-2400)</td>
</tr>
<tr>
<td><strong>Blood transfused</strong></td>
<td>4 units (1-6)</td>
<td>3 units (1-4)</td>
</tr>
<tr>
<td><strong>Hospital stay</strong></td>
<td>8 days (7-10)</td>
<td>14 days (10-16)</td>
</tr>
</tbody>
</table>

**Table 9.2:** Patient results. Continuous variables are represented as medians and interquartile ranges (IQRs) or * complete ranges.

**Assays**

All assays from all patients were included for statistical analysis. Therefore results from the three patients that died were included up until the time of their deaths. One of these patients was in established chronic renal failure at the time of surgery and continued haemodialysis up until death. There were therefore no urine specimens available for analysis and plasma assay results may have been influenced by dialysis in this patient. Table 9.3 demonstrates the peak assay values for the three patients that died and the patient who suffered a trashed foot.
Patient age, sex, operation and complication | Peak assay values with sample number in parentheses
---|---
 | IL-6 (pg/ml) | TNF-α (pg/ml) | sL-selectin (ng/ml) | NAGs (mol/Mm/hr) | 11-dhTx (pg/ml)
72 M, AMI, trash foot | 197.4 (9) | 44 (6) | 346 (7) | 162.2 (7) | 7740.2 (7)
72 M, AMI, MOF Died | 2118.5 (7) | 116 (4) | 477 (7) | 421 (4) | 18.1 (5)
74 M, Conventional bifurcate, MOF, Died | 1295.3 (9) | 27.5 (9) | 478 (9) | 447 (8) | 4968.6 (7)
61 M, AMI, small bowel perforation, Died | 603.4* (9) | 28.9 (12)* | 665.8 (9)* | * | *

Table 9.3: Peak assay values for the three patients that died and the patient with trashed foot. Demonstrating high levels of IL-6, TNF-α and urinary NAGs prior to death. (AMI aortomonoiliac, MOF multiple organ failure). * this patient was in established renal failure preoperatively requiring haemodialysis, therefore no urine assays were possible and postoperative dialysis may have influenced plasma assays.

Cytokines

The endovascular patients had significantly lower TNFα just after the start of the procedure (Median 14.8 pg/ml versus 28.1 pg/ml, CI (3.5,18.5), W=321, p=0.0082), prior to clamping (9.1 pg/ml versus 22.4 pg/ml, CI (5.9,23.0), W=381, p=0.0022), thirty minutes after reperfusion (11.1 pg/ml versus 22.5 pg/ml, CI (4.8,20.8), W=302, p=0.0074) six hours after surgery (11 pg/ml versus 27.5 pg/ml, CI (7.21.5), W=364.5, p=0.0004) and on days one, (9.8 pg/ml versus 16.6 pg/ml, CI (2.4, 14.5), W=298, p=0.007) and three postoperatively (10.2 pg/ml versus 23.6 pg/ml, CI (7.8,19.0), W=200, p=0.0043) (Figure 9.1). IL-6 concentrations were also significantly lower in this group on day one (Median 120 pg/ml versus 277 pg/ml, CI (51,243), W=425, p=0.0038) and day 2 after surgery (43 pg/ml versus 256 pg/ml, CI (71, 266), W=515, p=0.0002), (Figure 9.2). There was however no significant difference between the IL-1β results between the groups.
Renal Function

Analysis of urinary ACR and NAGs demonstrated significantly lower levels after endovascular surgery. Urinary NAGs were significantly lower at 5 minutes (Median 49.1 mol/mM/min versus 118 mol/mM/min, CI (38.9,105.5), W=353, p=0.0001) and 6 hours, (79.7 mol/mM/min versus 175.2 mol/mM/min, CI (29.8,289.1), W=369, p=0.0032) after reperfusion (Figure 9.3). Similarly the ACR was significantly lower after endovascular surgery at 5 (Mean 11.5 mg/mMol versus 23.1 mg/mMol, CI (3.8,35.5), W=413, p=0.0064) and 30 minutes (12.2 mg/mMol versus 30.5 mg/mMol, CI (3.7,31.8), W=417p=0.0082) after reperfusion (Figure 9.4).

White cell and platelet activity

Platelet activation as estimated by urinary 11-dehydro Thromboxane B2 was significantly higher in the endovascular group 6 hours (Median 3882 pg/ml versus 1105 pg/ml, CI (232,5006), W= 510, p=0.01) after reperfusion and demonstrated a trend towards higher values during the postoperative period in this group (Figure 9.5). There was no significant difference in white cell activation as measured by sL-selectin at any time point (Figure 9.6).

Haematological parameters

Full blood count and clotting analysis demonstrated significantly higher fibrinogen concentrations in the conventional group on the third postoperative day (Median 6.0 g/l versus 4.6 g/l, CI (0.5,2.4), W=207, p=0.0055) and a trend towards increased D-dimer (Figures 9.7 and 9.8). Despite significant changes in white cell and platelet counts and the haematocrits within each group there were no significant differences between the groups (Figures 9.9, 9.10 and 9.11).
Figure 9.12: Photograph showing a trashed right foot following endovascular AAA repair.
9.4 Discussion

Tissue reperfusion with oxygenated blood after conventional abdominal aortic aneurysm repair produces an acute inflammatory response with local and systemic effects (Khaira et al. 1996). Neutrophils are activated during aortic cross clamping in response to ischaemia and bind to microvascular endothelium (Zimmerman and Granger 1992). The neutrophils subsequently release cytokines and proteolytic enzymes damaging the endothelial cells and these in turn release further cytokines and arachidonic acid metabolites. Cytokines perpetuate the local and systemic injury by stimulating further neutrophil activation (Osborn 1990). In the present study we have demonstrated that endovascular aneurysm surgery results in significantly lower IL-6 and TNF-α profiles when compared to patients undergoing conventional repair. These results suggest that the neutrophil activation and endothelial cell damage associated with conventional AAA repair may be attenuated by endovascular techniques. These findings confer with our own previous investigations in a smaller series (Thompson et al. 1996a) and also the IL-6 findings of Swartbol et al (Swartbol et al. 1996) and Syk et al (Syk et al. 1998). The higher levels of IL-6 in 3 patients who subsequently died also corroborate the findings of Froon et al (Froon et al. 1996). If the assays performed on these patients had been excluded from analysis the differences demonstrated between the groups would have been greater as only one death occurred in the conventional group. The TNF-α results should however be interpreted with some caution as there was an apparent difference between the groups prior to surgery although this was not statistically significant. The sensitivity of the TNF-α assay used in this study was 3pg/ml with normal values less than 15pg/ml. The reason for the apparent abnormal TNF-α levels prior to surgery in the conventional group is unclear.

Acute renal failure is a well-recognized complication of elective aneurysm surgery, its incidence is however low and therefore, renal failure itself cannot usefully be used as an endpoint in human studies of this nature. Traditional tests of renal function are relatively insensitive, only altering significantly after large changes in function has occurred. Serum creatinine only rises out of its normal range when over half of functioning renal mass is lost (Gabriel 1986). Despite this, a normal post-operative creatinine has led some to suggest
that it is safe to cover renal artery ostia with aortic stents, based on this and CT scanning alone (Malina et al. 1997a). The systemic increase in vascular endothelial permeability following conventional aneurysm surgery causes transient organ dysfunction. In the kidney increased permeability allows larger molecular weight proteins to enter the glomerular filtrate. In particular urinary albumin and the albumin/creatinine ratio (ACR) provide sensitive indices of glomerular function and the systemic ischaemia reperfusion injury. Previous studies have demonstrated a rise in the ACR following aortic surgery, with the degree of increase predicting pulmonary dysfunction in the peri-operative period (Smith et al. 1994). Similarly urinary N-acetyl glucosamidase (NAG) levels are a very sensitive index of tubular renal impairment and have also been demonstrated to rise after conventional aneurysm surgery (Nicholson et al. 1996). Urinary albumin and NAG are therefore very sensitive indicators of renal glomerular and tubular injury respectively (Mogensen 1987; Price 1992) and are ideal variables to compare renal injury between these two surgical techniques. This study confirms that patients undergoing conventional aneurysm repair suffer significant subclinical renal damage, thus corroborating the findings of other authors (Smith et al. 1994; Nicholson et al. 1996). This renal injury was significantly attenuated by endovascular techniques, with the endovascular patients demonstrating lesser increases in both ACR and NAG. The precise mechanisms of renal injury during aneurysm surgery are undetermined. However, infrarenal aortic cross-clamping causes a rise in renal vascular resistance and a fall in renal blood flow. Subsequent reperfusion causes a reduction in glomerular filtration rate and exposes the kidney to activated neutrophils, cytokines and oxygen free radicals produced by the reperfused lower limbs. In addition microembolisation of the renal vascular bed may play a part. Interestingly we have demonstrated significantly higher levels of peripheral embolisation during endovascular aneurysm repair (Thompson et al. 1997c; Thompson et al. 1997e), perhaps suggesting that this is not the predominant mechanism.

The selectins are a family of cell surface glycoproteins that have important roles as adhesion molecules. L-selectin is expressed on the surface of lymphocytes, neutrophils and monocytes, and is rapidly shed (sL-selectin) from the surface of these cells following their activation (Lefer et al. 1994). Hence plasma sL-selectin can be used as an indirect measure of leukocyte activation (Spertini et al. 1992). In this study, there was no difference in sL-
selectin concentrations between the two groups. Flow cytometry may have been a more sensitive assay for assessing white cell activity. A previous study using plasma from patients undergoing conventional and endovascular AAA surgery demonstrated that endovascular repair stimulated a significantly higher adhesion molecule expression on donor white cells (Swartbol et al. 1997). The white blood cell counts increased from baseline in both groups after reperfusion, which is in keeping with previous work (Swartbol et al. 1997; Syk et al. 1998).

Platelet activation is followed by degranulation of a number of substances including thromboxane A2, which is rapidly metabolized to thromboxane B2 in the kidney. Assays of these two products in plasma have been used as a method to determine platelet activity in vivo. However the mechanical stimulation of blood cells during sampling has led to spuriously elevated and inaccurate results. 11-dehydrothromboxane-B\(_2\) is a prominent stable metabolite of thromboxane B2 in blood and urine and this therefore believed to be a far more reliable indicator of thromboxane production (Westlund et al. 1986) and hence platelet activity. 11-dehydrothromboxane-B\(_2\) activity increased after both conventional and endovascular surgery in this study. It has previously been demonstrated that the insertion of a synthetic vascular graft into the aorta causes a prolonged increase in the synthesis of thromboxane (Vesterqvist et al. 1987; Lewin et al. 1989). Perhaps the most interesting finding of this study is the significantly higher levels of 11-dehydrothromboxane-B\(_2\) demonstrated in the post-operative period in the endovascular group, suggesting significantly enhanced platelet activity. It may be hypothesised that this occurs as blood within the aneurysm sac organizes around the aortic endograft, a process that is not encountered during conventional surgery as thrombus within the sac is removed. A number of authors have described a post implantation syndrome in patients undergoing endovascular AAA repair in which patients develop a fever lasting for up to 10 days (Swartbol et al. 1996; Blum et al. 1997b). This study suggests increased platelet activity during aneurysm thrombosis may play an important part in this syndrome.

Alternatively these findings may reflect differences in graft material, the conventional grafts were all dacron, whilst the majority of the endovascular devices were ePTFE and had been constructed by serially dilating the graft with graded angioplasty balloons to 35mm (Thompson et al. 1997b). Recent work by Whiteley et al has suggested
that this process may permanently change the integral structure of ePTFE and perhaps make it more thrombogenic (Whiteley et al. 1997). However we have now implanted in excess of fifty of these devices and only encountered one case of intraluminal thrombus which was directly attributable to a technical error (Boyle et al. 1998).

Elective conventional AAA repair is associated with post-operative thrombocytopenia, followed by thrombocytosis in a significant percentage of patients. Similarly a peri-operative fall in fibrinogen levels is followed by hyperfibrinogenemia in the majority (Bradbury et al. 1997). We have confirmed these findings in this study and demonstrated a significantly lower rebound hyperfibrinogenemia in the endovascular group. The peri-operative thrombocytopenia, however, did not appear to be attenuated by endovascular techniques, although this may reflect the increased blood loss in this group. However a recent study by Syk et al also demonstrated no difference in platelet counts between two similar groups with significantly lower blood loss after endovascular surgery (Syk et al. 1998). Thrombocytopenia after conventional aneurysm repair has been significantly related to aortic cross-clamp time and not blood loss (Bradbury et al. 1997), and it may be hypothesised that the thrombocytopenia demonstrated after endovascular repair in this study was partly due to platelet sequestration in the aneurysm sac as suggested above. The blood loss in this study was significantly higher after endovascular repair reflecting the proportion of aortomonoiliac devices deployed in this group. As a result this group had significantly greater volumes of blood transfused, which may have confounded the assay results. However colloid and crystalloid infusions were similar and there were no significant differences in the haematocrits between the groups at any sample point.

There are some weaknesses with the data presented within this chapter, which are in keeping with those already alluded to in the early work. Patients were allocated to group according to individual aneurysm morphology, and therefore not randomized; patients unsuitable for endovascular repair were offered conventional surgery. At the start of the study, the technique of endovascular repair was at an early stage of development with unknown morbidity and mortality, and it was not felt justified to allocate patients to surgical treatment in a randomized manner. However, there were no preoperative differences in patient characteristics, biochemical or hematological indices between the
two groups and we have no reason to suspect the two groups to be physiologically different. Secondly the majority of the patients in the endovascular group underwent repair with an aortomonoiliac device with femoro-femoral crossover graft. The deployment of these devices required a significant period of common femoral cross-clamping and thus distal ischaemia, which was comparable with the aortic clamp time of the conventional group. It may be hypothesised that the differences between the two groups demonstrated in this study would have been greater if only tube or bifurcate endovascular prostheses were studied. Unfortunately the small number of tube (1) and bifurcated (3) devices deployed made meaningful comparison within the endovascular group impossible.

9.5 Summary

The main findings of this chapter demonstrate that endovascular techniques attenuate the subclinical renal injury and IL-6 response associated with conventional surgery. Interestingly there appears to be greater platelet activity after endovascular repair as measured by 11-dehydrothromboxane-B₂ activity which may result from thrombus organization within the sac. There is however, still a considerable response to endovascular aortic surgery, which may explain why the morbidity and mortality rates reported in the early published series are similar to those after conventional repair(Woodburn et al. 1998). These early figures may take into account a number of learning curves and one would expect that, in the light of this data, the incidence of multiple organ failure and mortality after endovascular AAA repair is likely to be lower.
## APPENDIX 9A Raw Data and Statistical analysis

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Conv. Mean (SD)</th>
<th>Stent Mean (SD)</th>
<th>T test p-value</th>
<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.38 (2.7)</td>
<td>1.95 (2.63)</td>
<td>0.55</td>
<td>0.19 (0-1.6)</td>
<td>1.25 (0-2.33)</td>
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<td>2</td>
<td>2.13 (2.1)</td>
<td>3.07 (2.91)</td>
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<td>1.75 (0.69-3.0)</td>
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<td>3</td>
<td>1.95 (2.77)</td>
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<td>4</td>
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*IL-1beta values presented as means and standard deviations and medians and interquartile ranges. p-values calculated by Student t-tests and Mann-Whitney U tests respectively.*
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<tr>
<th>Sample Number</th>
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<th>Conv. Median (IQR)</th>
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<th>M-W U test p-value</th>
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IL-6 values presented as means and standard deviations and medians and interquartile ranges (pg/ml). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
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<th>Stent Mean (SD)</th>
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<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
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TNF alpha values presented as means and standard deviations and medians and interquartile ranges (pg/ml). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Conv. Mean (SD)</th>
<th>Stent Mean (SD)</th>
<th>T test p-value</th>
<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
</tr>
</thead>
<tbody>
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*L-selectin values presented as means and standard deviations and medians and interquartile ranges (ng/ml). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
<table>
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<tr>
<th>Sample Number</th>
<th>Conv. Mean (SD)</th>
<th>Stent Mean (SD)</th>
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<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
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<tbody>
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FDPs values presented as means and standard deviations and medians and interquartile ranges (ng/ml). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
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<th>Conv. Mean (SD)</th>
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<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
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Fibrinogen values presented as means and standard deviations and medians and interquartile ranges (g/l). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
<table>
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<tr>
<th>Sample Number</th>
<th>Conv. Mean (SD)</th>
<th>Stent Mean (SD)</th>
<th>T test p-value</th>
<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
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WBC values presented as means and standard deviations and medians and interquartile ranges (x10⁹/l). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
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<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
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<td>171 (54)</td>
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<td>4</td>
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<td>163 (63)</td>
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<td>5</td>
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<td>153 (52)</td>
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<td>104 (46)</td>
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<td>100 (32)</td>
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<tr>
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<td>181 (52)</td>
<td>0.59</td>
<td>156 (129-189)</td>
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Platelets values presented as means and standard deviations and medians and interquartile ranges ($\times 10^9/l$). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
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<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
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<tbody>
<tr>
<td>1</td>
<td>0.403 (0.04)</td>
<td>0.423 (0.031)</td>
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<td>0.397 (0.374-0.44)</td>
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<tr>
<td>2</td>
<td>0.341 (0.033)</td>
<td>0.352 (0.034)</td>
<td>0.34</td>
<td>0.344 (0.308-0.361)</td>
<td>0.356 (0.329-0.382)</td>
<td>0.32</td>
</tr>
<tr>
<td>3</td>
<td>0.326 (0.047)</td>
<td>0.311 (0.038)</td>
<td>0.26</td>
<td>0.32 (0.292-0.341)</td>
<td>0.323 (0.282-0.342)</td>
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</tr>
<tr>
<td>4</td>
<td>0.31 (0.045)</td>
<td>0.305 (0.037)</td>
<td>0.83</td>
<td>0.299 (0.28-0.355)</td>
<td>0.304 (0.274-0.337)</td>
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<tr>
<td>5</td>
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<td>0.317 (0.046)</td>
<td>0.32</td>
<td>0.32 (0.302-0.364)</td>
<td>0.31 (0.284-0.358)</td>
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<tr>
<td>7</td>
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<td>0.346 (0.049)</td>
<td>0.031</td>
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</tr>
<tr>
<td>8</td>
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<td>0.31 (0.038)</td>
<td>0.016</td>
<td>0.354 (0.296-0.382)</td>
<td>0.305 (0.278-0.341)</td>
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<tr>
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<td>0.288 (0.027)</td>
<td>0.029</td>
<td>0.337 (0.287-0.37)</td>
<td>0.289 (0.263-0.312)</td>
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<tr>
<td>10</td>
<td>0.327 (0.033)</td>
<td>0.312 (0.04)</td>
<td>0.26</td>
<td>0.328 (0.302-0.342)</td>
<td>0.314 (0.276-0.345)</td>
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<td>0.323 (0.032)</td>
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Haematocrit values presented as means and standard deviations and medians and interquartile ranges (%). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
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<th>Stent Mean (SD)</th>
<th>T test p-value</th>
<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4.27 (6.63)</td>
<td>7.05 (7.84)</td>
<td>0.27</td>
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<tr>
<td>2</td>
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<td>0.05</td>
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<td>8.0 (4.5-16.7)</td>
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<td>19.19 (30.01)</td>
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<td>10.85 (5.85-21.95)</td>
<td>0.22</td>
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<tr>
<td>4</td>
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<td>0.0056</td>
<td>23.1 (14.65-61.6)</td>
<td>11.5 (5.5-22.67)</td>
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<td>14.74 (11.38)</td>
<td>0.0088</td>
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<td>3.2 (1.9-5.48)</td>
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<tr>
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<td>5.1 (2.5-13.2)</td>
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<td>3.7 (1.95-10.05)</td>
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<tr>
<td>11</td>
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<td>2.5 (2.1-6.4)</td>
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Albumin/Creatinine ratio values presented as means and standard deviations and medians and interquartile ranges (mg/mMol). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
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<th>Sample Number</th>
<th>Conv. Mean (SD)</th>
<th>Stent Mean (SD)</th>
<th>T test p-value</th>
<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
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<tbody>
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<td>23.7 (14.4)</td>
<td>0.15</td>
<td>27.5 (16.9-38.4)</td>
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<tr>
<td>2</td>
<td>79.9 (86.2)</td>
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<td>0.56</td>
<td>49.5 (34.5-90.1)</td>
<td>42.9 (16.2-74.5)</td>
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<tr>
<td>3</td>
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<tr>
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<td>49.1 (10.0-73.5)</td>
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<tr>
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<tr>
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<tr>
<td>8</td>
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</table>

NAGs values presented as means and standard deviations and medians and interquartile ranges (mol/mMol/hr). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
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<th>Stent Mean (SD)</th>
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<th>Conv. Median (IQR)</th>
<th>Stent Median (IQR)</th>
<th>M-W U test p-value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>759 (200-2400)</td>
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<tr>
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<td>7</td>
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<td>2069 (786-4285)</td>
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<td>3790 (4223)</td>
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<td>9</td>
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<td>0.99</td>
<td>1865 (666-3534)</td>
<td>2553 (572-3346)</td>
<td>0.87</td>
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</tbody>
</table>

11-dehydrothromboxane values presented as means and standard deviations and medians and interquartile ranges (pg/ml). p-values calculated by Student t-tests and Mann-Whitney U tests respectively.
APPENDIX 9B Figures 9.1-9.11
Figure 9.1  A graph plotting median plasma TNF-α concentrations (pg/ml) at the twelve time points. (1) Day before surgery, (2) start of procedure, (3) just prior to clamping (conventional) or balloon inflation (endovascular), (4) maximum ischaemia (just prior to clamp release or balloon deflation), (5) 5 minutes after reperfusion, (6) 30 minutes after reperfusion, (7) 6 hours, (8) 24 hours, (9) day 2, (10) day 3, (11) day 4 and (12) day 5) for the conventional (shaded) and endovascular (not shaded) patients with y-error bars corresponding to the 75th centile. Values were significantly lower in the endovascular group at time points 2 (p=0.0082), 3 (p=0.0022), 6 (0.0074), 7 (p=0.0004), 8 (p=0.007) and 10 (p=0.0043).
Figure 9.2  A graph plotting median plasma IL-6 concentrations (pg/ml) at the twelve time points with y–error bars corresponding to the 75th centile for the conventional (shaded) and endovascular (not shaded) patients. Values were significantly lower in the endovascular group at time points 8 (p=0.0038) and 9 (p=0.0002).
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Figure 9.4 A graph plotting median urine NAG activity (μmol/mMol/hr) at the eleven time points with y–error bars corresponding to the 75th centile for the conventional (shaded) and endovascular (not shaded) patients. Values were significantly lower in the endovascular group at time points 4 (p=0.0009) and 6 (p=0.0032).
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CHAPTER TEN

The changes in referral practice, workload and operative mortality following the establishment of endovascular abdominal aortic aneurysm surgery

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10.1 Introduction

The data presented in the previous three chapters has demonstrated that endovascular abdominal aortic aneurysm repair is less invasive than conventional surgery. The initial development of the technique was indeed based on this hypothesis and its use advocated as an alternative to conventional surgery, particularly in high-risk patients, in whom mortality may exceed 40% using the transperitoneal approach (Katz et al. 1994). Initially, the development of endovascular AAA surgery was confined to a number of specialty centres both world-wide and within the United Kingdom (Parodi et al. 1991; May et al. 1994a; Chuter et al. 1996; Balm et al. 1996; Nasim et al. 1996b). More recently within the UK a randomised trial of endovascular grafting has been established. The EVAR (EndoVascular Aneurysm Repair) study, however only recruits patients in a limited number of specified centres. These centres accept aneurysm patients, as tertiary referrals, with a view to endovascular treatment. It may therefore be hypothesised that these centres would experience an increase in workload and be exposed to a larger proportion of high-risk patients. The data presented in this chapter tested this hypothesis. We prospectively evaluated a consecutive series of elective AAA admissions between January 1994 and December 1996 following the initiation of an endovascular aneurysm program in early 1994. This patient cohort was then compared to a retrospectively analysed series of patients who underwent elective aneurysm repair between 1981 and 1993 (Sayers et al. 1997).

10.2 Patients and Methods

At the end of 1993, an endovascular aneurysm surgical program was established, with the first operation performed in March 1994. Patients with abdominal aortic aneurysms referred to the vascular unit at Leicester Royal Infirmary between January 1994 and December 1996 were evaluated prospectively. The source of referral, presentation, diagnosis, associated risk factors, treatment and outcome was prospectively recorded for each patient.
As a source of comparison, patients who underwent elective AAA repair at the same institution between January 1981 and December 1993 were identified from the Vascular Studies Unit audit, death certificate records and the records of the surgical wards, operating theatres and the intensive care unit. The case notes of these patients were reviewed to obtain data on presentation, diagnosis, associated risk factors, treatment, complications and survival (Sayers et al. 1997).

Elective surgical repair was defined as a planned procedure in a patient admitted from the vascular surgical waiting list. Mortality was any death during the initial hospital admission (either at the host institution or after transfer elsewhere) and up to thirty days after surgery. Co-morbidities included any of the following conditions that were being treated at the time of admission: ischaemic heart disease (IHD) (angina pectoris, myocardial infarction [MI], arrhythmias, congestive cardiac failure, coronary artery disease [CAD], coronary artery bypass), hypertension, chronic obstructive pulmonary disease (COPD), and renal failure requiring dialysis.

Endovascular repair entailed a detailed preoperative assessment, which was performed as a 2-day inpatient stay. The evaluation included cross-sectional imaging, radionucleotide ventriculography and anaesthetic consultation. Patients considered suitable for endovascular repair then underwent retrograde femoral aortography with a marker catheter to measure AAA dimensions.

**Statistical analysis**

Continuous data were expressed as the median and interquartile range (IQR). Differences in patient characteristics, referral patterns and mortality were examined with the chi-square test (df = 1) for proportions or the Wilcoxon's rank-sum test for continuous data. Differences between the groups were deemed significant for p < 0.05. All statistical tests were two-tailed and all data were analysed using the Minitab software package for the Apple Macintosh.
10.3 Results

Demographics

During the period from January 1981 to December 1993 315 patients underwent elective conventional AAA repair at Leicester Royal Infirmary, of which complete data were available on 304. The median aneurysm diameter was 5.8 cm (IQR 5.0 - 6.9 cm). In the period, January 1994 to December 1996, 213 patients (177 men) were admitted electively onto the vascular unit for preoperative assessment and cross-sectional imaging. From this cohort 142 underwent elective surgery, 101 via a transperitoneal approach, median aneurysm diameter 5.7 cm (IQR 5.2 - 6.85 cm), and 41 by endovascular techniques, median size 5.5 cm (IQR 5.0 - 5.63 cm). Of the remaining 72 patients 37 were treated conservatively, 19 referred back to their own centre for conventional treatment, 13 are on the waiting list for surgery, and 2 died, 1 while on the waiting list from an unknown cause and the other from an MI during assessment.

There was a significant increase in the median patient age in the two time periods studied, 69 years (range 45-86) to a median 71 years (range 54-86) for the 142 patients treated more recently (95% confidence interval [CI] = 2.001 to 3.001 years, p < 0.001). There were also significant rises in a number of co-morbid risk factors in the prospectively studied patients compared to the retrospectively studied cohort. The incidence of IHD rose from 32.2% (98 of 304) to 43% (61 of 142) (p < 0.05, chi-square value = 4.849) respectively. This was accompanied by a significant increase in the incidences of COPD from 4.6% (14 of 304) to 13.4% (19 of 142) (p < 0.01, chi-square value = 10.877) and hypertension from 36.8% (112 of 304) to 47.2% (67 of 142) (p < 0.05, Chi-square value = 4.308). There was also a slight increase in the incidence of cerebral vascular disease (transient ischaemic attack, amaurosis fugax, cerebrovascular accident, carotid endarterectomy) from 9.2% to 11.3% (chi-square value = 0.461). The risk factors in both groups are demonstrated in Table 10.2.

The median aneurysm diameter for the surgically treated patients in the first time interval was 5.8 cm (IQR 5.0-6.9), which did not differ from the patients who underwent
conventional surgery in the latter time period (5.7 cm [IQR 5.2-6.9]; 95% CI = -0.2001-0.3001 cm, p=0.41)

The aneurysm diameter in the endovascular group was significantly smaller than in the conventional group (5.5cm vs 5.7cm, p< 0.01), there was, however, no difference in size, in those patients undergoing conventional surgery, between the two time periods (5.8cm vs 5.7cm p= 0.41).

**Tertiary Referrals**

Since the instigation of the endovascular program the unit has dealt with an increasing number of tertiary referrals. A total of 89 (41.8%) patients were referred, of whom 52 patients underwent surgery. This represents 36.6% of the total surgical workload, which represents a significant increase on the 9.5% (29 of 304) during 1981-1993 (p< 0.01, Chi-square value = 47.756).

The median age of the tertiary referrals was 73 yrs (IQR 67-78 yrs) equal to that of the Leicestershire patients 73 yrs (IQR 67-76yrs), but higher than both surgical cohorts, reflecting the fact that older patients were more likely to be treated conservatively. The incidence of major risk factors was also higher in the tertiary referral group. Ischaemic heart disease (angina pectoris, myocardial infarction, arrhythmias, congestive cardiac failure, coronary artery bypass) (46.1% vs 43.4%), hypertension (53.9% vs 47.2%), COAD (18% vs 14.5%) and renal failure (haemodialysis, peritoneal dialysis) (6.7% vs 0.8%) were all greater than the percentages seen in the Leicestershire patients.

**Risk Factors**

There were significant rises in a number of co-morbid risk factors. The incidence of IHD rose from 32.2% (98 of 304) to 43% (61 of 142) (p< 0.05, Chi-square value = 4.849), this was accompanied by a significant increase in the incidence of COAD from 5% (14 of 304) to 13.4% (19 of 142) (p< 0.01, Chi-square value =
Table 10.1  Risk factors before and after the establishment of the endovascular program, ischaemic heart disease (IHD), hypertension (HT), chronic obstructive airways disease (COAD), cerebrovascular disease (CVD), diabetes mellitus (DM) and renal failure (RF).

* angina pectoris, myocardial infarction, arrhythmias, congestive cardiac failure, coronary artery bypass.

** transient ischaemic attack, amaurosis fugax, cerebrovascular accident, carotid endarterectomy.

10.877) and also a significant increase in the incidence of HT from 36.8% (112 of 304) to 47.2% (67 of 142) (p< 0.05, Chi-square value = 4.308). The was also a slight increase in the incidence of cerebral vascular disease (transient ischaemic attack, amaurosis fugax, cerebrovascular accident, carotid endarterectomy.) from 9.2% to 11.3% (Chi-square value = 0.461). The risk factors in both groups are demonstrated in Table 10.1.

Mortality

There was a significant increase in the number of operations performed per year after the establishment of the endovascular program (Medians 50 vs 23, Mann-Whitney p< 0.05). The increase in workload was accompanied by a significant increase in mortality from 6.7% to 12% (p< 0.05, Chi-square Value = 4.383). There have been 17 deaths since 1994, 14 (13.9%) were after conventional surgery and 3 (7.3%) in the endovascular group. Two of the conventionally treated patients required additional surgical procedures at the first operation, one had a renal artery stenosis that was reconstructed, and one required re-implantation of, both a lower polar renal
<table>
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<tr>
<td>73 male</td>
<td>Conventional</td>
<td>IDDM, MI, IHD</td>
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<td>HT, COAD</td>
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<tr>
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<td>81 male</td>
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<td>IHD</td>
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**Table 10.2** Causes of death after establishment of endovascular program
artery and the inferior mesenteric artery. In addition 6 of these 17 patients required further surgical intervention at a latter stage. The indications for re-exploration were, unexplained acidosis (3), peritonitis (1), intra-abdominal haemorrhage (1) and lower limb ischaemia (1). The operations performed included bilateral femoro-popliteal bypass grafting (1) and sub total colectomy (1). The causes of death are listed in Table 10.2. Causes of death in the historical group have been detailed in a previous publication(Sayers et al. 1997). A number of factors may have influenced the increase in mortality, there was a significant increase in the age of the patients from a median of 69 yrs (range 45-86 yrs) to a median 71 yrs (range 54-86 yrs) (p< 0.001 Mann-Whitney) in the two time periods studied and also the significant rise in the number of comorbid risk factors described above.

**10.4 Discussion**

The incidence of abdominal aortic aneurysms increased 20 fold in men between 1950 and 1984(Fowkes et al. 1989). Part of the increase may result from improvements in the accuracy of diagnosis and the aging population(O'Hara et al. 1995). However it seems likely that a true increase in incidence has occurred. The increase in elective workload demonstrated in this study may partly reflect this, and similar rises in operation rates have been described elsewhere(Samy and MacBain 1993). The dramatic increase in tertiary referrals since the start of the endovascular program, however, is the major factor in the unit's higher turnover.

The development of endovascular aneurysm surgery has been confined to a small number of centres both within the United Kingdom and worldwide(Parodi et al. 1991; May et al. 1994a; Chuter et al. 1996; Balm et al. 1996; Nasim et al. 1996b). The initial assumption and recent evidence(Thompson et al. 1996b; Boyle et al. 1997) that the procedure is less invasive than conventional transperitoneal surgery has lead to referral of so called high-risk patients to these centres for consideration of endovascular repair. This study confirms that in our experience tertiary referrals are not only older but have higher incidence of co-morbid factors than the abdominal aortic aneurysm population as a whole.
The most disturbing finding of this study is the increase in 30-day mortality from 6.7% during the previous 13 years to 12% in the 3 years following the establishment of the endovascular program. The mortality resulting from conventional surgery group was entirely responsible for this rise. There are a number of factors that may have played a part in the higher mortality. Two of the patients undergoing conventional surgery had simultaneous renal artery reconstruction, a procedure known to carry a mortality of 25% (Nypaver et al. 1993). The process of selection of patients for endovascular repair identifies those with adequate proximal neck length for stent fixation (Armon et al. 1997), it therefore follows that the majority of patients with longer necks will undergo endovascular repair, leaving the technically more difficult juxta-renal AAAs and those requiring renal revascularisation to be treated conventionally. Most (73%) of the patients treated by a transperitoneal approach in this series had been assessed for endovascular repair and were thought not to be suitable for this technique.

The significant increase in the co-morbid risk factors, ischaemic heart disease, chronic obstructive airways disease and hypertension observed in this study may also explain the increase in mortality. Mortality for elective AAA repair has been significantly reduced over the past 30 years with various centres reporting rates between 2-8% (Fielding 1981a; Naylor 1988; Greenhalgh 1990; Akkersdijk et al. 1994; Katz et al. 1994; Johnston 1994; Chen et al. 1996). However the mortality in patients with severe co-existent cardiac, renal or pulmonary disease is reported to be as high as 40% in some series (Gardner 1978; Katz et al. 1994). Coronary artery disease has been identified as a leading cause of perioperative mortality (Hertzer et al. 1984; Roger et al. 1989) and accounted for 6 of the 17 deaths in this series. The other causes of death included the commonly described multi organ failure, renal failure and respiratory failure. Two of the endovascular deaths were the result of microembolisation, a clinical entity that has been described by a number of authors and is said to carry a mortality of greater than 90% (Marin et al. 1995; Parodi 1995) and is thought to result from the increased visceral embolisation observed during endovascular surgery (Thompson et al. 1997f).

Some authors have advocated conservative treatment for AAA patients with severe co-existent medical disease, Bernstein and Chan reporting only a 4% mortality from rupture or after surgery, in a group of 99 high risk patients with small aneurysms
compared with 34% who died from causes unrelated to their unoperated aneurysms (Bernstein and Chan 1984). The authors calculated a cumulative 5-year survival of 63.7% in high-risk patients with aneurysms less than 6cm in size at presentation. Similarly Scott et al have demonstrated encouraging results after the conservative management of high-risk patients with small AAAs (Scott et al. 1993). However Szilagyi et al have reported the prognosis for patients denied surgery as dire, with 43% dying from rupture within two years (Szilagyi et al. 1972) and a five year survival of 17.2%. Yet, when considering only those aneurysms less than 6cm in size the 5-year survival rose to 47.8%. The logical conclusion from these studies is to treat high-risk patients expectantly with serial ultrasound scans if they present with aneurysms of less than 6cm diameter. The ideal management of the high-risk patient with AAA bigger than 6cm, nevertheless, remains controversial. The mortality in this study may have been lower if the higher risk patients were denied surgery, however the long-term aim of elective aneurysm surgery must be to reduce the rate of rupture in the community which carries an overall mortality of 90% (Drott et al. 1992). The benefits of elective surgery must be balanced against the risks; surgery can only be justified when the perioperative and late postoperative mortality is less than the mortality caused by the natural history of the disease. Geroulakos and Nicolaides suggested that even if allowing for a 10% perioperative mortality the benefit in terms of long-term survival outweighed operative loss (Geroulakos and Nicolaides 1992).

In offering an endovascular aneurysm repair centres must be aware that at present there is no evidence that it is a safer technique than conventional surgery. The unit must be prepared to assess high risk patients and manage the 45% (Armon et al. 1997) who are found not to be suitable for this technique. In our experience it is sometimes difficult to persuade a high-risk patient who is not suitable for endovascular repair that they are not fit for conventional surgery. After referral from other centres and extensive investigations patients develop a good understanding of their condition and are well aware of the poor outcome after rupture. They, therefore, are willing to undergo conventional surgery even when quoted a perioperative mortality of 40%. It may therefore be necessary to re-adjust the threshold for surgery in high-risk patients.
The endovascular program also has a number of resource implications. During their assessment, patients undergo a number of investigations including computerized tomography, magnetic resonance angiography and intra-arterial angiography. The endovascular devices are also considerably more expensive than conventional grafts. The faster post-operative recovery and shorter hospital stay offset some of these costs (Boyle et al. 1997). The increased number of tertiary referrals contributes to both the endovascular and conventional workload and despite additional funding, lengthens the waiting list. This may have a detrimental effect on the waiting time for other less urgent vascular conditions, unless provision is made for extra theatre time. In conclusion the establishment of an endovascular abdominal aortic aneurysm program has considerable clinical implications for the hospital concerned.

10.5 Summary

In summary the establishment of an endovascular aneurysm repair program has resulted in an increased caseload passing through the Vascular Unit, a considerable proportion of which is due to a rise in extra-contractual referrals. In addition the patients are older and sicker than in previous years probably reflecting both the aging population and the minimally invasive nature of the technique. The most disturbing finding of this chapter, is the rise in mortality after elective conventional aneurysm repair, which may be partly explained by an increase in high-risk patients undergoing surgery. This finding however has important implications for both preoperative assessment and patient selection prior to conventional surgery.
CHAPTER ELEVEN

Final Discussion, Conclusions and Future work
Abdominal aortic aneurysms are a common cause of death and the incidence of the disease appears to be rising in the Western world. This rise in incidence is in contrast to that of other cardiovascular disease and is too large to be explained by an aging population, greater clinical awareness and improved imaging alone. The evidence suggests that there is a true age specific rise in prevalence of AAA, which will translate to an increased workload for the vascular surgeon. The data presented in Chapter 10 confirms a year on year rise in patients undergoing AAA repair at Leicester Royal Infirmary and this may reflect both a rise in prevalence of disease and the provision of endovascular surgery.

The work presented in this thesis has addressed two current problems in the management of abdominal aortic aneurysms. Firstly the in vitro work has explored therapeutic options for the treatment of small AAA. Secondly the clinical studies aimed to investigate the hypothesis that endovascular AAA repair is less invasive than conventional surgery and thus is better management option for AAA repair in high-risk patients.

There is little doubt that the establishment of AAA screening programs will result in an increased number of patients with small AAA presenting to the vascular surgeon. The conclusions drawn from the recently published UK small aneurysm trial suggest that these patients should be managed conservatively until the aneurysm diameter is greater than 5.5cm at which time patients should be offered AAA repair if fit for surgery (Anonymous, UK Small Aneurysm Trial). Clearly a therapeutic approach aimed at inhibiting or arresting aneurysm growth in those patients who present with small aneurysms is desirable.

Although the cause of aneurysmal degeneration is still unclear, chronic inflammation and destruction of aortic wall connective tissue is integral to the disease process. Whilst numerous connective tissue proteinases have been implicated in aortic wall degradation, the matrix metalloproteinases are believed to play a crucial role and in particular the elastolytic MMPs-2 and-9. It has been suggested that these proteinases provide potential therapeutic targets aimed at reducing AAA growth. The tetracycline family of antibiotics has been demonstrated to possess anti-metalloproteinase properties
and the work presented in Chapter Four of this thesis demonstrated that doxycycline inhibited metalloproteinase activity and subsequent elastin degradation in a porcine aortic organ culture model of aneurysmal disease. These data corroborate the findings previously described in the rat aneurysm model (Petrinec et al. 1996) and have together stimulated further interest in metalloproteinase inhibition as a potential therapeutic avenue for small AAA treatment. Recent work in both models has demonstrated that chemically modified tetracyclines and hydroxamate based MMP inhibitors also inhibit aneurysmal degradation (Curci et al. 1998; Bigatel et al. 1999; Treharne et al. 1999). The results presented in Chapter Four stimulated the study of marimastat, a hydroxamate based MMP inhibitor, in the same porcine organ culture model in our centre. Therapeutic concentrations of marimastat significantly reduced elastin degradation within the aortic wall and, as one would expect with a competitive inhibitor, this was associated with a significant reduction in active MMP-2 at a supratherapeutic concentration (Treharne et al. 1999). Similarly work with batimastat in the rat elastase perfusion model, has demonstrated a reduction in aortic dilatation and preservation of medial elastin in the treated group (Bigatel et al. 1999).

The promising results of these animal studies, particularly those with doxycycline, have also recently led some investigators to perform preliminary human studies aimed at quantifying tetracycline penetration of the aortic wall and its subsequent effect on MMP activity and collagen turnover. Franklin et al administered an intravenous bolus of 500mg of tetracycline to 5 patients undergoing elective infrarenal AAA repair on induction of anaesthesia and demonstrated rapid penetration of the aortic aneurysm wall (Franklin et al. 1999). They also demonstrated in a separate experiment that tetracycline reduced MMP-9 secretion in aortic aneurysm explant cultures, however this in turn had no effect on protein turnover. They concluded that although tetracycline penetrated the aortic wall, the concentration achieved may be insufficient to limit protein turnover through reduced MMP production and activity. However patients in this study only received a single dose of tetracycline and any potential pharmacotherapy aimed at reduced AAA growth is likely to require prolonged treatment. In another recent study five patients were treated with doxycycline 100mg bd. for one week prior to elective AAA repair and compared with an untreated group. The aortic wall tissue demonstrated a 3-fold reduction in aortic
wall expression of MMP-2 and a 4-fold reduction in expression of MMP-9 in the treated group (Thompson and Baxter 1999). It remains to be seen whether this MMP inhibition will prevent human aortic wall degradation and thus potentially reduce aneurysm expansion. The authors suggested that this question can only be resolved by a properly designed prospective randomised clinical trial. The design of such a trial is currently underway, using doxycycline as the MMP inhibitor (Thompson and Baxter 1999).

However there remain a number of unanswered questions that may need to be clarified prior to undertaking such a trial. Firstly doxycycline acts as an MMP inhibitor by a number of different mechanisms, these require further delineation and the active domain of the doxycycline molecule needs identification. The discovery that tetracyclines had anti-MMP activity was serendipitous, and therefore further pharmacological research may allow the development of more specific agents with improved pharmacokinetics and tissue penetration to better target the aneurysm. The organ culture model described in this thesis rapidly mimics the changes seen in aneurysmal disease and provides the ideal setting for further investigation of the mode and site of action of doxycycline. As previously alluded to tetracyclines appear to inhibit MMP activity at a number of different levels, RT-PCR may allow differentiation between direct MMP-inhibition and reduced MMP expression in this model. The investigation of further MMP inhibitors or inhibitors of other proteinases is also easily possible using this technique.

In order to mimic the medial inflammatory cell infiltrate observed in human aneurysmal tissue the porcine aortic organ culture model has been developed further by the addition of autologous leukocytes to the culture medium. These have been shown to accelerate matrix degradation. Further work in this advanced model will allow investigation of the anti-inflammatory effect of doxycycline on macrophage function (Amin et al. 1997).

The results presented in Chapter 5, demonstrate that amlodipine, a calcium antagonist commonly prescribed for hypertension and angina, potentiates metalloproteinase activity and accelerates elastin degradation in the same model. These findings are potentially of great importance as the widespread use of these of these drugs in populations at risk of aneurysmal disease may explain the rising disease prevalence. These however are very preliminary findings and need to be interpreted with some
caution. The study could easily be corroborated, by investigating the effect of amlodipine in the elastase infusion rat model, and both models will also allow the study of other calcium antagonists. Calcium antagonists are in common use in the elderly population, for the treatment of hypertension and angina. Therefore it may be possible to assess whether, growth rates of small AAAs are influenced by calcium antagonists in a screened population, or whether calcium antagonist use is an independent risk factor for aneurysmal disease. Clearly this work is at a very early stage and the effect of calcium antagonists on aneurysmal disease needs clarifying further.

The clinical data presented in this thesis in Chapters 7, 8 and 9 aimed to prove or refute the hypothesis that endovascular AAA repair is less invasive than conventional surgery. As such it concentrated on two comparative cohorts undergoing conventional and endovascular AAA repair and compared cardiovascular, respiratory, renal and metabolic parameters between the groups. The data presented in Chapter 7 demonstrated significantly better respiratory function after endovascular repair than after conventional surgery as assessed by spirometry and overnight pulse oximetry and also reduced morphine consumption in this group. This is the first and as yet only study comparing respiratory function in two such groups. The potential weakness of this study is that the patients were unrandomised, however they were well matched for co-morbid factors. The real strength of this study is the tight anaesthetic control, all the patients received exactly the same general anaesthetic and were managed by independent intensivists in the immediate postoperative period. It is unlikely in the absence of a randomised controlled trial aimed specifically at comparing respiratory function between two such groups that comparable data will become available again, as the rapid development of endovascular technology has led to endograft deployment under regional anaesthesia in the radiological suite.

The cardiovascular data presented in Chapter 8 similarly demonstrates that cardiac index and heart rate were higher during conventional AAA repair and endovascular repair was associated with a significantly lower rise in adrenaline. The differences observed in the haemodynamic variables with cross clamp application and release may have been greater without the maintenance of intravascular volume and the use of inotropes and vasodilators. However we did not feel it was appropriate to clamp
and release the aorta without attempting to attenuate the potentially harmful haemodynamic affects that would follow. The data however imply that aortic occlusion by cross clamp is more invasive than that of intraluminal balloon inflation and these findings may result in lower cardiac morbidity and mortality after endovascular AAA surgery.

The results presented in Chapter 9 demonstrate that endovascular repair is associated with significantly less subclinical renal injury, as assessed by the urinary albumin/creatinine ratio and NAGs, than conventional surgery. There is also a reduced cytokine response in this group, which corroborates the findings of other authors (Swartbol et al. 1996; Syk et al. 1998). The interesting finding of this study was the significantly greater platelet activity after endovascular repair which may relate to organization of thrombus within the aneurysm sac during the perioperative period and could explain the post implantation syndrome described by some authors. Again these findings may translate to a lower incidence of morbidity and mortality after endovascular surgery but these hypotheses need to be addressed by a randomised-controlled trial.

Further data presented in Chapters 7-9 demonstrates that endovascular procedures took significantly longer than conventional repair, had a significantly shorter aortic occlusion time and that patients had a reduced hospital stay. These results concur with those described in other comparative series (May et al. 1998; Brewster et al. 1998). However both these studies did not control for anaesthetic technique as tightly as in this thesis. Interestingly crude mortality and morbidity rates associated with both techniques appear to be similar (Woodburn et al. 1998), May et al quoting a mortality rate of 5.6% in both groups, however the endoluminal patients in this study had significantly greater co-morbidity (May et al. 1998). The recent EUROSTAR data reported a 3.2 % mortality after endoluminal repair in a group of 899 patients of whom 12% were unfit for conventional surgery (Cuypers et al. 1999), approximately half the rate reported after conventional repair by the small aneurysm trial (Anonymous, UK Small Aneurysm Trial 1998). It is likely that as learning curves are overcome and endovascular technology advances morbidity and mortality rates will be lower than for conventional surgery, particularly when the data presented in this thesis is considered.
Recently a randomised control trial has started recruiting patients in the UK. The trial is split into two arms. EVAR (EndoVascular Aneurysm Repair) I, which is randomizing patients suitable for endovascular repair and fit for conventional surgery to one technique or the other. And EVAR II which randomizes patients, unfit for conventional surgery, to best medical management alone, or endovascular repair and best medical management. The results of this trail will not be available for a number of years but should produce accurate morbidity and mortality rates for both techniques.

Since starting work on this thesis the enthusiasm for endovascular AAA repair has grown enormously. Research has been undertaken into many aspects of the procedure including morphological assessment, endograft design and technical success. Medium term results of the technique have now become available. Most authors have identified significant rates of early and late endoleak after endovascular AAA repair and the long-term durability of the technique remains unproven. It has not been the aim of this thesis to address the problems of endograft durability, however, clearly there is little point in developing a minimally invasive approach to AAA repair unless long term results are comparable to conventional surgery. We will probably need to wait for the results of the EVAR trials to resolve these issues. In the meantime the prospects of further improvements in graft technology and delivery system design and the perceived reduced risk of morbidity and mortality will continue to make endovascular AAA repair an attractive option to both patient and vascular surgeons alike.


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