Evaluation of the effect of uterine artery embolisation on symptomatic uterine leiomyomata and analysis of the vasculature associated with these benign tumours

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Abstract

Uterine leiomyomata (fibroids) are the most common tumours found in the female reproductive system. Whilst many women affected by these benign tumours are asymptomatic, those who do have symptoms may experience a considerable reduction in quality of life and impose a significant burden on the National Health Service. Surgery remains the established treatment for women with fibroids unresponsive to medical treatment. Hysterectomy is the gold standard for those women who have completed their family whilst myomectomy has been the preferred option for women who still wish to conceive in the future.

Uterine artery embolisation (UAE) is a minimally invasive angiographic technique which was first reported in 1995 as an alternative treatment for symptomatic fibroids. Since its introduction, more than 100,000 procedures have been carried out over the last decade. Studies evaluating the technique are encouraging, stating that embolisation is effective in reducing fibroid-associated symptoms such as heavy menstrual bleeding, pelvic pain and bulk-related problems. As a result, the procedure is becoming increasingly accepted worldwide as a uterine sparing alternative for the treatment of symptomatic fibroids. Embolisation, however, requires futher evaluation in terms of its safety and efficacy in comparison to established therapeutic options for symptomatic fibroids. In addition, longer term data is required in order to establish the durability of the procedure and potential for associated late presentation of complications and failures.

The mechanisms by which fibroids develop and grow is still unclear to us and thus, examination of the uterine vascular network in greater detail may assist further in our understanding of the biology of these benign tumours. This may, in

turn, assist us in our understanding of the reasons for success and failure of fibroid therapies such as uterine artery embolisation

Chapter one provides an introduction to all work in the thesis and initially discusses the histology, incidence and epidemiology of uterine leiomyomata. The pathophysiology of fibroids is also explored, with particular reference to genetic, hormonal, vascular and growth factor influences. Myometrial and fibroid vasculature is discussed in this chapter as well as fibroid symptomatology and the aetiology of menorrhagia. Finally, therapeutic options for fibroids including UAE are explored as well as the relevance of quality of life assessments in the evaluation of UAE and an overview of the currently available comparative data on embolisation versus surgery in the treatment of symptomatic fibroids.

Chapter 2 presents data from a prospective study evaluating the effect of UAE on menstrual blood loss (MBL) and uterine volume. Main outcome measures were post-embolisation MBL (objectively measured using the alkaline haematin technique) and uterine volume changes. This study concluded that UAE is associated with a statistically significant reduction in objectively measured MBL which is maintained up to 48 months after treatment and a statistically significant reduction in uterine volume at six months.

Chapter 3 describes subjects and methods used to evaluate the effects of UAE on health-related quality of life (HRQoL). Using the Short Form 36 (SF 36) questionnaire, we concluded that health status is significantly improved at 3 and 6 months following UAE. In addition, this improvement appears to be maintained up to 60 months after treatment. It was also concluded that the observed increase in SF 36 scores provides evidence for the efficacy of UAE.

Chapter 4 sets out to investigate the evolution of inflammatory markers after UAE in order to monitor the normal course following the procedure. It was anticipated that the results would provide further insight into the aetiology of the post-

embolisation syndrome, a syndrome which occurs approximately 7-21 days after UAE and is associated with pelvic pain and flu-like symptoms. We found that uncomplicated UAE is associated with a significant rise in inflammatory markers, specifically white cell count (WCC), C-reactive protein (CRP) and interleukin 6 (IL-6). The peak rises occur at 3 days with normalisation of marker levels at one month. No correlation was found between pain scores at 24 hours and individual inflammatory markers at that time. These findings do not assist in providing an explanation for the post-embolisation syndrome.

Chapter 5 aims to evaluate and compare the safety and efficacy of UAE to standard surgical treatments for symptomatic fibroids. This is addressed in the setting of a multicentre randomised controlled trial. The primary outcome measure was health related quality of life at one year as assessed by the SF 36. The study demonstrated there were no significant differences between the embolisation and surgical arms of the trial, in all components of the SF 36 at one year. Embolisation, however, was associated with a shorter duration of hospitalization and a shorter period of time until resumption of normal activities whilst surgery was associated with better symptom scores at one year. Serious adverse events and treatment failures were accounted for in this trial and we concluded that the faster recovery after embolisation must be weighed against the need for further treatment in a minority of patients.

Chapter 6 highlights a late complication after uterine artery embolisation and presents the data in the form of a case report.

Chapter 7A aims to compare human myometrial and fibroid vasculature using stereological and morphometric analysis. A decreasing gradient of vascular smooth muscle from outer to inner myometrium was found in normal uteri, with no corresponding gradient in capillary tissue fraction. An association between vascular luminal size, amplitude and frequency of vessel bending was also

established. Fibroids, however, were found to lack structured or muscularized vasculature. We concluded that a quantitative gradient exists within the myometrial vascular system which is absent in fibroids. These structural differences between normal and diseased tissue may explain the distribution of embolic material and thus the mechanism by which uterine artery embolisation achieves its therapeutic purpose.

Finally, *Chapter 7B* describes experimental work involving vascular perfusion techniques and magnetic resonance imaging (MRI) of fresh human uterus ex-vivo. It was anticipated that this study would demonstrate better resolution of the microvascular network of the uterus, but we did not find this to be the case.

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Declaration

I declare that this thesis has been composed by myself. I have been responsible for patient recruitment, tissue collection, laboratory studies and data analysis, unless otherwise acknowledged.

I confirm that the contents of this thesis have not been submitted elsewhere for any degree, diploma or other professional qualification.

Aradhana Khaund

Glasgow, May 2007

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I am very grateful for the help of Dr David Millan, who facilitated the retrieval of archival material used in chapter 7a. In addition, Dr Millan was of great assistance to me when selecting the appropriate pathology specimens for my study. I would also like to give my thanks to the radiographers at Gartnavel General Hospital who were kind enough to perform MRI and MR-angiography on fresh human uteri out of hours, and also, to Dr Andrew Fagan, for both his very helpful advice regarding high intensity MRI and permission to the use of the 7-Tesla MRI at Glasgow Vetinary School.

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I dedicate this thesis to my dear parents, Dr RR Khaund and Mrs C Khaund, who have always encouraged me in all that I do.

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Abbreviations

ADP – adenosine diphosphate

ATP – adenosine triphosphate

bFGF – basic fibroblast growth factor

cm - centimetre

CK – creatine kinase

CRP – C-reactive protein

FBC – full blood count

FGF – fibroblast growth factor

FH – fumarate hydrase

GM-CSF – granulocyte-macrophage colony-stimulating factor

GnRH – gonadotrophin-releasing hormone

Hb – haemoglobin

HLRCC – hereditary leiomyomatosis and renal cell carcinoma

HRQoL – health related quality of life

IL-6 – interleukin 6

IL-18 – interleukin 18

IMA – ischaemia-modified albumin

IUS – intra-uterine system

IVF – in-vitro fertilisation

MRA – magnetic resonance angiography

MRI – magnetic resonance imaging

MPA – medroxy progesterone acetate

MBL – menstrual blood loss

mRNA – messenger ribonucleic acid

MVD – microvascular density

ml – millilitres

NICE – National Institute of Clinical Excellence

NSAIDs – non-steroidal anti-inflammatory drug

OCP – oral contraceptive pill

OD – optical density

PBAC – pictorial blood loss assessment chart

PBS – phosphate buffer solution

PDGF – platelet derived growth factor

PVA – polyvinyl alcohol

RCT – randomised controlled trial

REST – randomised controlled trial comparing embolisation to surgery for the treatment of fibroids

SAE – serious adverse event

SF 36 – short form 36

TAH and BSO – total abdominal hysterectomy and bilateral salpingoophorectomy

TNF-alpha – tumour necrosis factor alpha

TGFβ – transforming growth factor beta

UAE – uterine artery embolisation

UEA-I – ulex europaeus agglutinin 1

UFS-QOL – uterine fibroid symptom and quality of life

VEGF – vascular endothelial growth factor

WCC – white cell count

Chapter 1

Introduction

1.1 **Fibroids**

Uterine leiomyomata (fibroids, myomas, fibromyomas) are the most common benign tumours of the female genital tract. They can develop at various sites within the body, but are most frequently found to affect the uterine myometrium, arising from neoplastic transformation of single smooth muscle cells. The vast majority are found in the corpus (body) of the uterus (Stewart 2001). Less commonly, these benign growths occur in the cervix, uterine ligaments and ovary.

In 1852, Sir James Paget introduced the familiar name "fibroid", referring to a mass that resembles fibrous tissue in both texture and to the naked eye (Sampson JA 1912). However, the term does not imply that the tumour is composed of fibrous tissue microscopically. In fact, the presence of significant collagen within a leiomyoma is unusual. Nonetheless, the term "fibroid" is both universal in its use and understood by all, despite its unscientific origin.

Leiomyomas usually occur within the myometrium (uterine muscle) as well-circumscribed, firm, rubbery tumours with a characteristic white whorled appearance on cross-section (Shaw, Soutter & Stanton 1992). They are paler than the surrounding myometrium and there is usually a very sharp line of demarcation between the tumour and the normal uterine muscle **(Figure 1)**. Histologically, leiomyomata are typically composed of spindled smooth muscle cells arranged in fascicles with bland, uniform, cigar-shaped nuclei, arranged in interlacing bundles, showing little or no mitotic activity. They have abundant eosinophilic cytoplasm and the collagenous extracellular matrix tends to be prominent (Shaw, Soutter & Stanton 1992;Stewart 2001).

The size of a fibroid uterus is described in the same fashion as a pregnant uterus, in menstrual weeks. Unlike the pregnant uterus, however, it is usually irregular in shape **(Figure 2)**.

Figure 1

Cut surface of fibroid uterus – *demonstrates pale appearance of fibroid and line of demarcation between normal myometrium and fibroid tissue*

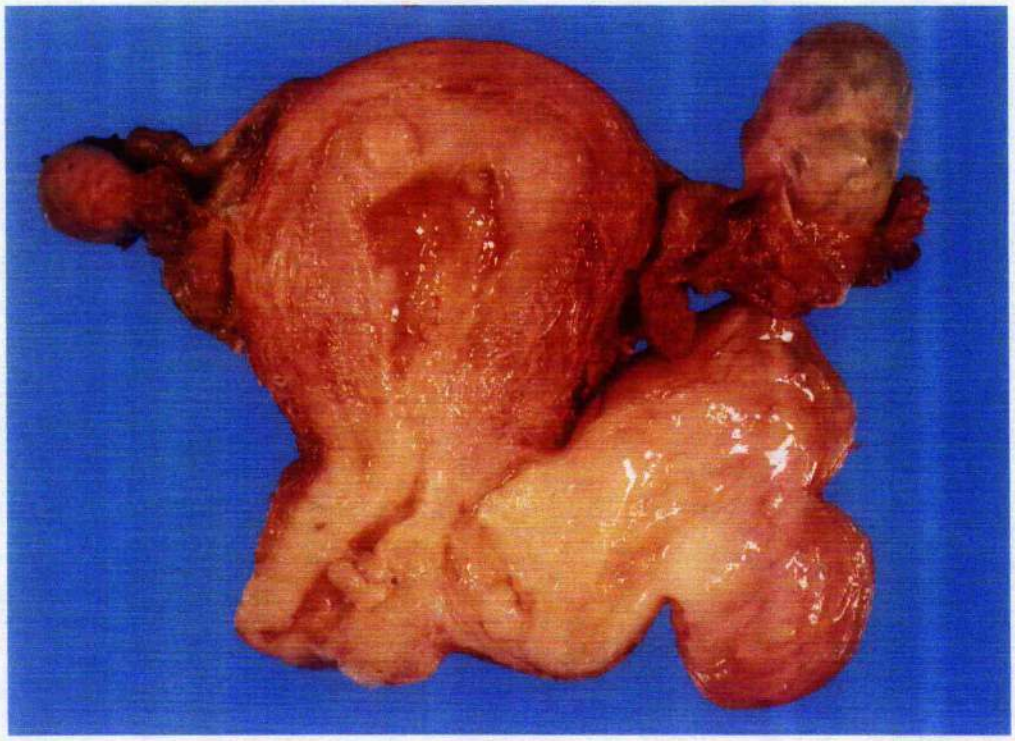
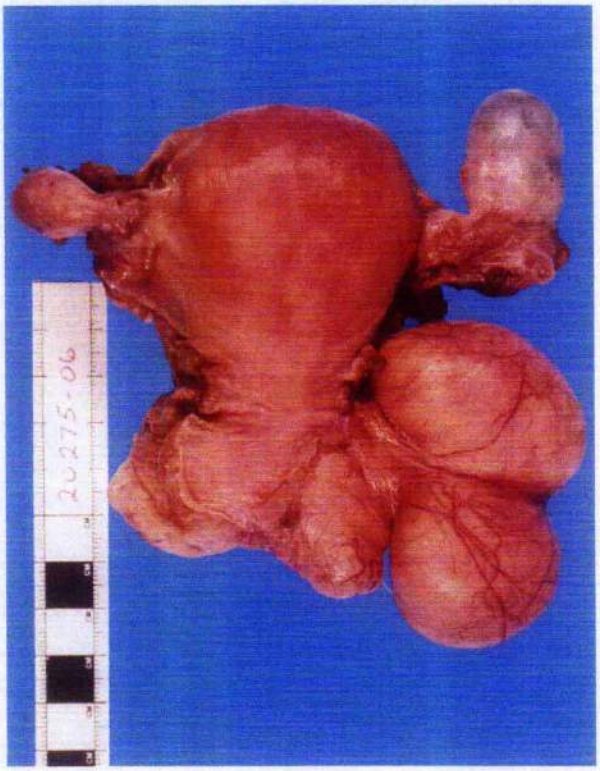


Figure 2

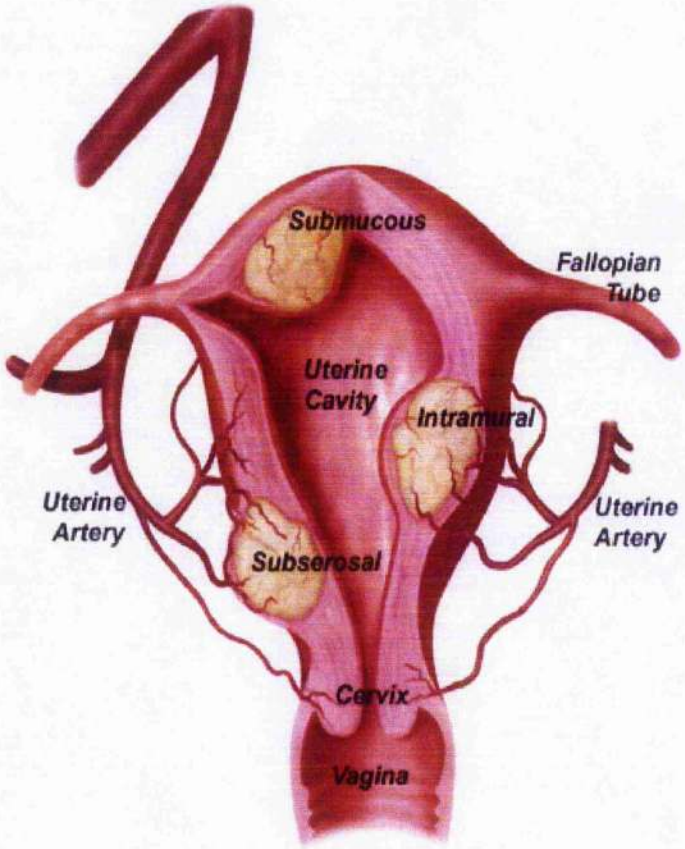
Gross specimen of fibroid uterus



Fibroids may be single, but are commonly multiple and can be further classified according to their location (**Figure 3**). The four clinical subgroups that we recognise include intramural, subserosal, submucous and cervical fibroids. Further descriptive classification include pedunculated fibroids, where a fibroid is attached to the normal myometrium of the uterus by a stalk and the rare parasitic fibroid, where the fibroid has developed an alternative blood supply, has separated from the uterus and become attached to another structure in the pelvis (Shaw, Soutter & Stanton 1992).

Figure 3

Location of fibroids - classification



The true incidence of fibroids is uncertain as many women with these tumours are asymptomatic and thus fail to reach clinical attention. Prevalence rates tend to be based on rates of diagnosis in symptomatic individuals and following pathological assessment of hysterectomy specimens. Whilst such estimates represent the morbidity associated with fibroids, it is likely that we significantly underestimate the true prevalence of these uterine lesions. Nonetheless, we are aware that fibroids occur in 20-30% of women during reproductive life, rising to 40% in women above the age of fifty years who are still menstruating (Buttram & Reiter 1981).

Leiomyomata respond to the gonadal steroids oestrogen and progesterone and their epidemiology parallels life-cycle changes in these reproductive hormones. These tumours have not been described in prepubertal (Stovall 2001) girls and are very rarely seen in adolescents. They are most commonly reported in women between the ages of thirty and fifty years and once women reach the menopause, fibroids tend to shrink, when both steroid hormone concentrations and menstrual cyclicity wane (Stewart 2001). However, some studies have shown an increase in risk of fibroids and their associated symptoms with the use of hormone replacement therapy (HRT) in the post-reproductive period, especially in those women who have taken HRT for 8 years or longer (Schwartz 2001) and have a body mass index (BMI) of less than 24 kg/m^2 (Reed et al. 2004). Certainly, with the use of HRT, they are less likely to decrease in size after the menopause, but larger epidemiological studies are required before we can make definite conclusions regarding the relationship between fibroids and HRT use during the menopause.

Further support of the role of oestrogen and progesterone in the pathogenesis of fibroids is the finding that the earlier the age of menarche, the greater the risk of developing fibroids (Marshall et al. 1998a).

There are significant racial differences in the incidence of uterine leiomyomata. Afro-Caribbean women have a 2-9 fold greater risk of developing fibroids and tend to present with these tumours at a younger age at the time of diagnosis when compared to Caucasian women. Not surprisingly, they subsequently undergo definitive treatments such as hysterectomy at earlier ages. In addition, Afro-Caribbean women tend to have multiple fibroids, higher uterine weights and are more prone to both anaemia and severe pelvic pain (Kjerulff et al. 1996;Schwartz 2001;Stewart 2001;Wise et al. 2004). This excess rate of fibroids in the latter group of women cannot simply be attributed to a higher prevalence of risk factors and is more likely to be due to a genetic predisposition.

Reproductive factors also influence the risk of fibroids and there certainly appears to be a reduction in the chance of developing fibroids in parous (having one or more pregnancies extending beyond 24 weeks) women (Luoto et al. 2000;Marshall et al. 1998a;Schwartz 2001;Stewart 2001;Wise et al. 2004). This reduction in risk increases with each liveborn child and decreases with an increasing interval of time since last birth (Chen et al. 2001;Luoto et al. 2000;Marshall et al. 1998a;Schwartz 2001;Stewart 2001;Wise et al. 2004). The true incidence of fibroids in pregnancy is unknown although rates of 0.1% - 12.5% have been reported. Despite the occurrence of red degeneration in early pregnancy, pregnancy has little or no effect on the overall growth of fibroids (Cooper & Okolo 2005).

Whilst there appears to be an association between the risk of developing fibroids and steroid hormone concentrations, studies linking the use of the oral contraceptive pill (OCP) and incidence of fibroid formation are conflicting (Chen et al. 2001;Marshall et al. 1998a;Schwartz 2001;Stewart 2001). It may well be that the timing of exposure to these exogenous hormones is the critical event which determines whether or not fibroid growth is suppressed or encouraged.

Overall, it appears that use of the OCP outwith the teenage years has a protective effect against fibroid formation and growth, and this effect is directly proportional to the duration of use (Marshall et al. 1998a;Stewart 2001). OCP use and pregnancy are both associated with high steroid hormone concentrations, suggesting that mechanisms other than hormones, influence the development of fibroids.

Depot medroxyprogesterone acetate (MPA) is an injectable progestin-only contraceptive. A study in Thailand showed a strong inverse association between a history of depot medroxyprogesterone acetate use and the risk of surgically confirmed uterine leiomyomata (Lumbiganon et al. 1995). In particular, the reduction in risk was most pronounced among women who had been using depot MPA for 5 or more years and who were currently using this contraceptive method. Studies correlating the risk of fibroids and obesity are inconsistent. However, we do know that obesity is associated with higher levels of endogenous oestrogen and generally, it has been shown that there is a tendency towards an increased risk of developing fibroids with increasing BMI (Luoto et al. 2000;Marshall et al. 1998b;Schwartz 2001;Wise et al. 2004). It may also be possible that obesity does not directly influence the development of fibroids, but rather, promotes the development or severity of fibroid – associated symptoms and / or reduces the effectiveness of non-surgical therapeutic options for these tumours (Schwartz 2001).

It has been reported that there is an association between diabetes and the risk of uterine fibroids (Faerstein, Szklo, & Rosenshein 2001). Hyperinsulinaemia and insulin-like growth factors (IGFs) have been implicated in the past, for many malignant and benign genital tract pathologies (Macaulay 1992;Yu & Rohan 2000). We know that insulin resistance and hyperinsulinaemia, both of which are associated with clinical diabetes, may also be causes for hypertension,

hyperlipidaemia and obesity. All of the aforementioned factors may cause atherosclerosis (Reaven 1988). Bearing this in mind, it has been suggested that the development of fibroids may share some factors with the development of atheromatous plaques which also involves a smooth muscle cell proliferation of monoclonal origin (Mashal et al. 1994).

Environmental factors also influence the risk of fibroid development. Independent of BMI, smoking appears to decrease the risk of fibroid development (Parazzini et al. 1996;Schwartz 2001) as does a high intake of green vegetables and independently, a low consumption of red meats (Chiaffarino et al. 1999;Schwartz 2001).

1.2 Pathophysiology of fibroids

The pathophysiology of fibroids remains poorly understood, although it is clear that gonadal steroid hormones and their receptors play a vital role in formation and growth of these tumours. Fibroids, themselves, contain both oestrogen and progesterone receptors and therefore are responsive to hormonal stimulation. The clinical course of women with symptomatic fibroids strongly suggests that the growth of these benign tumours is promoted by both oestrogen and progesterone signalling (Maruo et al. 2004). However, sex steroids are not the only regulators of fibroid growth since oestrogen and progesterone levels tend to be normal in almost all women with symptomatic fibroids. As yet, it is not clear as to whether sex steroid signalling directly causes the formation of leiomyomata founder cells either by transformation of normal myometrial lineage cells or by clonal expansion of mesenchymal cells.

Historically, fibroids have not been considered a genetic disease, but now, however, we know that genetic predisposition, gene dysregulation and growth

factors important in both fibrotic processes and angiogenesis, also play roles in fibroid actiology (Stewart 2001).

Initial support of a genetic liability came from twin studies where it was seen that in women undergoing hysterectomy for symptomatic fibroids, a strong element of heritability occurred (Snieder, MacGregor, & Spector 1998;Stewart & Morton 2006). Twin studies prior to that established a concordance rate for hysterectomy (for all indications) in monozygous twins that is twice that observed in dizygous twins (Treloar et al. 1992). It has also been shown that women with a positive family history of fibroids have a 1.5 – 3.5 fold greater risk of developing these tumours than those women who do not (Ligon & Morton 2001;Schwartz 2001;Van Voorhis, Romitti, & Jones 2002).

It is postulated that there are at least two components to fibroid development; the first process which is quite common, involves transformation of normal myocytes into abnormal myocytes whilst the second process involves their growth into clinically apparent tumours. The latter process occurs via clonal expansion (Mashal et al. 1994;Stewart 2001).

We know that 60% of fibroids are chromosomally normal with a 46, XX karyotype (Rein et al. 1991). Of the 40% that have an abnormal karyotype, certain consistent patterns are found suggesting that genes important in the formation and development of fibroids are likely to be found in these disrupted regions. There is, however, major discordance in the karyotypic abnormalities found in fibroids and in leiomyosarcomas, suggesting that benign and malignant uterine/myometria tumours arise via different pathogenetic pathways (Stewart 2001).

The first chromosome aberrations in fibroids were described in 1988 when the t(12;14) translocation was shown to be a specific abnormality (Gibas, Griffin, & Emanuel 1988;Heim et al. 1988;Turc-Carel et al. 1988). The first gene to be discovered in fibroid biology was HMGA2 (previously HMGI-C), dysregulated

by translocations between chromosomes 12 and 14. HMGA1 (previously HMGI(Y)), associated with rearrangements of chromosome 6, is a related gene, also found to have a role in fibroid biology (Stewart & Morton 2006;Williams et al. 1997).

Several hereditary syndromes have been reported which involve multiple types of smooth muscle tumours, including uterine fibroids (Ligon & Morton 2001). Many of these display Mendelian inheritance and thus facilitate relatively straightforward approaches to gene discovery for these benign tumours. Presently, the most clinically important syndrome known is *hereditary leiomyomatosis and renal cell carcinoma* (HLRCC) owing to its associated risk of malignancy (Kiuru et al. 2001;Stewart & Morton 2006). This is an autosomal dominant disease where both cutaneous and uterine fibroids are present. Both men and women may present with this phenotype and all who are affected are at risk of papillary renal cell carcinoma (Launonen et al. 2001). Women, however, appear to have an increased risk of renal cell carcinoma when compared to male family members. They are also at risk of developing leiomyosarcomas pre-menopausally, the malignant form of fibroids or leiomyomas (Launonen et al. 2001;Toro et al. 2003).

Reed's syndrome also known as *multiple cutaneous and uterine leiomyomata* (MCUL1) is often reported as an individual syndrome, however, it is now clear that both Reed's syndrome and HLRCC are one disease (Stewart & Morton 2006). Both syndromes became associated when it was found that they involved the fumarate hydratase (FH) gene (Alam et al. 2003;Colgan et al. 2003). The latter gene codes for the Kreb's cycle enzyme that converts fumarate to malate. It appears that fumarate hydratase acts via a loss of function or tumour suppressor mechanism for uterine fibroids. Many different mutations of FH exist, all of which result in an absent, truncated or non-functional protein (Alam et al.

2003;Tomlinson et al. 2002). Family members who are affected by fibroids and cutaneous lesions should therefore be screened for FH mutation as they are at increased risk of developing papillary renal cell carcinoma and if female, are at increased risk of developing uterine sarcoma too. Currently, however, testing for this gene is not commercially available. The FH gene has also been shown to play a role in some women with non-syndromic fibroids (Gross et al. 2004;Lehtonen et al. 2004).

Clinical studies are now beginning to identify DNA polymorphisms that influence fibroid risk (polymorphisms are small sequence variations that are clinically silent because there is no amino acid change) and the development of sib-pair analysis has allowed us to search all chromosomes for genes involved in uterine fibroids (Stewart & Morton 2006).

The genetics of uterine fibroids therefore play a vital role in our understanding of the pathophysiology of these tumours. We are aware that certain treatments for fibroids have varying efficacies in individuals and so understanding the different phenotypes and genotypes associated with fibroids, is clinically very relevant.

Abnormalities in uterine vasculature and angiogenic growth factors are also involved in the pathogenesis of uterine fibroids. We are aware that the fibroid uterus has increased numbers of arterioles and venules and it is likely that molecular changes are responsible for increased vessel formation and alterations in vessel function (Stewart & Nowak 1996).

Leiomyomata are associated with higher amounts of messenger ribonucleic acid (mRNA) for basic fibroblast growth factor (bFGF) - an angiogenic growth factor - than matched myometrium (Stewart 2001). bFGF has been shown to stimulate proliferation of leiomyoma cells in culture (Lee et al. 1998). There are also differences seen in bFGF receptors of normal and myomatous uteri. In addition, a reservoir of bFGF protein is stored in the extracellular matrix that characterises

fibroids, a finding which is consistent with the role of bFGF as a heparin-binding growth factor (Mangrulkar et al. 1995). An abnormal expression of the type 1 bFGF receptor in the endometrium has also been demonstrated in women with fibroid-related bleeding, a finding not seen in women who have normal menstrual cycles (Anania et al. 1997). Platelet-derived growth factor (PDGF), another angiogenic growth factor, is expressed in similar levels in both normal myometrium and fibroid tissue. Its expression, however, is increased in the myometrium during pregnancy, suggesting that PDGF has a role in smooth muscle hypertrophy at this time (Hague et al. 2000). Both bFGF and PDGF are heparin-binding growth factors.

Fibroid growth and development can be viewed as a fibrotic process, where specific upregulation of collagen types I and III can be seen. The latter are major components of the extracellular matrix that distinguishes fibroid tissue from normal myometrium (Stewart et al. 1994). As in other fibrotic processes throughout the body, there is also evidence that transforming growth factor beta (TGFβ) and granulocyte–macrophage colony–stimulating factor (GM-CSF) may be involved in fibroid pathophysiology. Greater amounts of TGFβ and TGFβ-receptor mRNA and protein have been identified in fibroids than in normal myometrium, during the secretory phase of the menstrual cycle (Dou et al. 1996). In addition, fibroids from women who have been previously treated with a gonadotrophin-releasing hormone (GnRH) agonist, a treatment shown to cause reduction in fibroid size, have significantly reduced TGFβ concentrations (Dou et al. 1996). It may be that by regulating its own expression and that of TGFβ, GM-CSF also induces the tissue fibrosis that we see in fibroids (Chegini, Tang, & Ma 1999).

1.3 Myometrial and fibroid vasculature

The human uterus is supplied by the uterine, ovarian and vaginal arteries, all of which anastomose, giving the organ a rich blood supply (Ramsey 1994). The right and left uterine arteries penetrate the myometrium giving rise to circumferentially orientated arcuate arteries which in turn, branch radially, to form radial arteries (Farrer-Brown, Beilby & Tarbit 1970b). The latter traverse the myometrium and penetrate the endometrium as small calibre spiral arterioles. Referring exclusively to the endometrial vessels as spiral is somewhat of a misnomer because the whole uterine arteriolar system is in fact tortuous and spiral in character. In contrast, the thinner walled venous system, although radial in orientation, does not exhibit coiling, but instead, runs a relatively straight course with smaller cross-communicating venules (Farrer-Brown, Beilby & Tarbit 1970c).

Development of the embryonic vascular system results from a continual remodelling process which spans the development of small vessels of the primary plexus to the formation of large muscularized elastic arteries (Drake, Hungerford, & Little 1998;Hungerford et al. 1996;Risau 1997). There is early distinction between arteries and veins, with both linear and radial growth of the vascular tree (Burke, Wang, & Jones 1994;Drake, Hungerford, & Little 1998;Kohnen et al. 2000). Linear growth facilitates increase in size of the embryo whilst radial growth allows both increases in wall thickness and luminal diameter (Drake, Hungerford, & Little 1998). Microangiography of perfused uterine slices demonstrates that spiralling of vessels occurs when axial growth of the vessel exceeds that of the surrounding tissue (Farrer-Brown, Beilby & Rowles 1970a; Farrer-Brown, Beilby & Tarbit 1970b). Similarly, growth of the endometrial arterioles, during the menstrual cycle, follows a similar pattern. Gross vascular changes occur in the female genital tract during adulthood, and in pregnancy, the uterus, with its unusually structured vascular supply, accommodates both stretch

and a vast increase in the linear dimensions of uterine volume (Fleming & Bell 1997;Gunja-Smith & Woessner 1985).

The vascular pattern in human myometrium and fibroids has been studied for a number of years and dates back to as early as 1912 (Sampson JA 1912). Fibroid vasculature has been studied in a quantitative manner only recently. Prior to this, most studies have been qualitative, using a variety of techniques to compare fibroid and normal myometrial vasculature. The earliest work in this area focused on describing the structure of fibroid vasculature, using injection of either pigment or radio-opaque dyes to outline the vessels (Faulkner 1944;Holmgren B 1938). These studies demonstrated that the arteries supplying the fibroids were dilated in appearance when compared to those supplying the myometrium, suggesting that there was an increase in fibroid vasculature relative to normal uterine tissue.

In the 1970s, Farrer-Brown and colleagues went on to find differences in fibroid vasculature in fibroids of different size. They carried out uterine macroangiographic studies which initially focused on describing structural arrangements, illustrated by projection images (Farrer-Brown, Beilby & Rowles 1970a; Farrer-Brown, Beilby & Tarbit 1970b; Farrer-Brown, Beilby & Tarbit 1970c; Farrer-Brown, Beilby & Tarbit 1970d; Farrer-Brown, Beilby & Tarbit 1971). The earlier studies involved injection of a radio opaque medium into the blood vessels of the uterus at both physiological pressure and temperature. Radiography of five millimetre thick transverse uterine slices was subsequently carried out. Selected slices were then cut in half or into slices between 75 and 500 micrometers thick, prior to visualisation under the microscope. This simple perfusion technique demonstrated the macrovasculature of the uterus and revealed a level of detail that had not been previously demonstrated. It was also used to study myometrial fibroids and demonstrated substantial differences between normal myometrium and fibroid tissue (Farrer-Brown, Beilby & Tarbit 1970b;

Farrer-Brown, Beilby & Tarbit 1970c; Farrer-Brown, Beilby & Tarbit 1970d). Arterial vessels of uterine fibroids were typically increased in size, especially in association with larger tumours whilst the degree of arterial vascularity was variable. It was also observed that small fibroids were usually, although not always, less vascular than the surrounding myometrium, whilst larger fibroids tended to be more vascular than their surrounding myometrium. In addition, it was demonstrated that there was no intrinsic vascular pattern within fibroids. Instead, the vascular arrangement of fibroids was thought to represent a localized expansion of the vasculature of the corresponding myometrium, with the vessels within these tumours orientated in the direction of the muscle cell bundles thus accommodating localised proliferation of smooth muscle. We would now interpret this as tumour associated angiogenesis. Changes in the vasculature distant to the tumour were also seen; in particular, dilated and congested vessels contralateral to the site of the fibroid were observed.

Unlike studies carried out thirty years earlier, no venous lakes were demonstrated within the normal myometrium (Farrer-Brown, Beilby & Tarbit 1970c), but dilatation of myometrial veins around the periphery of a fibroid is a feature demonstrated repeatedly by Farrer-Brown and colleagues. Within the inner myometrium, close to the endo-myometrial junction, there appears to be a relatively "anaemic zone", first noted by Sampson in 1912. This corresponds to newer data which reveal a difference between inner and outer myometrium. The network of veins draining the inner myometrium gradually increase in size as it runs outwards in a radial direction and joins the plexus of circumferentially running arcuate veins (Farrer-Brown, Beilby & Tarbit 1970c).

Since then, studies involving corrosion casting and scanning electron microscopy (Skopichev & Savitski 1992) have been carried out. This method involves injection of a resin which fills the whole vascular bed, including capillaries.

Thereafter, successive corrosion of the tissue unmasks the vascular network which is then amenable to examination with electron microscopy. The authors found that small fibroids were avascular whilst larger ones (3-10 millimetres in diameter) were surrounded by a dense and compressed vascular network with penetration of the fibroid tissue by small vessels originating from the fibroid capsule itself. The authors also found that the larger the fibroid, the greater the vessel density although this was never greater than that of its surrounding myometrium.

Blood flow and perfusion studies have also been useful in providing further information about fibroid vasculature (Forssman 1976a;Forssman 1976b). Forssman demonstrated that leiomyomata had significantly lower blood flow than the surrounding myometrium, leading to the hypothesis that the lower blood flow associated with fibroids may be responsible for the degenerative changes often seen in these benign tumours. MRI studies using gadolinium enhancement to assess fibroid and myometrial vascularity, confirm the findings of reduced arterial perfusion in leiomyomata (DeSouza & Williams 2002). However, not all studies concur with the finding of reduced blood flow in fibroids; colour Doppler ultrasound has been shown to demonstrate an increased blood velocity in the uterine arteries of women with fibroid uteri when compared with women with normal non-fibroid uteri controls.

More recently, quantitative studies of human uterine and fibroid vasculature have been carried out. Morphometric analysis (i.e. linear or area measurements made on tissue sections) of normal and diseased myometrial tissue suggest that fibroids lack the well developed vascular pattern of normal uterine muscle (Casey, Rogers, & Vollenhoven 2000;Gargett et al. 2002;Hague et al. 2000;Ponnelet et al. 2002;Weston et al. 2003). Casey et al carried out a quantitative analysis of microvascular density (MVD) using immunohistochemical techniques which involved staining of normal myometrial and fibroid tissue with a variety of

endothelial cell markers. They found that the MVD (i.e. vessels per mm^2) and proportional vascular area (proportion of each tissue staining as blood vessel) of myometrium are greater than that of fibroids, both large and small. Whilst this does support the blood flow studies of Forssman and de Souza and Williams, it contradicts the findings of both Sampson and Farrer-Brown et al who demonstrated increased vascularity in large fibroids compared with the adjacent myometrium. A more recent immunohistochemical study also supports the finding of increased MVD and vascular area in myometrium when compared to fibroid tissue (Poncelet et al. 2002).

The three-dimensional architecture of uterine microvasculature may be demonstrated with more precision, using stereological analysis. Stereology involves estimations of three-dimensional quantities from sections, using spatial probes of lines or points. The three-dimensional nature of the microvascular network on cut surfaces of fresh human uterus has been visualised and demonstrated recently (Hamid, Daly, & Campbell 2003), by diffusion labelling. Using a fluorescent lectin, Ulex Europaeus Agglutinin I (UEA-I), which labels endothelial cells by diffusing into tissue and binding to the endothelial cells, Hamid et al were able to visualise the endothelium of the uterus directly. The use of an endothelial marker, in this case, UEA-I, highlighted the vascular differences between normal myometrium and fibroid tissue. In particular, fibroids were noted to have a less dense distribution of vessels when compared to normal tissue. In an extension of this technique, a further study was carried out, using anti-smooth muscle antibodies to label the surrounding smooth muscle. The study demonstrated that vessels in fibroids were less curved and had fewer branches, when compared to normal myometrium. The fibroids also displayed considerable variation in the structure of their muscle bundles; this was thought to be due to a

combination of the plane of visualisation and the pathological characteristics of the fibroids themselves (Hamid et al. 2006).

In addition to variations in vascular organization, myometrial muscle also appears to be layered and displays gradient properties. Discontinuity within the myometrium has been demonstrated with the use of MRI and time-lapsed ultrasound (de Vries et al. 1990;Kunz et al. 1996;Lesny & Killick 2004). MRI, using T2-weighted sequences, demonstrates a distinct inner layer within human myometrium although the significance if this observation remains unclear (Brown et al. 1991). Time-lapsed observations of transvaginal ultrasound recordings demonstrate functional differences between inner and outer myometrium. In particular, slow peristaltic activity has been shown to emanate from the inner myometrium (Lesny & Killick 2004;Masui et al. 2001). These waves vary in amplitude, direction and frequency with menstrual cyclicity and are noted to be absent from outer myometrium. This functional observation is consistent with a previous report which demonstrated a decreasing gradient of elastin distribution from outer to inner human myometrium (Metaxa-Mariatou et al. 2002) Whilst distinct layering was not observed, findings certainly suggest that the inner myometrium may be less elastic than the outer myometrium. The tissue components thought to make up this gradient include vessel wall, perivascular tissue and myometrial smooth muscle. All of these observations suggest the existence of distinct inner and outer layers of the human myometrium.

The mechanisms by which fibroids develop and grow remains unclear and thus, examination of the uterine vascular network in greater detail may assist further in our understanding of the biology of these benign tumours. This may, in turn, assist us in our understanding of the reasons for success and failure of fibroid therapies such as uterine artery embolisation.

1.4 Fibroid symptomatology

Whilst the prevalence of uterine fibroids is high, many women with these benign tumours are asymptomatic and do not require medical intervention. In fact, it is estimated that only 20-50% of women with one or more fibroids will experience symptoms that are directly attributable to them. Symptoms associated with these tumours can be variable, ranging from mild to severe, causing distress and impinging significantly on HRQoL. These include abnormal uterine bleeding and bulk-related problems such as pelvic pain and pressure, urinary frequency and constipation. Reproductive dysfunction is also associated with fibroids although the exact mechanism by which this occurs is poorly understood.

The incidence and severity of symptoms associated with fibroids is related to the location, size and number of lesions present within the uterus, and it is very common for an individual to have more than one fibroid present. We are aware that these lesions respond to hormonal stimulation and thus it is not surprising that symptoms may vary in both severity and character throughout reproductive life, paralleling the growth or shrinkage of these tumours. The duration of time, however, in which a change in symptoms occurs, may be of significant length. Fibroids tend to be fairly slow growing tumours and rapid growth is always a cause for concern. Nonetheless, their malignant potential is less than 1% and thus the main aim of treatment is to provide symptomatic relief and improve QoL.

The most commonly reported symptom associated with fibroids is abnormal vaginal bleeding. This may be regular or irregular. The two most common patterns associated with fibroids, however, are menorrhagia and metrorrhagia. Menorrhagia is defined as excessive menstrual blood loss (MBL) in excess of 80 millilitres (ml) per cycle. This figure represents the 95th percentile, with a mean between 30 and 40 ml (Cole, Billewicz, & Thomson 1971;Hallberg et al. 1966a). Metrorrhagia refers to prolonged vaginal bleeding. These may occur individually

or in combination, and whilst such patterns of bleeding are not diagnostic for fibroids, it has been shown that women who present with abnormal uterine bleeding are more likely to have a uterine fibroid (Clevenger-Hoeft et al. 1999). Excessive bleeding may result in iron-deficiency anaemia and all its associated problems and can disrupt a woman's social life significantly. Studies have shown that small fibroids are associated with an increased risk of heavy bleeding and this risk increases with size. This fibroid-associated bleeding has been demonstrated not only with submucosal fibroids (Sulaiman et al. 2004), but also with those that are not submucosally located (Wegienka et al. 2003). The pathophysiology of fibroid-associated bleeding will be discussed later.

The second most common fibroid-associated symptom is pelvic pain and / or pressure. The site and severity of the latter tends to be related to the location and size of the fibroid(s). They may compress abdominal and pelvic structures; for example, a fibroid originating from the anterior wall of the uterus may compress the bladder causing urinary frequency and / or incomplete bladder emptying whilst a posterior wall fibroid may cause backache. Occasionally, large fibroids may outgrow their blood supply and in this situation, tissue ischaemia occurs, resulting in necrosis which in turn can lead to pelvic pain, often acute and severe. Ischaemic necrosis of fibroids is more commonly associated with pedunculated fibroids and those which occur during pregnancy. In the latter situation, the degeneration process is known as "red degeneration", where haemorrhage occurs within the fibroid, usually causing significant pain (Makar et al. 1989). This pain may settle with analgesia and conservative measures, but occasionally, surgical intervention is required.

The effect of fibroids on reproductive function is difficult to assess and remains unclear. It has been suggested that fibroids are the sole cause of infertility in only 2-3% of cases when all other factors have been ruled out (Buttram & Reiter

1981). We are aware that both subfertility and fibroids increase in incidence with age, and thus it is not surprising that they are more commonly associated with women of advanced reproductive age. Just as increased maternal age is associated with increased chromosomal abnormalities and subsequent pregnancy loss, fibroids are also associated with early pregnancy loss (Casini et al. 2006;Cooper & Okolo 2005a). The effect of a single entity such as fibroids on reproductive function, however, is difficult to determine when overall function involves the interplay of numerous factors including ovulation, fallopian tube function and fertilization. The location of a fibroid or fibroids is important in determining tumour-related effects on fertility. Certainly in the past, fibroids with a submucosal or intracavity (uterine) component have been implicated, in particular (Pritts 2001). Studies looking at in-vitro fertilisation (IVF) outcomes in women with known fibroids suggest that fibroid location, followed by size, are the most important factors in determining the impact of fibroids on IVF outcomes. Any distortion of the endometrial cavity seriously affects IVF outcomes, and myomectomy is indicated in this situation. Myomectomy should also be considered for patients with large fibroids, and for patients with unexplained unsuccessful IVF cycles (Rackow & Arici 2005). Other studies looking at the effect of fibroids on IVF implantation rates also support the notion that patients with intramural fibroids have a lower implantation rate per cycle (Benecke et al. 2005).

Despite the limited data on the effects of fibroids on fertility, several mechanisms by which these tumours are thought to interfere with normal reproductive function have been postulated. Firstly, they may interfere via mechanical mechanisms. For example, fibroids occurring in the region of the cornual portion of the uterus may occlude the fallopian tube causing reduced fecundity. Less commonly, multiple fibroids may cause bilateral tubal occlusion (Stovall 2001). It has also been

hypothesised that the rhythmic uterine contractions which facilitate sperm motility through the uterus may be disrupted and reduced in quality, by large fibroids (Coutinho & Maia 1971). Fibroids, either submucous or intramural, may also distort the uterine cavity causing reproductive dysfunction. Similarly, a large fibroid within the uterine cavity, may cause miscarriage, intrauterine death, premature rupture of membranes, preterm delivery and / or malpresentation of the fetus, simply by mass effect, causing a reduction in the compliance of the pregnant uterus.

Implantation of the embryo may also be negatively affected by fibroids. Factors responsible for this include impaired gamete transfer, distortion of the uterine cavity, impairment of the vascular supply to the endometrium and atrophy or ulceration of the endometrium (Eldar-Geva et al. 1998). It has also been hypothesised that endometrial growth factors may play a role (Benecke et al. 2005).

1.5 Aetiology of fibroid-associated menorrhagia

The pathogenesis of fibroid-associated abnormal uterine bleeding is poorly understood although a classic theory, first suggested by Sampson in 1912, states that local dysregulation of the vascular structures in the uterus, is responsible for it (Sampson JA 1912). Since then, a number of theories have been postulated **(Table 1)**.

Table 1

Postulated mechanisms for abnormal fibroid-associated bleeding

1. Increased vascularity and blood flow to the uterus
2. Increased surface area of endometrium
3. Endometrial ulceration over the surface of a submucous fibroid
4. Interference of normal uterine contractility
5. Compression of myometrial venous plexus by fibroid(s)

More recent evidence, however, suggests that dysregulation of growth factors or their receptors in the fibroid uterus, which have direct effects on vascular function and angiogenesis, provides the molecular mechanism which underlies vascular abnormalities such as menorrhagia (Stewart & Nowak 1996). Factors which may prove to be important in this process include basic fibroblast growth factor, platelet-derived growth factor, transforming growth factor-beta, parathyroid hormone-related protein and prolactin.

Another mechanism by which a fibroid may cause excessive vaginal bleeding includes the rare situation where a pedunculated submucous fibroid prolapses though the cervix, resulting in ischaemic necrosis and subsequent haemorrhage. Fibroids, however, do not cause abnormal vaginal bleeding via ovulatory

dysfunction as they do not influence ovarian function, ovulation or sex steroid synthesis.

1.6 Menstrual blood loss estimations

Heavy menstrual bleeding is one of the most common causes of ill health during reproductive life. Between the ages of 30 and 49 years, 5% of women consult their general practitioner each year for advice regarding this problem. Excessive menstrual blood loss accounts for 12% of gynaecological outpatient referrals and previously, 60% of these women were likely to undergo hysterectomy within five years (Myers et al. 2002;Stewart 2001). Now, however, with the increasing use of minimally invasive surgical and non-surgical techniques for the treatment of menorrhagia, the hysterectomy rate for this problem has significantly reduced.

Menorrhagia can be defined both objectively and subjectively. The definition of objective menorrhagia is a total measured MBL in excess of 80 ml per cycle. As mentioned previously, this definition is taken from population studies carried out forty years ago (Hallberg & Nilsson 1966b). Subjective menorrhagia is defined as a complaint or woman's perception of excessive MBL throughout several consecutive cycles during reproductive life.

A number of different methods have been used in the past to estimate menstrual blood loss, some subjective and some objective. Most techniques have their limitations, however. It is useful, though, not only to be able to measure MBL for the purpose of evaluating a woman's menstrual symptoms or disease progression, but also, to use such estimations as a measure of the efficacy of a treatment (e.g. UAE and its effect on fibroid-associated bleeding).

There are a number of subjective methods of MBL estimation. A woman's own subjective estimation of her blood loss may be of some use as a screening tool, but

is not particularly accurate in making a diagnosis of menorrhagia. Studies comparing this subjective method with more objective techniques found that only 38-76% of women who complained of heavy MBL actually had objective menorrhagia (Chimbira, Anderson, & Turnbull 1980;Rybo 1966;Van Eijkeren, Scholten, & Christiaens 1986). Counting the number of days of menstruation, to assess MBL is another fairly inaccurate method of quantification. Based on this method, a diagnosis of heavy menstrual bleeding is made if menses last more than seven days per cycle (Chimbira, Anderson, & Turnbull 1980). It has been demonstrated that 78% and 91% of the total MBL of one cycle occurs by the second and third days, respectively, of menstruation (Rybo 1966). It has also been reported that women with menorrhagia are more likely to experience the greatest volume of blood loss during the first three days of their cycle (Haynes et al. 1977). Not surprisingly, it has been found that only 45% of women who bleed for more than seven days, have objective menorrhagia (Rybo 1966). This test, with its low sensitivity, is therefore not deemed to be diagnostic for menorrhagia.

Another method for quantification of MBL is counting the number of sanitary protection products used during menstruation. Again, this is a poor tool for making a definitive diagnosis of menorrhagia. Firstly, it has been shown that the most important determinants for the number of sanitary products utilised were a woman's personal hygiene practices, her frequency of attention to menstrual flow and her financial resources (Higham, O'Brien, & Shaw 1990) Secondly, sanitary products, depending on the brand, vary widely in terms of their absorbency, thus further influencing the number of products required during menstruation.

Weighing sanitary protection, by subtracting the weight of the unused from the used product, can also facilitate quantification of MBL. Again, this method has its limitations, as it accounts for not only menstrual blood, but also, menstrual fluid from sources such as endometrial glands and tissue exudates, cervical and vaginal

secretions. It has been shown that the proportion of blood in menstrual fluid varies widely amongst women and blood is thought to constitutes only an average of 36.1% of the total menstrual loss (Fraser et al. 1985). Another drawback of the last two methods of MBL estimation is that women may find the process of collecting used sanitary products unpleasant, inconvenient or unacceptable.

The "menses cup" has also been used to quantify MBL. It involves the use a latex seal which completely covers the vagina and collects all menstrual blood and fluid without the need for additional sanitary protection. There are two types of cup described, both of which have proven to be easy to insert into the vagina, but difficult to remove whilst successfully retaining all the collected menstrual loss (Cheng et al. 1995;Gleeson et al. 1993). In addition, removal of the intact cup appeared to be more difficult for those women with a heavy MBL (Cheng et al. 1995)

The pictorial blood loss assessment chart (PBAC) is a visual representation of MBL from which a numerical score is derived (Higham, O'Brien, & Shaw 1990). It was first introduced in 1990 and comprises a series of diagrams representing light, moderate and heavy soiling of sanitary protection in the form of tampons and sanitary towels. In addition, the passage of clots and flooding were accounted for, the former, by equating the size of clot to that of UK coinage. Increasing scores were assigned for light to heavy soiling of sanitary protection. It was reported that when using the PBAC as a diagnostic clinical tool, scores of 100 or more diagnosed menorrhagia with a sensitivity and specificity of more than 80% (Higham, O'Brien, & Shaw 1990). However, a number of factors were not accounted for, including the assessment of extraneous and large volume blood loss. In addition, the types of sanitary protection used in the study are now no longer available, thus further reducing the accuracy of this tool (Reid, Coker, & Coltart 2000).

The menstrual pictogram is a modified version of the PBAC. Additional icons have been created to represent further variation of blood loss on sanitary protection, variation in size of blood clot and variation in volume of blood lost in the toilet whilst changing sanitary towels and tampons. Distinctions are also made between the different levels of absorbency of sanitary protection in order to reduce the inaccuracies of MBL estimations at the higher ranges. Another difference from the PBAC is that the score is calculated in millilitres which in turn, is equivalent to the actual volume of blood lost (Wyatt et al. 2001). A significant positive correlation has been shown between a woman's ability to estimate her blood loss on sanitary protection using this technique and her actual objective MBL calculated using the alkaline haematin technique (Hallberg, Hogdahl & Nilsson 1966b;Wyatt et al. 2001). Unlike the PBAC, the menstrual pictogram has the additional advantage of accounting for estimation of extraneous blood loss. By including the latter assessment, the incidence of objective menorrhagia increased from 36% to 74% of women who were found to have menorrhagia as assessed by the menstrual pictogram. It is important to remember that studies validating the use of the menstrual pictogram involved the use of standardised brands of sanitary wear and it may well be that conversion charts are required when using different brands of sanitary protection.

Menorrhagia is one of the most common causes of anaemia in premenopausal women and thus it has been suggested that a full blood count (FBC) may assist in the diagnosis of this problem. However, it has been shown that after comparing women's haemoglobin (Hb) levels with their objective MBL measurements (using the alkaline haematin technique), anaemia is 74% predictive for having menorrhagia (Janssen, Scholten, & Heintz 1995). Haematocrit, serum iron and protoporphyrin levels are inversely related to MBL (Janssen, Scholten, & Heintz

1995). Nonetheless, all these haematological indices should not be solely relied upon to make a conclusive diagnosis or exclusion of menorrhagia.

The alkaline haematin technique is the gold standard for objective quantification of MBL and was first reported in 1964 (Hallberg & Nilsson 1964). It involves dilution of sanitary protection in a 5% sodium hydroxide solution which extracts haem from the menstrual fluid. Whilst very precise, this method is labour intensive and time consuming for the clinician. In addition, the collection of used sanitary protection may be considered unpleasant and cumbersome by some women. This technique, therefore, has limited use in clinical practice and tends to be reserved for use in a research setting. It has, however, been modified over time, in an attempt to make it simpler to perform (Gannon et al. 1996). Like other methods mentioned previously, the alkaline haematin technique fails to account for extraneous MBL which can be significant in women with heavy menses. It is also a concern that newer sanitary products may interfere with the absorbance of haem, leading to underestimations of haemoglobin concentrations.

1.7 Treatment of fibroids

The management of fibroids very much depends on the symptoms that they cause and their effects, if any, on general health and lifestyle. Those women who are asymptomatic or perceive their symptoms to be very mild, do not require definitive treatment. They may be followed up regularly with clinical review and serial pelvic imaging. Should any deterioration in symptoms or increase in fibroid size occur, however, appropriate treatment may then be offered.

The traditional treatment of symptomatic fibroids is surgical, namely hysterectomy and myomectomy. Hysterectomy ensures complete resolution of fibroid-associated menstrual symptoms such as heavy menstrual bleeding and

dysmenorrhoea. It also guarantees removal of all fibroids and no recurrence of these lesions. Not surprisingly, it is associated with high patient satisfaction rates and improvements in overall quality of life. However, it is also associated with significant morbidity, a relatively long hospital stay and guarantees infertility (Lumsden 2002;Vessey et al. 1992). Hysterectomy may be performed via abdominal laparotomy, vaginally or as a laparoscopically assisted vaginal procedure. Most hysterectomies for fibroids are currently performed by the abdominal route. With better training, however, it may be that the vaginal approach will be increasingly the choice method of hysterectomy for those women with a moderately enlarged fibroid uterus.

Myomectomy, whilst aiming to preserve fertility, is also associated with morbidity, a hospital stay of a few days and carries the risk of proceeding to emergency hysterectomy. In addition, it may further compromise reproductive potential and provide clinicians with difficulties in long term management owing to the high recurrence rate of fibroids and adhesion formation resulting from previous surgery (Stewart 2001). The procedure tends to be reserved for those women who wish to retain their fertility. The indications for myomectomy in infertile women, however, are controversial. As mentioned previously, it is recommended that those infertile women with a fibroid distorting the endometrial cavity should have the tumour removed. However, the management of those women with subserosal or intramural fibroids without the distortion of the uterine cavity, remains unclear (Smith & Uhlir 1990;Verkauf 1992). Myomectomy may be performed as an open procedure (via laparotomy – the classical method) or laparoscopically, as a laparoscopically assisted myomectomy (using a minilaparotomy to assist with the laparoscopic procedure). It may also be performed vaginally or hysteroscopically, using the vaginal route. Whilst the latter procedure is the gold standard for the treatment of submucous myomas where

reproductive function is an issue (Kolankaya & Arici 2006). surgical alternatives to the classical method of myomectomy must be evaluated in prospective studies, before being recommended for routine use. Currently, most myomectomies tend to be performed by the classical method as many women have more that one fibroid for resection. Women should also be made aware of the conversion rate of minimally invasive myomectomy procedures to laparotomy.

Medical treatments such as the levonorgestrel intra-uterine system (*Mirena IUS*) and gonadotrophin-releasing hormone (GnRH) analogues have been used to treat fibroid-associated menorrhagia and pain, with limited success. The Mirena IUS is only useful if the fibroids are small and there is minimal distortion of the uterine cavity (Grigorieva et al. 2003). GnRH analogues, whilst proven to be effective in shrinking fibroids and greatly improving menstrual symptoms, are limited by their side effect profile, in particular, osteoporosis and unpleasant menopausal symptoms (Golan 1996). Even when used in conjunction with addback therapy, they are usually only used as a short term treatment solution for symptomatic fibroids. Analogues are, however, useful in reducing peri-operative blood loss, when administered for short periods of time pre-myomectomy (Lethaby, Vollenhoven, & Sowter 2000).

With the evolution of minimally invasive surgical and non-surgical techniques, and changing attitudes of both women and clinicians towards uterine preservation, the popularity of conservative treatment options has escalated over the last decade. Uterine artery embolisation (UAE) is a minimally invasive angiographic procedure which is increasingly being used as an alternative to surgery for symptomatic fibroids (Ravina et al. 1995). Other conservative therapies include laparoscopic myolysis (Donnez et al. 2000), MRI guided percutaneous laser ablation (Hindley et al. 2002), interstitial laser photocoagulation (Visvanathan et al. 2002) and high intensity focused ultrasound energy (Chan et al. 2002;Hesley et

al. 2006;Stewart et al. 2003;Stewart et al. 2006). With the exception of UAE, the latter modalities are relatively new and existing data on their clinical outcomes is based on studies that are relatively small and of variable quality. Until their safety and efficacies are adequately assessed, they can not be fully validated, and thus we are unable to offer these conservative therapies to women routinely.

1.8 Uterine artery embolisation

UAE is a well established minimally invasive procedure performed by interventional radiologists as an alternative to surgery for symptomatic fibroids. It has been used in the management of acute pelvic haemorrhage in both obstetric and gynaecological settings, for more than two decades (Greenwood et al. 1987;Heaston et al. 1979). Its use, however, in the treatment of symptomatic fibroids, was first reported in France by Ravina, in 1995 (Ravina et al. 1995). Since then, thousands of UAE procedures have been performed in the UK and it is now estimated that more than 50,000 have been carried out worldwide. With this increasing experience of fibroid embolisation, we now have a greater understanding of the indications, patient selection, pre- and post-procedural imaging, potential risks and clinical outcomes associated with this technique.

Optimal pre-procedural selection of women is vital for high clinical success rates and avoidance of complications following UAE. Women who have symptomatic fibroids in the absence of other pelvic pathology are suitable candidates. Exclusion of women with adenomyosis is necessary as this particular uterine pathology responds less well than fibroids to embolisation (Jha et al. 2003). Those with pedunculated subserosal fibroids should also be excluded owing to the risk of ischaemic necrosis and potential for the fibroid to disintegrate and become free in the abdomen. The latter may be further complicated by causing peritoneal

irritation, infection and possibly even bowel adhesions (Pelage et al. 2000). Confirmed or suspected pelvic infection, an undiagnosed pelvic mass, the immunocompromised and severe contrast allergy are other contraindications. Previous pelvic irradiation or surgery and coagulopathies are relative contraindications to the procedure. UAE is particularly useful in women who are not ideal candidates for surgery. Such patients include the morbidly obese, diabetic and those with multiple medical problems. Embolisation is also a useful alternative to surgery in women who refuse blood transfusions. These women, however, must be counselled regarding the risk of emergency hysterectomy.

Pregnancy is an absolute contraindication to UAE and until the effects of embolisation on reproductive potential have been fully established, the procedure should be offered to women who desire future fertility with caution.

A thorough pre-procedural evaluation should be made on all women prior to undergoing UAE. Detailed gynaecological and general medical histories should be taken and a gynaecological examination performed. All women should have a separate consultation with a gynaecologist prior to referral to an interventional radiologist. This team approach is the key to a successful outcome. Baseline FBC, ferritin levels and a coagulation screen should be performed. A hormone profile should also be considered, to provide information about ovarian reserve. Screening for infection with vaginal swabs and a serum c-reactive protein (CRP) estimation is only required in women with suspected pelvic infection. Endometrial biopsy should be performed where appropriate, to exclude endometrial hyperplasia and malignancy. For example, women with irregular cycles, constant or intermenstrual bleeding and those with prolonged menses or heavy menstrual bleeding above the age of 45 years, should all undergo endometrial evaluation prior to UAE. This can be done with an outpatient pipelle endometrial biopsy or during formal hysteroscopy and endometrial biopsy.

Uterine imaging must be performed, firstly to confirm the diagnosis of fibroids and secondly, to provide information on their location, size and number. It also assists in excluding other pelvic pathology which may be responsible for a woman's symptomatology. Imaging should also assess the viability of the fibroid. Those tumours with a poor blood supply (e.g. calcified fibroids or degenerative fibroids with cystic or haemorrhagic necrosis) are more likely to respond poorly to embolisation (DeSouza & Williams 2002;Marret et al. 2004b). Whilst Doppler ultrasound is used in some centres to fulfil these tasks, most radiologists prefer to use contrast-enhanced magnetic resonance imaging (MRI) where available **(Figure 4a).**

Figure 4a

Pre-embolisation MRI (sagittal T2-wighted image) - normal sized uterus displaced upwards by large cervical fibroid

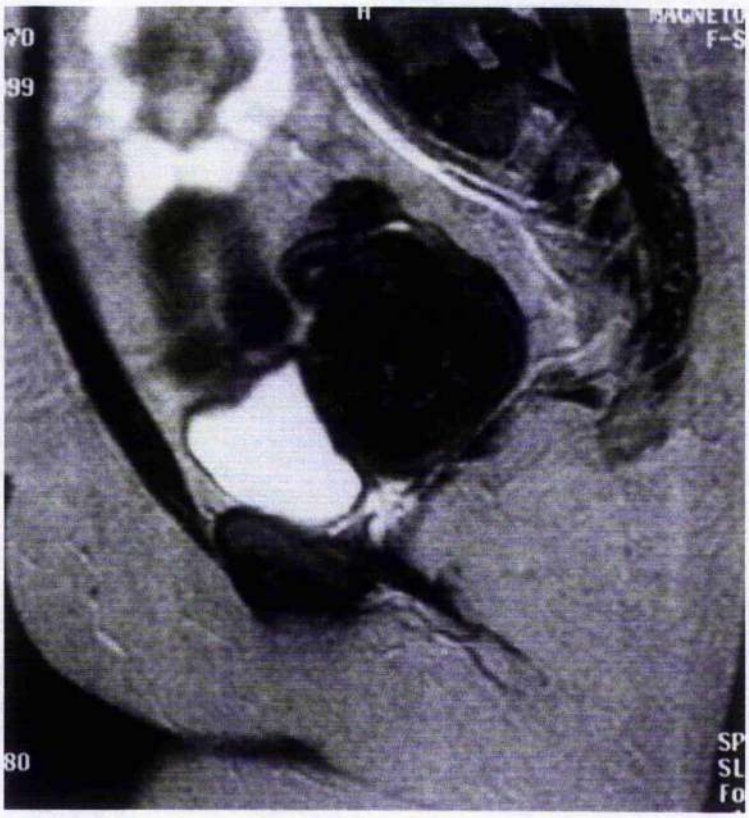
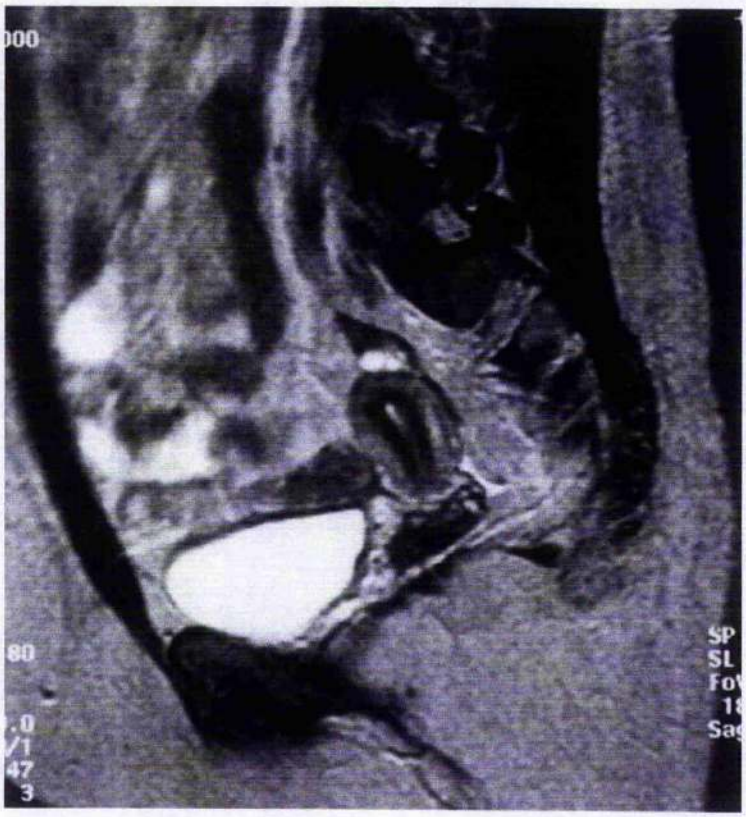


Figure 4b

Post-embolisation MRI (sagittal T2-wighted image) - note marked reduction in cervical fibroid volume and the return of the body of the uterus to its correct anatomical site



Bilateral UAE is performed by appropriately trained interventional radiologists using a standard technique. The procedure is usually carried out under conscious sedation although spinal or epidural anaesthesia have been used as alternatives. Antiemetics are administered and local anaesthetic is infiltrated into the groin prior to percutaneous catheterisation of each of the femoral arteries. Thereafter, both internal iliac arteries are selectively catheterised in turn, allowing catheterisation of their respective uterine arteries **(Figure 5a).** The latter is facilitated by contrast enhancement and digital fluoroscopy **(Figure 6a).** Once catheter placement is confirmed and the vascular supply to the fibroids and uterus demonstrated, multiple small particulate emboli in the form of polyvinyl alcohol particles (PVA, 500-710um in diameter) are then injected into the circulation **(Figure 5b).**

Figure 5a

Transfemoral catheterisation of the uterine arteries

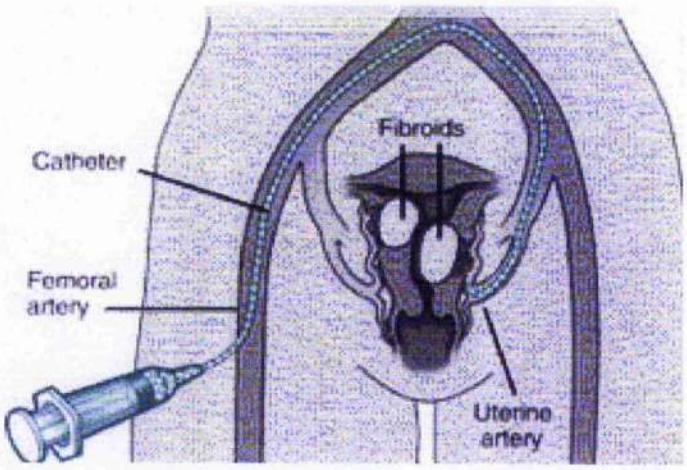
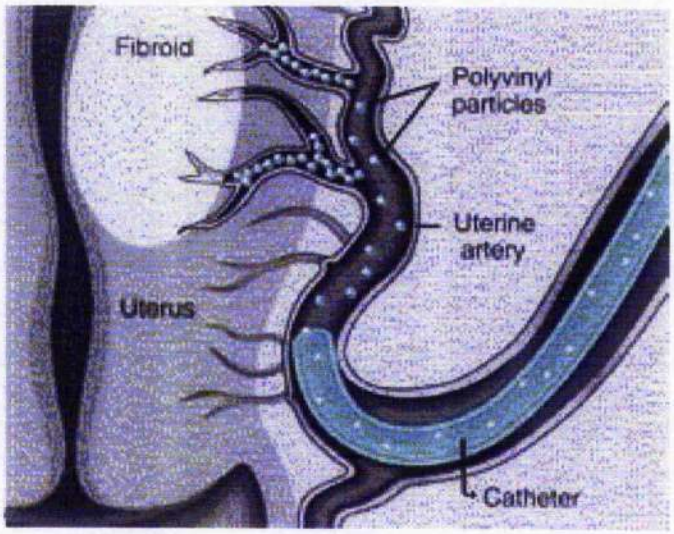


Figure 5b

Injection of poly vinyl alcohol particles into the circulation to effect embolisation



The aim of the embolic material is to selectively occlude both uterine arteries, resulting in fibroid devascularization and subsequent fibroid shrinkage. Owing to the rich pelvic collateral circulation, however, the normal myometrial tissue revascularises. Initially, embolisation was considered complete when there was virtually no demonstrable flow in the distal uterine artery **(Figure 6b)** (Pelage et al. 2000;Ravina et al. 1995;Walker & Pelage 2002).

Figure 6a

Pre - embolisation angiogram demonstrating simultaneous catheterisation of both uterine arteries and the tortuous branches of the uterine arteries supplying the fibroid uterus.

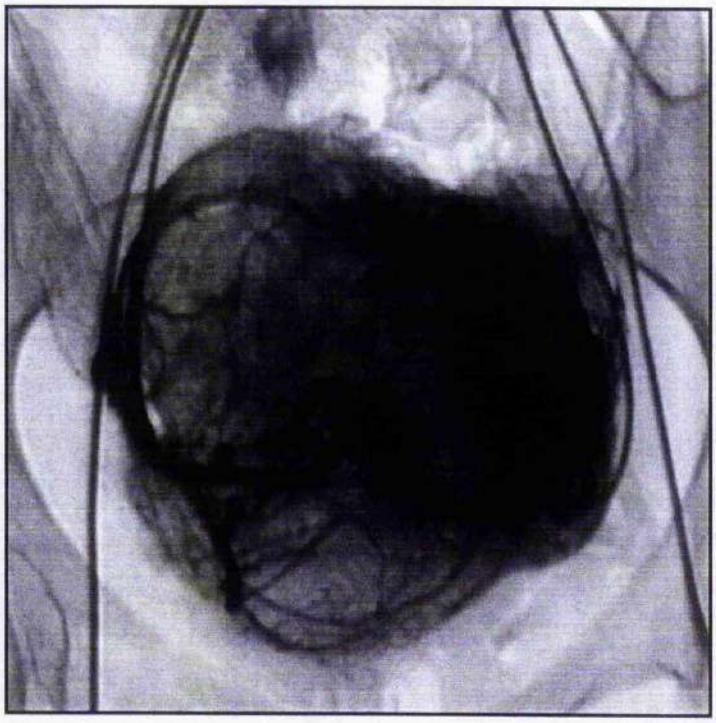
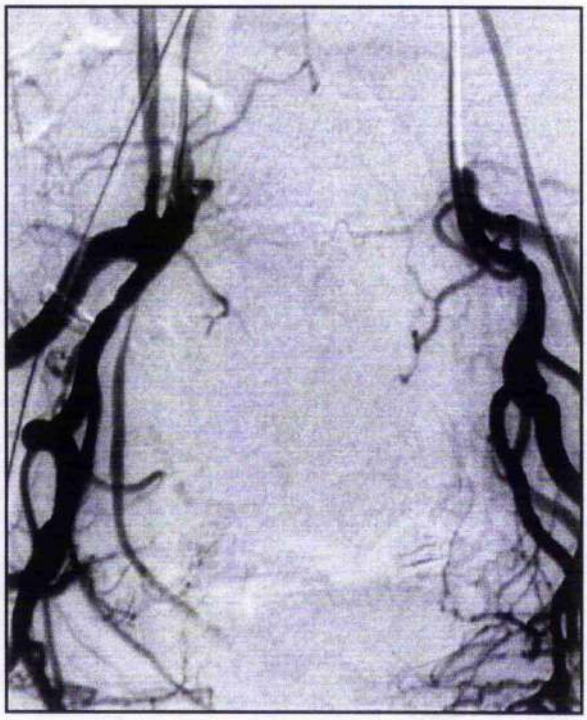


Figure 6b

Post - embolisation angiogram showing virtually no demonstrable flow in the distal uterine arteries, thus highlighting that the embolic procedure is complete.



With increasing experience and the evolution of embolic materials, however, the degree of arterial occlusion has become more precise and most radiologists now aim to achieve arterial *blushing* rather than stasis, where the main arterial trunk is left patent at the end of the procedure (Pelage et al. 2003). This improved precision allows more targeted fibroid embolisation with concurrent reduced unnecessary devascularization of the myometrium and ovarian vessels.

Individual procedures take approximately 45 minutes to complete and every effort is made to keep the total fluoroscopy time and number of image sequences taken to a minimum, thus reducing the radiation penalty to the ovaries. The technique of simultaneous catheterisation of both femoral arteries and synchronous embolisation of the uterine arteries may be used to further reduce the duration of the procedure and thus, total radiation exposure.

Most patients experience a degree of pain after the second uterine artery is embolised. This may be accompanied by nausea and vomiting which responds to intravenous antiemetics. Pelvic or abdominal pain usually peaks at six to twelve hours post-procedure and it tends to be self-limiting over a further twelve hours. For this reason, most women undergo this procedure either as a day case or with a single overnight hospital stay where they can receive adequate pain control. A clear pain management protocol should be in place to facilitate this. A combination of oral non-steroidal anti-inflammatory drugs (NSAIDs) and a paracetamol-codeine based preparation can provide adequate pain relief post-embolisation, but usually, opiate analgesia is required in the immediate post-operative period.

It is not uncommon to experience mild lower abdominal cramps for the next seven to fourteen days and most women return to normal activities within a couple of weeks. They should be made aware of all potential complications and side effects of the procedure and have an emergency contact number in their possession, prior

to discharge from hospital. Such complications include allergy to the contrast medium used during MRI. This is rare and occurs in approximately 1% of patients. Puncture site complications may also occur (in less than 1% of women) following femoral artery catheterisation and these include bleeding, haematoma formation and infection. The radiation penalty to the ovaries appears to be minimal and as mentioned earlier, occurs during digital fluoroscopy at the time of arterial catheterisation. Radiologists aim to keep individual fluoroscopic time to a minimum with a radiation dose exposure which is similar to that of a barium enema (Glomset et al. 2006;Vetter et al. 2004).

Non-target embolisation or misembolisation may occur either as a result of poor procedural technique or due to the presence of aberrant arteries. It can cause premature ovarian failure and ischemia or necrosis of neighbouring organs (Andrews & Binkert 2003).

The post-embolisation syndrome may occur in up to 10% of women, typically 7-21 days after UAE. The syndrome manifests as general malaise, a low grade fever, pelvic pain, nausea and vomiting. It is associated with a leucocytosis and is often very difficult to distinguish from clinical infection. The syndrome has been thought in the past, to result from the release of inflammatory mediators from fibroid tissue that has been rendered ischaemic. Management includes adequate pain control, hydration, prophylactic antibiotics and reassurance (Walker & Pelage 2002).

Transcervical fibroid expulsion has been reported in up to 5% of women (Spies et al. 2002c). This may occur as a result of uterine shrinkage forcing an intramural fibroid into the uterine cavity. Expulsion is more common in the presence of submucous fibroids. Intense abdominal pain can occur just prior to fibroid expulsion when the cervical canal is obstructed. Occasionally, fibroids are partially extruded and require hysteroscopic myomectomy for complete removal

(Marret et al. 2004a). Persistent vaginal discharge occurs in approximately 4% of women after embolisation and may persist for a few weeks to many months (Walker & Pelage 2002). It is due to the expulsion of fibroid necrotic tissue and if not self-limiting, may require antibiotic prophylaxis or (hysteroscopic) endometrial curettage.

Transient amenorrhea occurs in 5-10% of women after UAE whilst permanent amenorrhoea occurs in up to 15% of women above the age of 45 years and in 1% of younger women. This premature menopause is usually a consequence of embolic material entering the ovarian arterial circulation via uterine-ovarian anastomotic vessels (Goodwin et al. 1999;Spies et al. 1999). The resultant reduced ovarian perfusion eventually leads to ischaemia. All women should be thoroughly counselled about this important potential side effect and its consequences, prior to undergoing embolisation.

Infection is potentially the most serious complication following UAE (Pron et al. 2003;Spies et al. 2002c). This may span from mild infection requiring a course of oral or intravenous antibiotics, to pelvic sepsis, necessitating emergency hysterectomy (Aungst et al. 2004). The latter occurs in less than 1% of cases and can be life-threatening. Two cases of death secondary to fatal septic shock following UAE have been reported in the literature, to date (De Blok et al. 2003;Vashisht et al. 1999). Any patient, therefore, presenting with worsening pelvic pain, fever, vaginal discharge and leucocytosis following embolisation should be admitted as soon as possible. A full infection screen and uterine imaging should be performed urgently and the appropriate treatment administered promptly.

All women should undergo post-embolisation uterine imaging (**Figure 4b**), in order to assess fibroid shrinkage and vascularity. This tends to be performed six months after the procedure. In addition, women should be reviewed by both

gynaecologist and interventional radiologist within the six month post-embolisation period.

The effect of embolisation on fertility and pregnancy is still not fully established. However, successful conceptions, pregnancies and deliveries have been reported in women following the procedure (Carpenter & Walker 2005;Pelage et al. 2000;Pron et al. 2005;Walker & Pelage 2002). It has been suggested by some authors that the rates of miscarriage, intra-uterine growth restriction, preterm delivery and post-partum haemorrhage are higher following UAE, all of which are complications thought to be due to alterations in uterine blood flow after the procedure (Pron et al. 2005). It should also be borne in mind that women undergoing embolisation tend to be older and have large or multiple fibroids, factors which also influence fecundity. Reassuringly, MRI of the uterus performed three to six months post-embolisation reveals rapid revascularization of the normal myometrium and an essentially normal appearance of the endometrium (DeSouza & Williams 2002;Katsumori, Nakajima, & Tokuhiro 2001;Pelage et al. 2004).

Whilst most women who undergo UAE have completed their family, the procedure may be offered to women who wish to retain their fertility with caution. The latter group of women must be thoroughly counselled about the potential risk of ovarian failure and be made aware that our data on fertility and pregnancy remains limited.

The efficacy of uterine artery embolisation can be determined by the degree of improvement or resolution of symptoms. Results from published studies and data presented at scientific meetings are similar. Most studies divide fibroid-associated symptoms into excessive menstrual bleeding, pelvic pain and bulk-related problems. Clinical success rates for treating these complaints range from 81-96%, 70-100% and 46-100%, respectively (Gupta et al. 2006;Katsumori, Kasahara, &

Akazawa 2006;Ravina et al. 1995;Smeets et al. 2006;Spies et al. 1999;Walker & Pelage 2002).

As mentioned previously, uterine volume reduction and thus fibroid shrinkage can be measured by ultrasound or MRI. A 25-60% reduction in uterine volume has been reported between three and six months following UAE (Ravina et al. 1995;Spies et al. 1999;Walker & Pelage 2002). It should be noted, however, that reductions in fibroid volume do not always reflect an improvement in clinical symptoms.

Three prospective clinical trials comparing the outcome of UAE and hysterectomy have been published to date, the first two being randomised controlled trials (Hehenkamp et al. 2005;Pinto et al. 2003;Spies et al. 2004;Spies et al. 2005). UAE has been shown to be associated with a shorter hospital stay and recovery time when compared to hysterectomy. Satisfaction rates are similar for both procedures. Authors state, however, that the higher minor complication rates and subsequent higher readmission rates after embolisation, highlight the need for careful post-procedural follow-up of women undergoing this procedure.

A prospective randomised controlled trial comparing UAE to myomectomy has also been carried out (Mara et al. 2006). The authors concluded that although the reproductive outcomes of UAE and myomectomy could not be sufficiently evaluated at the time of study publication (more than half of the study population were still trying to conceive), initial results indicate that both methods are clinically successful in the majority of cases and are not connected with a significant number of serious complications.

Longer term data on the effects of UAE on symptomatic fibroids is still required, with comparisons being made to standard surgical procedures for this benign condition.

1.9 Quality of life

Symptomatic leiomyomata can impose restrictions to women in terms of physical, social and occupational functioning which in turn, can lead to a reduction in overall HRQoL. The latter, as perceived by the patient, is an important variable in terms of the outcome of a particular treatment or intervention. Quality of life is a descriptive term that refers to an individual's emotional, social and physical wellbeing, and their ability to function in the ordinary tasks of living. HRQoL analysis aims to measure the impact of a disease process or subsequent therapy on these holistic areas within a person's life. The results of such measures allow us to inform patients about the burden of their disease, the likely effect of a treatment and to monitor the success of a treatment from the patient's perspective.

Despite the prevalence of symptomatic fibroids, little work has been done on the impact of symptoms on HRQoL until recently. When assessing the severity of a disease or the efficacy of a therapeutic intervention, clinicians often prefer to rely upon traditional objective clinical measures. In the case of symptomatic fibroids, such clinical assessments include objective menstrual blood loss measurements, haemoglobin estimations and radiological measures of fibroid shrinkage. However, bearing in mind that the main aim of treatment for benign disease such as fibroids is to improve symptoms and HRQoL, the relevance of such objective measures may be questioned by both patients who wish to decide their own plan of management, and clinicians. Whilst objective measurements are certainly useful, subjective assessments of self-perceived health status which take account of HRQoL have become increasingly important since the 1970s.

Quality of life tools may be divided in to generic and disease-specific categories. The former may be used to assess the impact of any disease process or intervention and include the Short form 36 (SF 36) and EuroQol (Jenkinson, Coulter, & Wright 1993;Kind P 1996) whilst the latter is designed for assessment

of a disease process or treatment for a particular condition. In the case of symptomatic fibroids, an example of a disease-specific questionnaire includes the more recently developed Uterine Fibroid Symptom and Quality of Life (UFS-QOL) questionnaire (Spies et al. 2002a). Quality of life tools should be short, easy to administer, acceptable to patients and fully validated.

Using such tools for the assessment of UAE for symptomatic fibroids, satisfaction rates of 97% have been reported in most published studies and this tends to parallel improvements in symptoms (Gupta et al. 2006;Walker & Pelage 2002). Combining the more recent UFS-QOL questionnaire with a generic questionnaire such as the SF 36 could provide us with further useful information regarding the efficacy of UAE. The former questionnaire, however, was not fully validated at the commencement of this research project and was therefore not used in our studies.

1.10 Comparison of UAE to surgery

UAE has been evaluated in the short term in a number of studies since its introduction in 1995 as a treatment for symptomatic fibroids. In the UK, the National Institute of Health and Clinical Excellence (NICE) issued guidance regarding the procedure in October 2004. They stated that UAE appeared safe for routine use and majority of patients experienced short term symptomatic relief (NICE 2004). The safety and efficacy of embolisation compared to other accepted surgical treatments for symptomatic fibroids, however, has not been evaluated until very recently. We therefore aimed to compare UAE to surgery in the setting of a randomised controlled trial (REST trial – *multicenter randomised controlled trial comparing embolisation to surgery for the treatment of fibroids*) where the primary outcome measure was quality of life at 1 year. Randomisation has the

advantage of removing patient selection bias and also has a tendency to yield comparable study groups. The REST trial commenced in November 2000 and since then, three other randomised controlled trials comparing UAE to surgery have been published (Hehenkamp et al. 2005;Mara et al. 2006;Pinto et al. 2003). The first, published in 2003 by a Spanish group, used a controversial randomised consent methodology (Zelen) where women randomized to the hysterectomy arm were not informed of the study or of the possible alternative treatment of UAE (Pinto et al. 2003;Zelen 1979). The trial was fairly small (n=57) and used length of hospital stay as the primary outcome measure. Those in the embolisation arm of the trial were found to have a significantly shorter hospital stay whilst the complications and satisfaction rates for both UAE and hysterectomy arms of the trial were similar.

The second trial, the EMMY trial was carried out by a Dutch group (Hehenkamp et al. 2005). They enrolled 177 women into the study (88 and 89 women were treated with UAE and hysterectomy, respectively). The primary endpoint of the study was elimination of menorrhagia at a follow-up period of two years, with UAE being considered equivalent to hysterectomy if menorrhagia resolved in at least 75% of women with no significant differences in major complications between the two arms of the trial. Whilst there was no significant difference in major complications between the two arms of the trial, the authors found that UAE was associated with a significantly higher minor complication rate during the first six weeks post-procedure; this in turn, was associated with a higher readmission rate. The authors also reported a technical failure rate of 5.3% and a high procedural failure rate of 17.3% (a technical failure being regarded as one where bilateral uterine arteries are not occluded and a procedural failure being regarded as one where bilateral UAE was not completed for any reason).

The third published randomised controlled trial compared UAE to myomectomy in women who wished to preserve their fertility (Mara et al. 2006). Sixty three women were recruited into this study (30 were treated with UAE and 33 were treated with myomectomy) where the mean follow-up period was 17 months. The authors found that UAE was associated with significantly shorter procedure length, hospital stay and recovery time. However, UAE was also associated with higher intervention rates and a lower rate of total symptomatic relief. Both arms of the trial did not differ significantly in terms of technical success rates, febrile morbidity, complication rates and hormone profiles at 6 months.

Whilst the above studies have answered a number of important questions, like any trial, they all have their flaws. No trial alone can be taken as a definitive statement on the early outcomes of UAE and hence the REST trial attempts to provide further information regarding the safety, efficacy and place of UAE as a uterine-sparing modality in the treatment of symptomatic fibroids.

1.11 Objectives and hypotheses of thesis

The aims of this thesis are to assess the effect of UAE on objective MBL, uterine volume and HRQoL. I also wish to assess the normal course of inflammatory markers after uncomplicated UAE, with particular reference to the post-embolisation syndrome.

The REST trial (multicenter *r*andomised controlled trial comparing *e*mbolisation to *s*urgery for the *t*reatment of fibroids) aims to compare UAE to surgery in terms of safety and efficacy. My role throughout the trial involved recruitment of women from hospital gynaecology clinics in the West of Scotland over a period of 2 years. I was not responsible for analysis of trial data.

Finally, I wish to provide some insight into the pathophysiology of uterine fibroids. By examining the vasculature of these lesions and the surrounding myometrium, I wish to provide information on the biology of vessel wall formation and maturation. Ultimately, I wish to gain some knowledge regarding vessel development which in turn, may contribute to the understanding of fibroid growth. Owing to time constraints, experimental work and data analysis carried out in chapter 7A were carried out by a BSc student.

My hypotheses for this thesis are as follows:-

1. UAE is efficacious in the treatment of symptomatic fibroids in terms of sustained reduction in MBL and uterine volume, and improvement in HRQoL.

2. The post-embolisation syndrome is associated with a rise in inflammatory markers.

3. UAE is as effective as surgery in terms of safety, efficacy and quality of life.

4. Myometrial vascular integrity is disrupted by the fibroid disease process

Chapter 2

Evaluation of the effect of uterine artery embolisation on menstrual blood loss and uterine volume

2.1 Introduction

The traditional treatment for symptomatic fibroids is surgical and medical therapies may be used in a select group of patients, usually in the short-term. These therapeutic options, however, are not without drawbacks and alternative treatment modalities are being sought. Uterine artery embolisation (UAE) is a useful alternative which has been gaining popularity over the last decade. Preliminary observational studies carried out in both Europe and the USA suggest that the procedure is effective in relieving fibroid-associated symptoms in 80-94% of women (Goodwin et al. 1999;Pelage et al. 2000;Spies et al. 1999;Walker & Pelage 2002). To date, however, none of these studies include objective measurements of menstrual blood loss (MBL) pre- and post-embolisation but instead, rely on subjective assessment of symptoms. Since the correlation between objective and subjective assessment of menstrual blood loss is poor, it is important to obtain objective information where possible (Chimbira, Anderson, & Turnbull 1980b;Fraser, McCarron, & Markham 1984), especially when evaluating a procedure in its early stages.

The definition of objective menorrhagia is a MBL exceeding 80mls/cycle (Hallberg et al. 1966b). To make such a diagnosis accurately, precise measurement of MBL is required. We therefore decided to carry out a prospective observational study to evaluate the effect of UAE on MBL using an objective measurement technique. With the use of MRI, we also aimed to evaluate the effect of UAE on uterine volume and thus, fibroid shrinkage.

2.2 Materials and methods

Local ethics committee approval was obtained prior to commencing this prospective observational study. Women with an established diagnosis of symptomatic uterine fibroids were referred for consideration for UAE from hospital gynaecology outpatient clinics in the West of Scotland. They had to have experienced either excessive menstrual blood loss, pelvic pain or pressure symptoms thought to be due to fibroids; some women had a combination of symptoms. The latter was confirmed by a gynaecologist on history taking. The women were given detailed written information on the risks, benefits and complications of embolisation prior to signing a written consent form agreeing to participation in our study.

The study group was requested to collect all sanitary protection from one menses prior to embolisation and at regular intervals thereafter (3, 6-9, 12-24, 24-36 and 36-48 months). Objective measurement of MBL was performed using the alkaline haematin technique, a method regarded as the gold standard for quantification of menstrual blood loss (Hallberg et al. 1966b). All menstrual protection from a single menses was soaked in a 5% sodium hydroxide solution (for 24 hours) in order to extract haemoglobin from menstrual blood. The optical density (OD) of the alkaline haematin supernatant was then measured using spectophotometry and compared with that of the patient's own venous blood; the ratio of the ODs facilitated calculation of menstrual blood loss.

Prior to UAE, women underwent a pelvic MRI scan, firstly to confirm the diagnosis of fibroids (including detail of number, site and size) and secondly, to exclude other pelvic pathology such as adenomyosis and ovarian disease. This was repeated at six months post-procedure. Imaging included transverse and sagittal T1- and T2-weighted images. During the latter part of the study, a T1-gadolinium enhanced sequence was also added to assess uterine vascularity.

Uterine volume estimations were made in each case, by taking uterine measurements in three dimensions. Using the prolate ellipse equation formula (D1 x D2 x D3) x 0.5 where D1, D2 and D3 were the transverse, oblique and vertical axes measurements of the uterus respectively, the volume was calculated. Measurements were calculated by four experienced radiologists, each based in one of four Glasgow city hospitals.

Bilateral uterine artery embolisation was performed by appropriately trained interventional radiologists using a standard technique previously described in chapter one, section 1.8, page 36.

Since the data for MBL are positively skewed, non-parametric statistical tests (Wilcoxon signed rank test) were used for analysis. Data are expressed as medians and ranges. $P < 0.05$ is considered statistically significant.

2.3 Results

Patients were aged between 29 and 54 years (n = 53). Of 41 women who complained of menorrhagia, 36 (88%) had a measured blood loss in excess of 80ml/cycle. The remaining 12 women who did not complain of menorrhagia complained of pressure and / or pain symptoms in the absence of bleeding problems. The median MBL was lower at all post-treatment time intervals when compared with pre-treatment levels (**Table 2**).

Table 2

Menstrual blood loss (ml) pre- and post-embolisation

	N	Range	Median
Pre-treatment	53	9-1339	160
3 months	35	0-767	59
6-9 months	36	0-1283	70
12-24 months	33	0-265	33
24-36 months	19	0-205	18
36-48 months	10	0-66	21

The median *reduction* in MBL at 3, 6-9, 12-24, 24-36 and 36-48 months was 78, 61, 125, 126 and 245 ml (95% CI 64-200, 52-175, 81-226, 74-252 and 142-472), respectively. **Figure 7a** demonstrates menstrual blood loss for individual patients pre- and at 3 months post embolsation and **Figure 7b** highlights the median percentage reduction in MBL at all post-treatment time intervals

Figure 7a

Menstrual blood loss for individual patients pre- and at 3 months post-embolisation N = 35

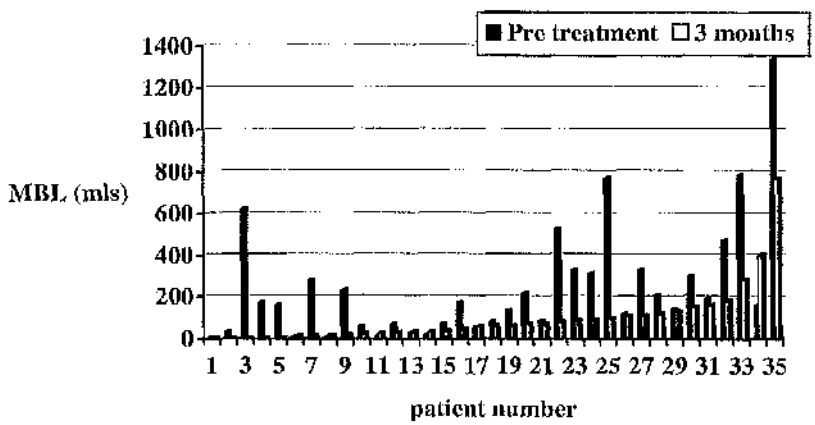
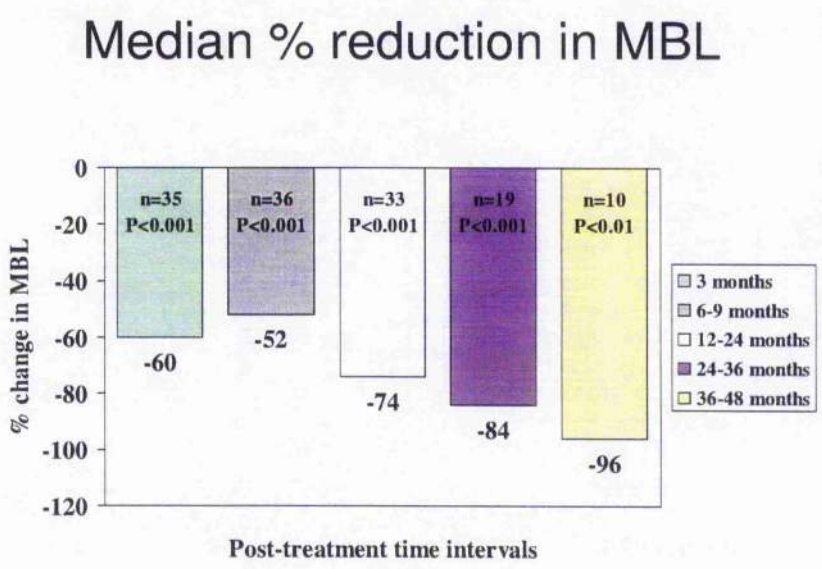


Figure 7b

Median percentage reduction in MBL at all post-treatment time intervals



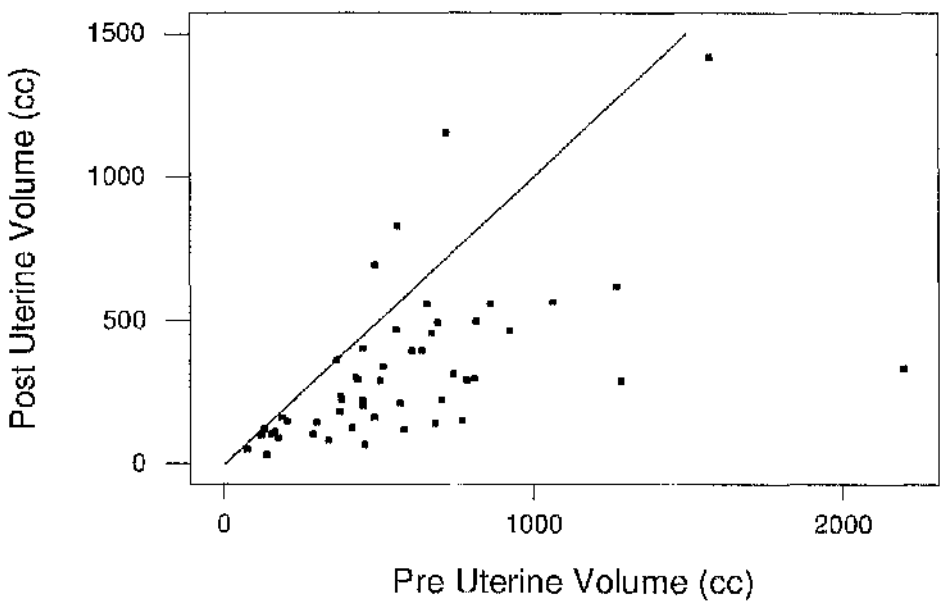
All post-embolisation reductions in MBL were statistically significant when compared with pre-treatment levels (3 months $P < 0.001$; 6-9 months $P < 0.001$; 12-24 months $P < 0.001$, 24-36 months $P < 0.001$; 36-48 months $P < 0.05$).

All but three women experienced reductions in uterine volume following embolisation (**Figure 8**). The median reduction in uterine volume at six months post-treatment was 41% (n = 51; 95% CI 34-49.7; $P < 0.001$). This did not correlate with the change in MBL at that time (Spearman's rank correlation coefficient $\rho = 0.134$; $P = 0.4$).

Figure 8

Scatter plot demonstrating pre- and post-embolisation uterine volumes for individual patients.

[Each dot represents an individual patient; dots lying below the *line of identity* represent all individuals with a reduced post-treatment uterine volume]



Six (11%) women underwent hysterectomy during the follow up period; four for inadequate reduction of MBL, one for persistent pressure symptoms with insufficient fibroid shrinkage and one for a combination of both persistent menorrhagia and pressure symptoms. Although a significant reduction in MBL occurred in three cases (median 61%; range 43-64%), the post-embolisation blood loss was still above the upper limit of normal in two of these cases. Two women have undergone myomectomy for insufficient relief of their pelvic pressure symptoms, despite good uterine volume reduction.

Seven (13%) women have become amenorrhoeic, six of whom have menopausal

hormone profiles. The median age at the time of menopause was 46 years (range = 40-54 years) whilst the median "treatment-menopause" time interval was 4.5 months (range = 3-36 months). One patient who became amenorrhoeic despite having a normal hormone profile was presumed to have developed post-infective intra-uterine adhesions, thus mimicking the clinical picture of Asherman's syndrome. None of these women desired a pregnancy in the future.

Two women have conceived spontaneously at the age of 45 years and 39 years, respectively. The first woman conceived 21 months following UAE and after an uneventful pregnancy, she delivered a healthy term baby vaginally. This particular patient experienced a 50% reduction in her MBL 12-18 months after treatment and a 51% reduction in her uterine volume at six months post- UAE. The second woman conceived 2.5 years after her embolisation procedure. In this instance, UAE was performed for bulk-related symptoms only. After an initial 35% reduction in uterine volume, her fibroids grew in excess of their pre-embolisation dimensions. In view of the risk of major peri-partum haemorrhage, elective caesarean section with prophylactic placement of balloon catheters in the uterine arteries was performed, culminating in an uneventful delivery of a healthy, but small for gestational age infant.

2.4 Discussion

The mechanisms whereby fibroids are thought to cause heavy menstrual loss remain poorly understood. Hysterectomy, myomectomy and medical treatments all have their limitations in the treatment of symptomatic fibroids and uterine artery embolisation appears to provide an efficacious alternative to women who wish to avoid surgery.

In the past, MBL has been assessed with the use of pictorial blood loss assessment

charts and more recently, using menstrual pictograms (Higham, O'Brien, & Shaw 1990;Wyatt et al. 2001). Previous studies assessing the efficacy of UAE have used menstrual diaries and questionnaires to determine changes in menstrual symptoms and most of these report a significant subjective improvement in such symptoms following embolisation (Andersen et al. 2001;Brunereau et al. 2000;Chimbira at al. 1980;Fraser, McCarron, & Markham 1984;Goodwin et al. 1999;Hallberg et al. 1966b;Hutchins, Jr., Worthington-Kirsch, & Berkowitz 1999;Pelage et al. 2000;Spies et al. 1999;Walker, Green, & Sutton 1999;West and Lunsden 1989). Walker and Pelage who were one of the first clinicians to publish a large prospective study evaluating UAE, report an 84% and 79% rate of improvement in menstrual bleeding and menstrual pain, respectively (Walker & Pelage 2002). It is important to remember, however, that there is little or no correlation between objective measurement and subjective assessment of blood loss (Chimbira, Anderson & Turnbull 1980;Fraser, McCarron, & Markham 1984). This emphasizes the need for objective tests such as the alkaline haematin technique, for the accurate assessment of menstrual bleeding. The problem with the latter method, however, is that it is cumbersome and time-consuming for both patient and laboratory investigator. Bearing in mind these impracticalities, it is usually reserved for use in a research setting such as the one we present here and more commonly, subjective assessment of fibroid symptomatology is relied upon. Ideally, two menstrual collections would have been collected pre-embolisation in our study, but for the reasons stated above, we thought it would be reasonable to collect sanitary protection from one menses, prior to treatment. At present, the alkaline haematin technique is the only practical means by which an accurate diagnosis of menorrhagia can unequivocally be established. However, a precise, but simpler method of menstrual blood loss quantification requires to be developed in the future.

The incidence of ovarian failure was high in our group of predominantly peri-menopausal women. All but one who became amenorrhoeic were over the age of 45 years and all had completed their family prior to treatment. Nonetheless, this is an important issue that must be discussed with all women considering embolisation, particularly those who wish to maintain their fertility. In fact, owing to the significant risk of ovarian failure following UAE, some gynaecologists and radiologists may feel that fibroid embolisation should not be offered to those women who desire future fertility.

Elimination of clinical symptoms is the main objective of UAE with uterine volume reduction and thus fibroid shrinkage being an additional benefit of treatment. It is important to note, however, that symptom relief can occur after embolisation in the absence of fibroid shrinkage. In this study, there was no relationship found between changes in MBL and uterine volume. Our observation reinforces the lack of understanding of the pathophysiology of fibroid-associated menorrhagia.

We have shown that UAE is a useful technique for treating uterine fibroids in terms of relief of menorrhagia. However, continuing studies with objective MBL assessments are required to establish the long term efficacy of this treatment.

2.5 Conclusion

Uterine artery embolisation causes a statistically significant reduction in menstrual blood loss which in maintained up to 48 months following treatment. It is also associated with a statistically significant reduction in uterine volume at six months. This study suggests that embolisation may prove to be an excellent long-term alternative to both surgical and medical treatments currently available to some women with symptomatic uterine fibroids.

Chapter 3

The Short Form 36 and health-related quality of life after uterine artery embolisation

3.1 Introduction

Symptomatic fibroids are a prevalent pathology amongst our population of women causing a significant disease burden to our health services. The chief objective of any treatment for benign disease such as symptomatic fibroids is to improve HRQoL by alleviating the symptoms associated with the particular condition. Despite this fact, little work has been done on the impact of fibroid symptoms on HRQoL. Over the last decade, subjective assessments of health have gained popularity when evaluating symptoms before or after a particular treatment intervention. Bearing this in mind, there is a need for the development and use of validated HRQoL tools.

The SF 36 was first introduced in the early 1990s and it is this tool that I have chosen to use for assessment of our group of women undergoing UAE. It is a previously validated questionnaire and has been used in a number of areas of medicine in the past (Brazier et al. 1992;Jenkinson, Coulter, & Wright 1993;Kiebzak et al. 2002;Ware J 1993). The questionnaire has the advantage of discriminating between symptom severity and health-related quality of life.

The purpose of this prospective observational study was to use the SF 36 to assess health status in a group of women with an established diagnosis of fibroids prior to and up to 60 months following uterine artery embolisation (UAE).

3.2 Materials and methods

The SF 36 comprises 36 questions (**Appendix 1**) assessing 8 dimensions of health encompassing physical, emotional and social status (**Table 3**). A further unscaled single item exists which assesses changes in the respondent's health over the past year.

Table 3

Dimensions of Health of the Short Form 36

PF-	Physical functioning
RP-	Role limitation as a result of physical problems
BP-	Bodily pain
GH-	General health
VI-	Vitality (the frequency of feeling full of energy versus feeling tired)
SF-	Social functioning
RE-	Role limitation resulting from emotional problems
MH-	Mental health

Local ethics committee approval was obtained prior to commencing this study. Women with an established diagnosis of symptomatic fibroids underwent UAE in one of four Glasgow City Hospitals. They were given detailed written information on the risks, benefits and complications of embolisation prior to signing a written consent form agreeing to participation in our study. Forty seven women aged between 29 and 47 years (median 43 years) completed the SF 36 health survey prior to UAE. Follow-up was completed by 35, 25, 18, 15 and 12 and 9 women at 3, 6, 12-24, 24-36, 36-48 and 48-60 months respectively.

All questionnaires were sent to women and returned by post. Patients who did not respond initially were sent another copy of the survey with a stamped addressed envelope one month later. The questionnaire took approximately 5-10 minutes to self-administer.

Normative data were taken from *Jenkinson et al* (Jenkinson, Coulter, & Wright 1993) for the purpose of comparison with our group of women with symptomatic fibroids. Pre- and post-embolisation scores were also compared. For each of the 8 variables of the SF 36, scores were coded, transformed and summed onto a scale ranging from 0 indicating the worst possible health status, to 100 indicating the

best possible level of functioning and well being. Paired t-tests were used to analyse whether the groups being compared differed significantly and $p < 0.05$ was considered to be statistically significant.

3.3 Results

Figure 9 demonstrates mean SF36 scores for women with symptomatic fibroids prior to treatment and compares them with those of age-matched women of the normal female population. For each of the 8 variables of the SF 36, women with symptomatic fibroids achieved lower scores, indicating the disease burden of fibroids.

Figure 9

Mean SF36 scores for women with symptomatic fibroids (pre-treatment) and age-matched women in the normal population

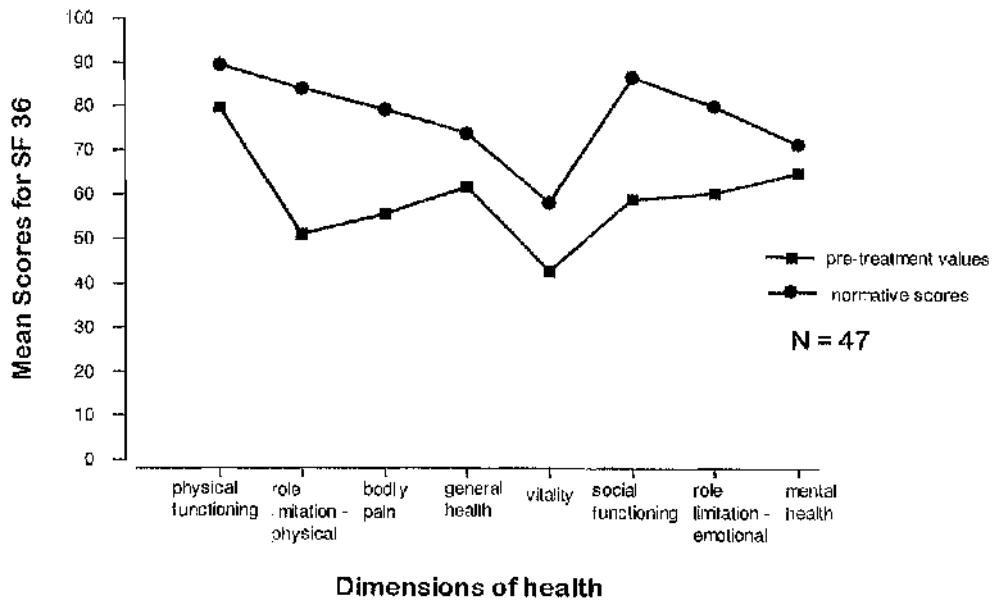
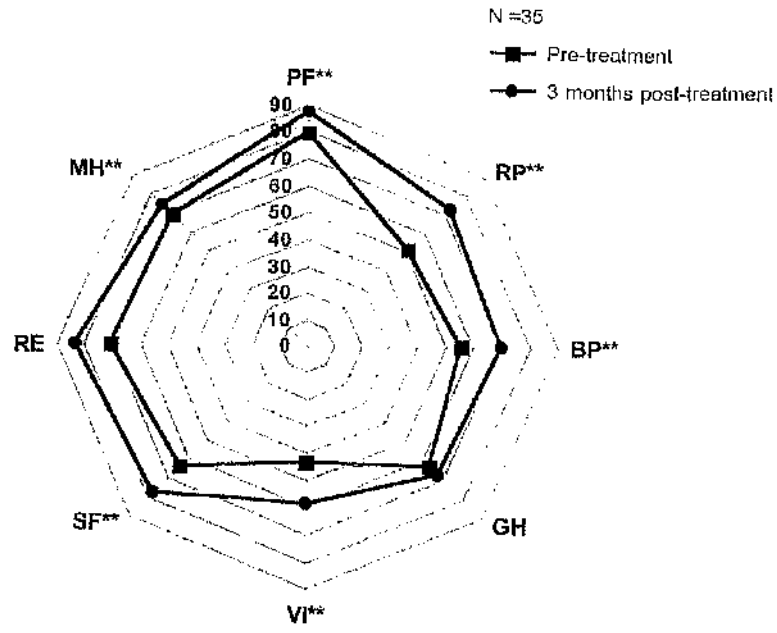


Figure 10 displays a radar plot comparing mean pre-treatment scores with those obtained from 35 women 3 months following embolisation. Mean scores increased in all dimensions of health after treatment indicating subjective improvement in health status. Changes in scores were statistically significant in all but two dimensions of health (GH and RE).

Figure 10

Radar plot comparing mean pre-treatment scores with scores 3 months post-embolisation



Each spoke on the plot represents a specific dimension of health of the SF36 (see **table 3**).
Plots are read along each spoke from the centre outwards.
Scores are shown on concentric circles beginning with 0 (at centre – indicates worst possible health status) and increasing to 100 (outer line – indicates best possible health status).
** = statistically significant difference at $P < 0.05$

Table 4 highlights the mean difference in scores and their respective confidence intervals, at this post-treatment time interval.

Table 4

Mean difference in pre- and 3 month post-treatment scores and their respective confidence intervals (** = statistically significant)

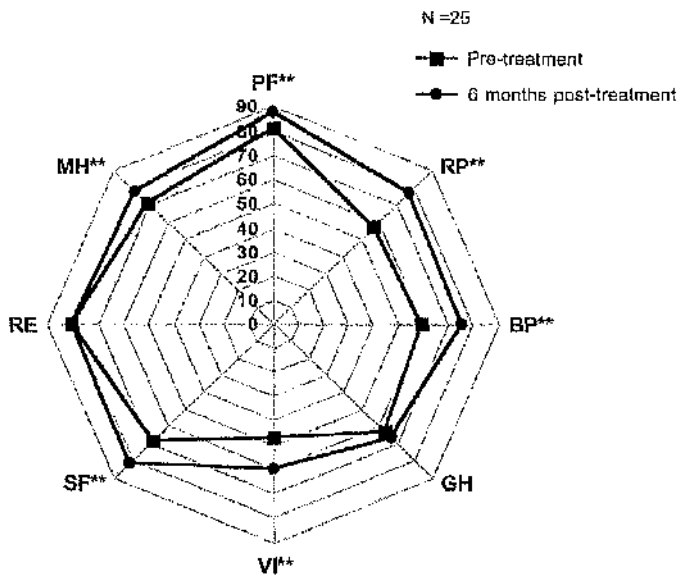
	Mean difference in scores	95% CI
PF**	8.0	(2.7, 13.3)
RP**	21.1	(4.6, 37.6)
BP**	14.8	(6.0, 23.5)
GH	4.4	(-0.5, 9.2)
VI**	15.4	(8.7, 22.1)
SF**	13.9	(5.7, 22.1)
RE	12.9	(-4.3, 30.1)
MH**	4.8	(0.2, 9.3)

(see **Table 3** for explanation of abbreviations)

At 6 months post-embolisation, 25 women completed the SF 36. Their mean scores obtained at this time interval were compared with pre-treatment scores **(Figure 11)**. Changes in scores at this time interval were statistically significant in 6 out of 8 dimensions of health.

Figure 11

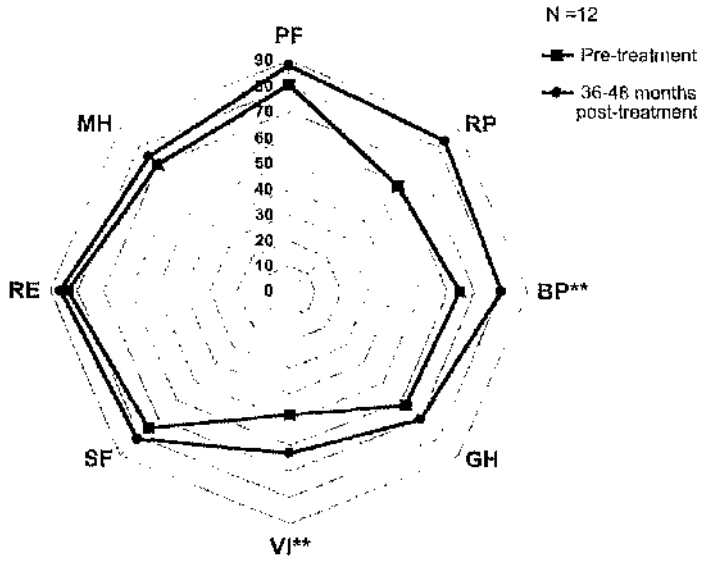
Radar plot comparing mean pre-treatment scores with scores 6 months post-embolisation



At 12-24, 24-36 and 36-48 months **(Figure 12)** after treatment, 18, 15 and 12 women respectively, completed the questionnaire; scores increased at these times in a very similar pattern when compared to previous post-treatment time intervals; these increases in scores were statistically significant in 2, 3 and 2 out of 8 dimensions of health, respectively.

Figure 12

Radar plot comparing mean pre-treatment scores with scores 36-48 months post-embolisation

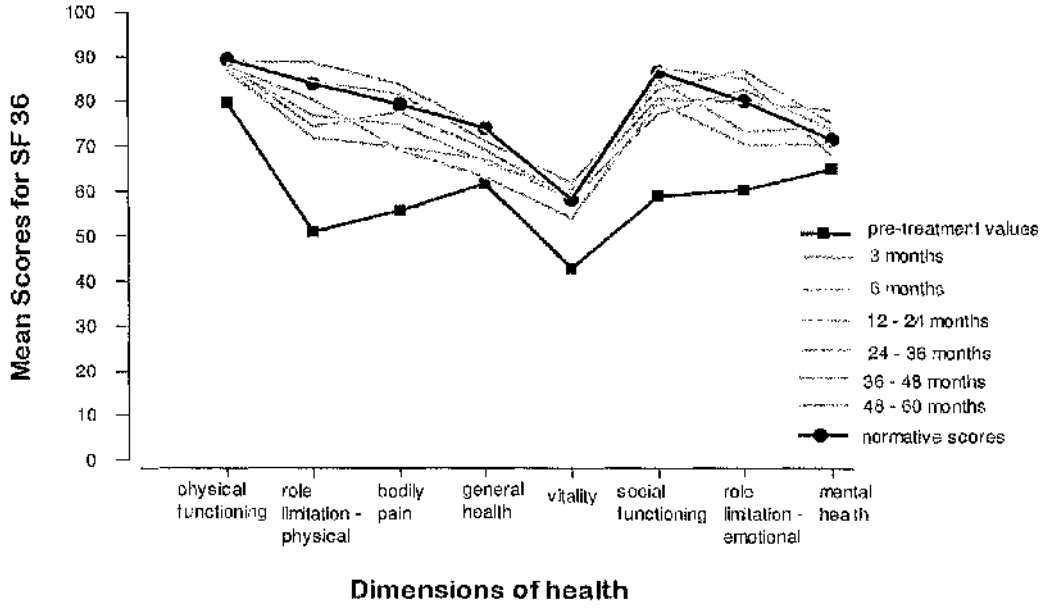


Finally, at 48-60 months after UAE, 9 women completed the SF 36 and once again, mean scores increased from pre-treatment values in all dimensions of health. Changes in scores for all dimensions of health at this time interval were not statistically significant.

Figure 13 displays mean scores at pre- and all post-treatment time intervals and compares these scores with those obtained for the normal age-matched female population. It is clear that following UAE, scores obtained at all post-treatment time intervals not only increase from pre-treatment levels, but also increase to levels similar to that of the normal female population, suggesting that overall functioning and wellbeing has improved towards normality after UAE.

Figure 13

Mean SF 36 scores at ALL time intervals



3.4 Discussion

In the past, subjective tools to assess symptomatic fibroids have been aimed primarily towards assessment of menstrual symptoms alone. Such tools include menstrual pictograms, menstrual diaries and questionnaires. Whilst these are useful in assessment of menorrhagia, neither the non-bleeding symptoms specific to fibroids are assessed and nor is the effect of such symptoms on health-related QOL.

The advantage of using the subjective SF 36 health survey over the aforementioned tools is that it takes into account the overall effect of fibroids on health status. In addition, unlike objective and subjective testing of menstrual blood loss, the survey is easy to administer and not especially time-consuming for

patients to complete. Nonetheless, our group of women who completed follow-up diminished as time went by due to lack of response or withdrawal from the study. Whilst each questionnaire takes approximately 5-10 minutes to self-administer, some women may have perceived that the completion of the questionnaire and the additional time taken to arrange its postage was too time-consuming to continue with. The lack of statistically significant changes in scores with increasing pre-treatment to post-treatment time intervals may be a reflection of the smaller numbers of women being assessed as time went by.

A disadvantage of the SF 36 is that it does not include variables which take quality and quantity of sleep and sexual function into account. Both sleep and sexual activity may be influenced by symptomatic fibroids and therefore are worth considering when assessing both the disease and the influence of a treatment intervention on this condition.

The advantage of the UFS-QOL questionnaire (Spies et al. 2002a) when compared to the SF 36 is that it not only aims to assess health-related QOL, but also, specific menstrual symptoms and bulk-related problems (e.g. urinary frequency and back ache), both of which are commonly experienced by women with uterine fibroids. The survey has a symptom severity scale comprising 8 questions and a HRQoL scale comprising 29 questions; these scales can be scored either separately or together thus allowing discrimination between symptom severity and HRQoL. The UFS-QOL questionnaire has been found to have a low to moderate correlation with the SF 36 and a moderate to strong correlation with the Menorrhagia Questionnaire (Lamping et al. 1998). As far as the SF 36 is concerned, the "bodily pain" variable of this questionnaire was shown by *Spies et al* to have the highest correlation to the UFS-QOL "activities scale". This survey, however, has only been available for use in the last five years (Smith et al. 2004) and was not fully validated at the time of this study. For this reason, we chose to

use the generic SF 36 questionnaire for assessment of HRQoL in our group of women with symptomatic fibroids.

Whilst the SF 36 general health survey does have its limitations, we have shown that it is a useful tool for documenting subjective improvements in HRQoL in women with symptomatic leiomyomata who have undergone UAE. In addition, this questionnaire provides further evidence for the efficacy of UAE as a treatment for symptomatic fibroids.

3.5 Conclusion

We have concluded from our study that health as assessed by the SF 36 questionnaire is significantly improved at 3 and 6 months following UAE. This improvement appears to be maintained up to 60 months after treatment. We have also concluded that the observed increase in SF 36 scores provides evidence for the efficacy of UAE.

Chapter 4

Inflammatory response to uterine artery embolisation

4.1 Introduction

Uterine artery embolisation is associated with significant post-procedural pelvic pain in most cases. Whilst this resolves in majority of women within 24-48 hours with the use of appropriate analgesia, approximately 10-15% of them experience further pain 7-21 days after their procedure. This pain tends to be accompanied by a flu-like illness characterised by general malaise, a low grade fever and nausea with or without vomiting. We presume that this second episode of significant pelvic pain is part of the post-embolisation syndrome (Pelage et al. 2002;Pelage et al. 2000;Walker & Barton-Smith 2006;Walker & Pelage 2002). It is similar to the syndrome which occurs following myocardial infarction (Erzen et al. 2006;Frangogiannis 2006) and has also been reported after both hepatic artery and renal artery embolisation (Bissler et al. 2002;Wigmore et al. 2003).

The aetiology of post embolisation syndrome, however, remains poorly understood. It has been postulated that the syndrome results from a release of inflammatory mediators from the fibroids which have undergone ischaemic necrosis as a result of UAE. Clinically, it is very difficult to distinguish the syndrome from infection and on a number of occasions, unnecessary hysterectomy after UAE has been performed for suspected pelvic infection when in fact the problem has been the post-embolisation syndrome. The latter is usually self-limiting and can be treated with adequate analgesia, anti-emetics, antibiotics, rehydration and reassurance.

In order to identify and treat this problem appropriately, we require a better understanding of the aetiology of this process. We are aware that an inflammatory response occurs in organs other than the uterus after embolisation and therefore we set out to investigate the evolution of inflammatory markers after UAE in order to monitor the normal course following the procedure. In addition we hoped

that our results would provide further insight into the aetiology of the post-embolisation syndrome.

The inflammatory markers that we chose to measure include white blood cell count (WCC), C-reactive protein (CRP), creatine kinase (CK), adiponectin, interleukin 6 (IL-6), interleukin 18 (IL-18) and tumour necrosis factor alpha (TNF-α).

White blood cells, also known as leucocytes, are cells of the immune system which defend the body against both infectious diseases and foreign material. Several types of white blood cells exist, all of which are produced and derived from a pleuripotent cell in the bone marrow known as a haemopoietic stem cell. These cells increase in response to inflammation or infection and are therefore useful markers of inflammatory processes. C-reactive protein is a protein produced by the liver that is present during episodes of acute inflammation or infection. This protein plays an important role in the body's immune defence mechanism and whilst it is not specific for a particular pathology, high levels of CRP in blood serum is a general indication of acute inflammation. Creatine kinase is an enzyme expressed by various tissue types. It catalyses the conversion of creatine to phosphocreatine, consuming adenosine triphosphate (ATP) to adenosine diphosphate (ADP), as well as doing the reverse. In skeletal muscle especially, and both brain and smooth muscle, phosphocreatine serves as an energy reservoir for the rapid regeneration of ATP, the major source of energy in biochemical reactions. Elevation of CK is an indication of damage and thus injury to muscle.

Adiponectin is a protein hormone secreted specifically by adipocytes. This protein regulates the metabolism of both lipids and glucose and influences the body's response to glucose. Adiponectin has anti-atherogenic and anti-inflammatory roles, and plasma levels of this hormone are decreased in patients with coronary

artery disease. Interleukin 6 has a major role in the mediation of inflammatory or immune responses; it is a pro-inflammatory protein secreted by T-cells and macrophages. IL-6 is released in response to infection, burns, trauma and neoplasia, and its functions range from key roles in acute-phase protein induction to B- and T-cell growth and differentiation. Thus, this protein has direct effects on cells and can be agonistic or antagonistic in conjunction with other cytokines. Interleukin 18 is a pro-inflammatory cytokine produced mainly by antigen-presenting cells. It is an important regulator of both innate and acquired immune responses, playing a key role in autoimmune, inflammatory and infectious diseases. Finally, tumour necrosis factor alpha, named for its antitumour properties, is a cytokine produced primarily by monocytes and macrophages. It plays a critical role in normal host resistance to infection and the growth of malignant tumours, serving as an immunostimulant and mediator of the inflammatory response. Over-production of TNF-alpha has been implicated in a number of conditions including septic shock, septicaemia, autoimmune disorders and cachexia (progressive wasting).

4.2 Materials and methods

Local ethics approval was obtained prior to recruiting 13 women into this prospective observational study. The women were scheduled to undergo UAE for symptomatic fibroids in one Glasgow city hospital following either direct referral for the procedure or via the embolisation arm of a randomised controlled trial comparing UAE to surgery for the treatment of symptomatic fibroids (REST trial). All of them signed a consent form agreeing to venepuncture prior to UAE, 24 hours post-procedure and at intervals of 3 days, 7 days and one month thereafter. The latter three intervals required a 20 minute additional visit to the

hospital. Blood was taken in order to measure WCC, CRP and CK. Each of the latter was measured by North Glasgow University Hospitals haematology and biochemistry laboratory staff using a standard technique. WCC was measured by the Sysmex SE9500 analyser whilst CRP and CK were measured using the Beyer, Advia analyser.

Additional blood samples were taken at the aforementioned time intervals in order to quantify levels of IL-6, IL-18, adiponectin and TNF-α. These blood samples were spun by centrifuge (Beckman GS-6KR Centrifuge) at 3000 revolutions per minute for 10 minutes at 4 degrees centigrade, in order to separate plasma from blood cells. This was carried out within two hours of venepuncture. Measurements of IL-6, IL-18, adiponectin and TNF-α were then carried out in the Vascular Biochemistry Laboratory of North Glasgow University Hospital, by Dr Lynne Crawford (Senior Research Technician) and Pauline Watt (Research Technician).

IL-6 was measured using an R & D kit (19 Barnton Lane, Abingdon Science Park, Abingdon, OX14 3NB, UK) – catalogue number D6050. IL-18 was measured using a MBL kit supplied by R & D (catalogue number 7620). Adiponectin and TNF-α were measured using R & D kits (catalogue numbers DRP 300 and HSTA00C, respectively).

Pain scores at twenty four hours were recorded by each patient on a linear analogue scale (ranging from 0 [no pain] – 10 [worst possible pain]) and this was correlated with change from baseline in inflammatory markers at that time interval.

Data are expressed as mean ± SD and one-way analysis of variance (ANOVA) and paired t-tests were used to analyse results. $P < 0.05$ was considered statistically significant.

4.3 Results

Ten women had a full set of blood results for all time intervals. Following uterine artery embolisation, WCC and CRP values increased significantly from baseline pre-treatment levels up to seven days post-procedure. Levels peaked at 3 days with average maximums of $11.95 \pm 3.50 \times 10^{-9}$ / L and 62.60 ± 43.53 mg / dL, respectively; thereafter, levels declined sharply for both markers, normalising towards baseline levels at one month **(figures 14 and 15)**.

Figure 14

Mean white cell count (WCC) at all time intervals

P = 0.012

[error bars represent standard deviation (SD)]

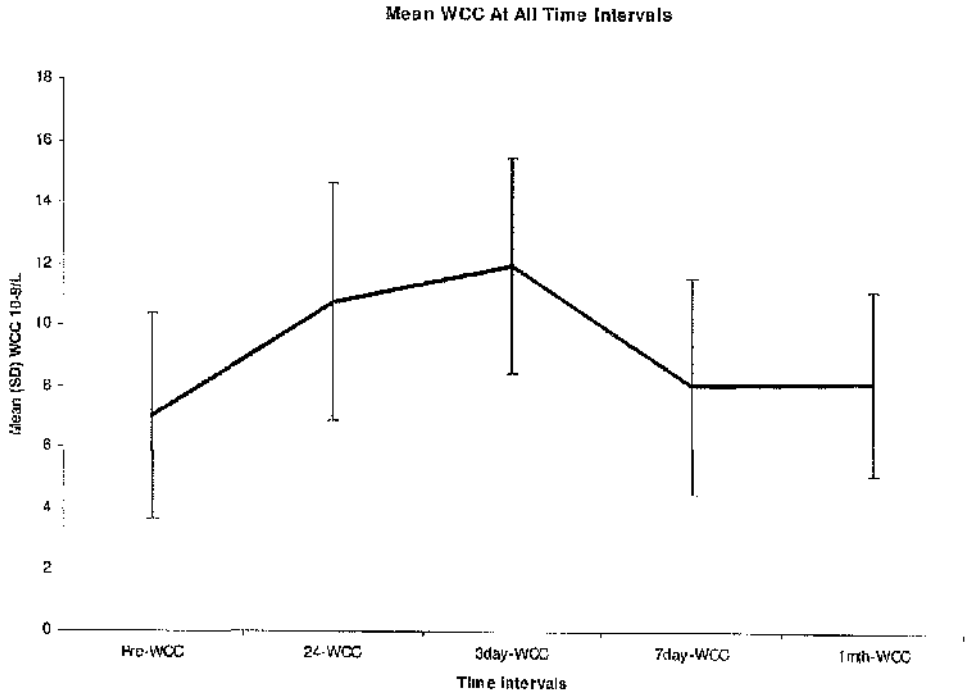
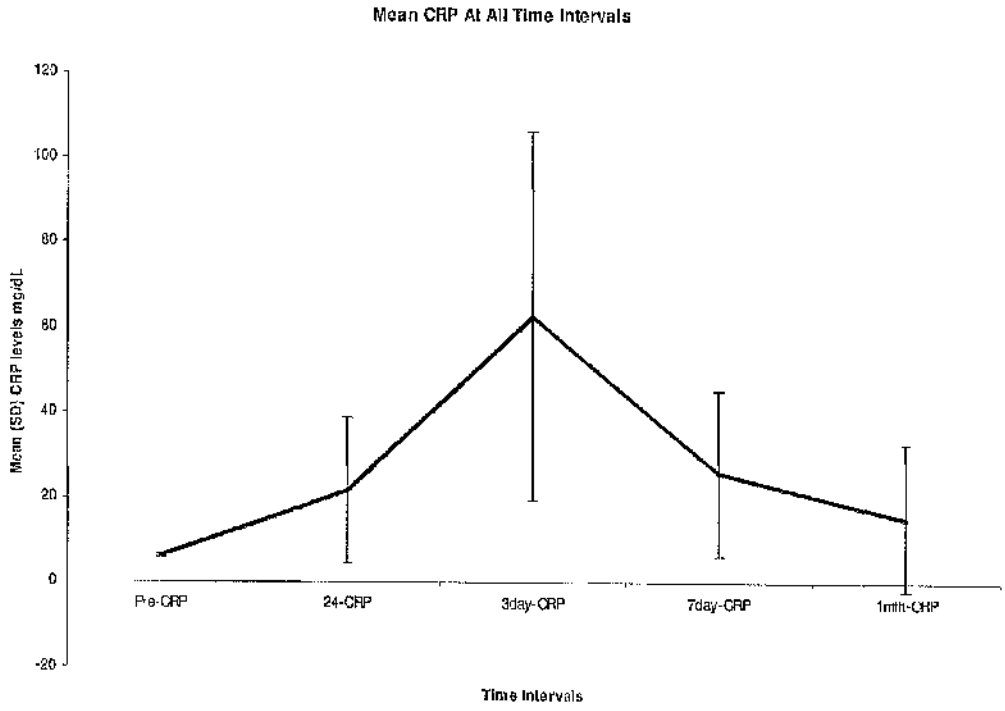


Figure 15

Mean C-reactive protein (CRP) at all time intervals

$P < 0.0001$

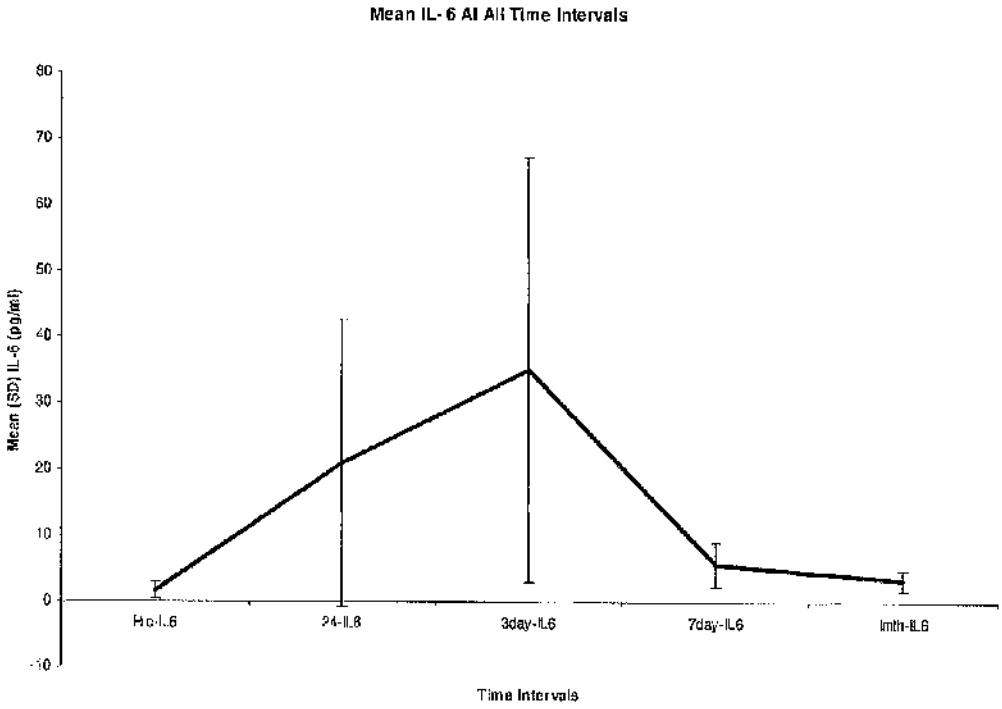


IL-6 levels increased significantly from baseline up to one month post-embolisation. Once again, levels peaked at 3 days with an average maximum of 34.97 ± 32.04 pg / ml, declining thereafter towards baseline levels at one month **(figure 16)**.

Figure 16

Mean interleukin 6 (IL-6) at all time intervals

$P < 0.0001$



Creatine kinase, adiponectin, IL-18 and TNF-α levels did not alter significantly from baseline after UAE and did not appear to show the typical 3-day peak of other markers (**figure 17, 18, 19 and 20**).

Figure 17

Mean creatine kinase (CK) at all time intervals

P = 0.76

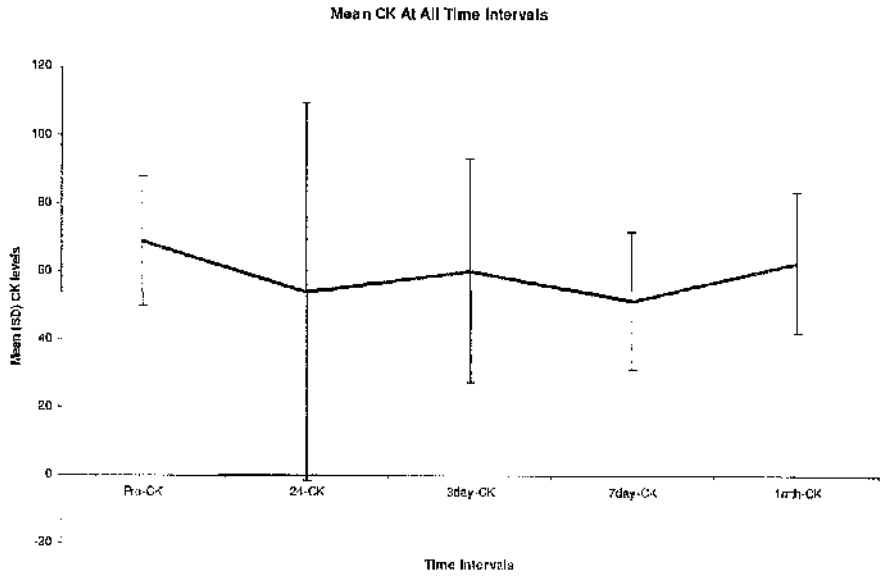


Figure 18

Mean adiponectin at all time intervals

P = 0.83

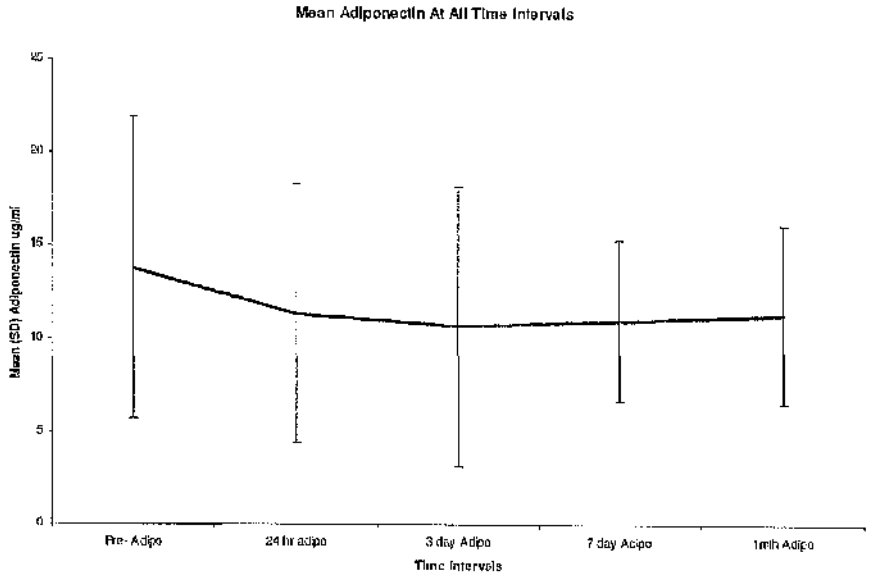


Figure 19

Mean interleukin 18 (IL-18) at all time intervals

P = 0.58

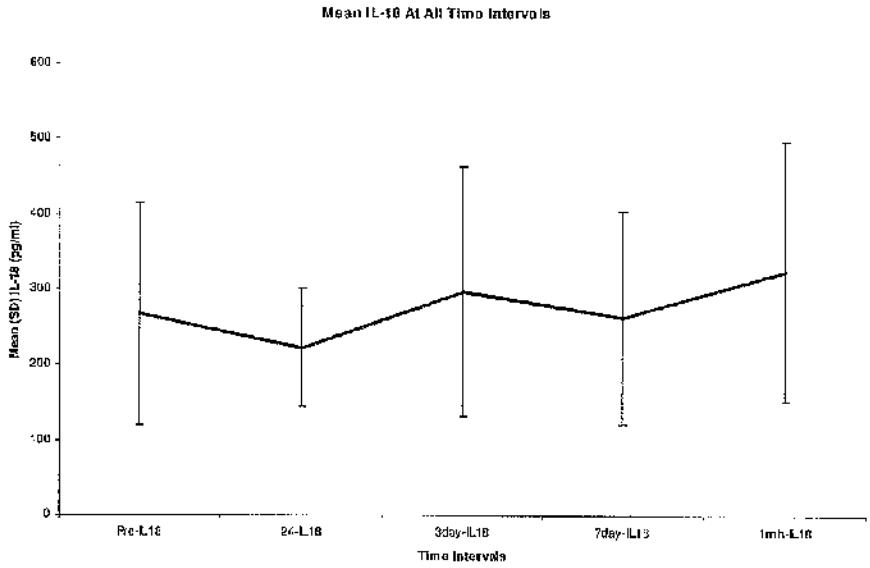


Figure 20

Mean tumour necrosis factor alpha (TNF-α) at all time intervals

P = 0.51

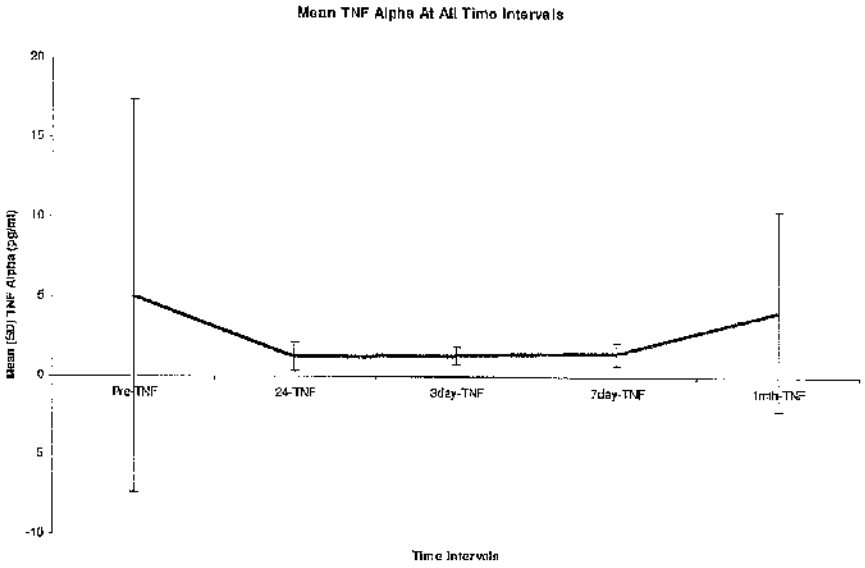
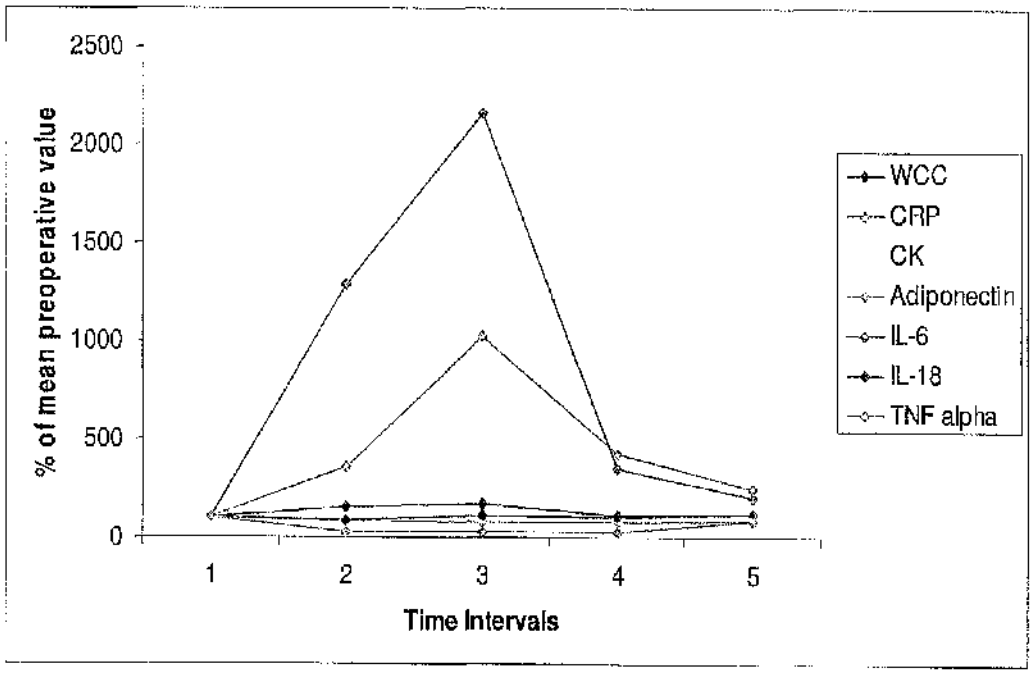


Figure 21 demonstrates all markers at all time intervals, expressing each marker value over time as a percentage of its baseline pre-treatment value.

Figure 21

All markers at all time intervals



Mean pain score at 24 hours was 4.85 ± 3.12 (n = 10). No correlation (Pearson's correlation coefficient) was found between pain score and each of the individual inflammatory markers at this time (WCC - r=0.006, P=0.99; CRP - r=0.037, P=0.92; CK - r=0.203; P=0.57, Adiponectin - r=0.247, P=0.49; IL 6 - r=0.236, P=0.51; IL 18 - r=0.153, P=0.67; TNF alpha - r=0.002, P=1).

4.3 Discussion

The results of this study indicate that an inflammatory response occurs following uncomplicated UAE. However, the peak rises in inflammatory mediators did not occur at the time intervals that we expected. Levels of WCC, CRP and IL-6 peaked at 3 days post-embolisation, with normalisation at one month after treatment. To our knowledge, only two studies have been published with regard to the inflammatory response after UAE. The first was by a German group who measured WCC and CRP in twenty women before and up to five days after fibroid embolisation (Vorwerk et al. 2003). The authors concluded that uncomplicated UAE is associated with a steep and significant increase in CRP between 48-96 hours after treatment, with a decline in levels thereafter. They also demonstrated a mild increase in WCC, peaking at 72 hours and normalising by the fifth day post-embolisation. Both these trends in inflammatory markers were thought not to indicate an infected fibroid.

More recently, markers of muscle ischaemia, necrosis and inflammation following UAE have been studied by Banu et al (Banu et al. 2007). A number of markers were quantified, including CK, ischaemia-modified albumin (IMA) and CRP. The study group were randomised to either the UAE treatment arm (n=14) or the abdominal myomectomy arm (n=11), and blood markers were measured before, immediately after, 18-24 hours and six weeks after treatment. Inkeeping with our study, no significant change was seen in CK levels after UAE. However, whilst a rise in CRP was seen 24 hours after embolisation, the rise in this inflammatory mediator was significant only six weeks after treatment. No significant rise in IMA was observed after UAE. The authors concluded that no significant ischaemia or necrosis occurs in association with UAE, suggesting that the procedure does not significantly damage the myometrium.

The findings from our study do not support our initial hypothesis that the post-embolisation syndrome results from a release of inflammatory mediators from fibroids which have undergone ischaemic necrosis as a result of UAE. If this was the case, we would have expected to see significant rises or peaks in inflammatory markers 7-21 days after embolisation, in women who experienced flu-like symptoms and pelvic pain at this time interval. Thus an alternative explanation must exist for the pathogenesis of the post-embolisation syndrome. The lack of correlation that we found between levels of pain experienced by women and individual inflammatory markers 24 hours after UAE also suggests that fibroid ischaemia and inflammation are not the only causes of pelvic pain experienced by women after embolisation.

All women in our study experienced variable degrees of pelvic pain in the immediate few hours and days after treatment. As expected, most women were discharged 24 hours after their procedure and reported both resolution of pain over 7 days and resumption of normal activities within two weeks of embolisation. Four women complained of mild flu like symptoms (in the absence of a temperature) and pelvic pain 7-21 days after UAE; their symptoms were thought to be due to the post-embolisation syndrome, but inflammatory markers were normal at the time when they reported their symptoms. One woman, however, required admission for severe pelvic pain 7-10 days after UAE and again, three-four weeks post-procedure. Her clinical symptoms also occurred in the absence of a temperature. White cell count and CRP levels were persistently elevated at these times. It was presumed that this woman's symptoms (which fully resolved six weeks after UAE with conservative measures and reassurance) were due to the post-embolisation syndrome.

This study had a number of limitations. The number of women recruited was fairly small as it was difficult to find women who were willing to undergo

additional blood tests necessitating extra hospital visits. Also, some women who initially agreed to entry into this study, subsequently defaulted from a number of the necessary hospital visits; these women had to be excluded from the study. It may have been useful to measure our chosen markers more frequently over a one month period and it may well be that measurement of inflammatory markers other than those that we chose to analyse, would have provided us with additional information regarding the course of uncomplicated UAE and the post-embolisation syndrome.

4.5 Conclusion

Uncomplicated UAE is associated with a significant rise in inflammatory markers, specifically WCC, CRP and IL-6. The peak rise occurs at 3 days with normalisation of marker levels at one month. No correlation was found between pain scores at 24 hours and individual inflammatory markers at that time.

Chapter 5

Comparison of uterine artery embolisation with surgery for the treatment of symptomatic leiomyomata

5.1 Introduction

Uterine artery embolisation is starting to become accepted as a routine uterine-sparing modality for the treatment of symptomatic fibroids. Nonetheless, there is still a need to compare it to established and accepted therapeutic options for this condition. Whilst most published studies are encouraging, few present comparative data assessing the safety and efficacy of the procedure. The REST trial is a Scottish multicenter randomised controlled trial comparing embolisation to surgery as a treatment for fibroids. It aims to address these issues with a primary outcome measure of quality of life at one year. Long term follow-up is due from 2007.

5.2 Materials and methods

The trial commenced in November 2001 in seven hospitals in Scotland, but later expanded to include a total of 27 hospitals in the United Kingdom, two of which were based in England. Recruitment was completed in May 2004 and twelve month follow-up was completed in September 2005. Ethics committee approval was granted by the Multicentre Research Ethics Committee and local approval granted at every centre. All subjects provided written informed consent. The potential recruits were provided with written information which described the study and the potential risks of treatment including the unknown effects of embolisation on any subsequent pregnancy. A trial coordinator was appointed to supervise the trial and 4 research nurses and a research fellow in gynaecology (myself), carried out recruitment and follow-up of patients within the 26 centres participating in the trial. Appropriately trained interventional radiologists carried out the embolisation procedures, with referral to specialist centres from district units when required. They used a standard technique for embolisation, as

described in chapter 1, section 1.8, page 36. Surgery was in the form of either hysterectomy or myomectomy and was carried out at each local centre.

Patients were recruited at the local hospital gynaecology clinics. Women were eligible for entry into the trial if they had one or more fibroids > 2cm in diameter which could be adequately imaged using MRI and caused sufficient symptoms (e.g. menorrhagia, pelvic pain and pressure) to justify surgical treatment. Exclusion criteria included a contraindication to MRI, severe allergy to iodinated contrast media, the presence of subserosal pedunculated fibroids, other pelvic pathology such as endometriosis or adenomyosis, current pregnancy, recent or ongoing pelvic inflammatory disease and chronic illness. No restrictions were made regarding the size or number of fibroids.

All women underwent a baseline MRI scan prior to treatment, with T2 sagittal, T2 coronal oblique and T1 sagittal images being taken. During the mid to latter stages of the trial, gadolinium enhancement was used; the aforementioned protocol for imaging was therefore carried out before and after blood vessel enhancement. Imaging was repeated at 6 months following treatment in those women who were recruited into the embolisation arm of the trial, using the same protocol. All women who required further intervention following either UAE or myomectomy were asked to have a further MRI scan prior to the intervention. The number of fibroids, the diameter of the largest fibroid and uterine volumes (measured using the standard technique described in chapter 2, section 2.2, page 54) were recorded together with the presence of any other abdomino-pelvic pathology.

Those patients whose MRI findings fitted the trial criteria were then randomised into one of two study groups according to a computer generated schedule (permuted blocks) held by the study co-ordinator. Randomisation was stratified by centre and used a 2:1 ratio with twice as many women being allocated to the new

treatment (embolisation) group as to the surgical group. This allowed us to obtain as much information as possible on the novel procedure with minimal reduction in statistical power of the study.

The method of surgery i.e. hysterectomy or myomectomy was left to the discretion of the individual operator who took into consideration the woman's reasons for wishing to preserve her fertility and / or uterus and MRI findings. Both operations were included since virtually all surgical procedures for fibroids are performed by the open route, allowing appropriate comparison of outcomes.

Likewise, the precise technique for embolisation was not specified and left to the discretion of the operator although both uterine arteries had to be embolised and the particle size of the embolic agent was standardised at 500-710 microns. Most centres used non calibrated polyvinyl alcohol (PVA) (Cook Inc, UK) and Trufill (Cordis Ltd, UK) although latterly a few patients were embolised with Bead Block (Biocompatibles UK Ltd, Surrey, UK).

The primary outcome measure was quality of life at 12 months as assessed by the SF 36. As mentioned previously, this generic tool has been previously validated in women with menorrhagia (Jenkinson, Peto, & Coulter 1996). Secondary outcomes included quality of life as measured by the EuroQol 5D QoL questionnaire (an instrument used to measure preferences for certain health outcomes), a tool with a range of scores paralleling those of the SF 36. Other secondary outcomes were the time taken to reach functional milestones after the procedure, an 11 point symptom score (-5 [markedly worse] to + 5 [markedly better]) and a satisfaction score asking whether the women would recommend her treatment to a friend (recorded as either yes, no or unsure). Pain score at 24 hours was also measured using a 10-point linear analogue scale with documentation of the analgesic requirement at that time. The presence or absence of complications was also recorded as well as treatment failures, defined as the need for subsequent

intervention for symptom control, including hysterectomy or repeated embolisation.

Complications were graded using the Society of Interventional Radiology (SIR) classification, recommended in their "Standards of Practice" (Goodwin et al. 2001), as follows:

Society of Interventional Radiology Complication Grading

1 No therapy, no consequence

2 Nominal therapy, no consequence, includes overnight admission for observation only

3 Requires therapy, minor hospitalisation (<48 hours)

4 Requires major therapy, unplanned increase in level of care, prolonged hospitalisation (>48 hours)

5 Permanent adverse sequelae

Grades 1-2 and 3-5 were subsequently grouped as minor and major complications, respectively. The categorization of complications was carried out by two of the investigators independently – one a Consultant Gynaecologist and the other, a Consultant Radiologist. In discordant cases, the worse grade was used.

Major serious adverse events (SAEs) included any major complication, life threatening events, hospitalisation (initial or prolonged), disability, an intervention required to prevent permanent impairment or damage and death. SAEs also included treatment failures requiring readmission for repeat embolisation or surgery.

A fixed analgesia protocol was suggested for the UAE arm of the trial which included rectal diclofenac, parenteral morphine or diamorphine, benzodiazepine sedation and antiemetics; all preparations were given 1 hour prior to embolisation. Further opiates were administered during the procedure as single doses and over the next 24 hours, women received analgesia via a patient controlled analgesia pump (PCA).

Surgical patients all had their procedures performed under general anaesthetic. Post-operative pain was managed by the administration of opiates via a PCA pump according to the standard procedures in that unit. The total opiate consumed within the first 24 hours was recorded for both groups.

After discharge from hospital, outcome measures were assessed at 1, 6, 12, and 21 months, and annually thereafter. Twelve month results are presented here, with the exception of major adverse events requiring hospitalisation and subsequent intervention for treatment failure, which are reported up to September 2005 (maximum follow-up 58 months).

Statistical analysis was based on intention to treat (i.e. patients were analysed in the group to which they were randomly assigned, irrespective of the treatment they actually received). Analysis of covariance was used to compare quality of life scores (SF36 and EuroQol) between groups, adjusting for baseline values. Two sample t- and Mann Whitney tests, and chi-squared tests were also used to make comparisons between groups, the former for continuous data and the latter for categorical data.

The original power calculation required the enrolment of 200 patients to give a power of 90% to detect a difference of 10 points in the SF36 score at 12 months (the primary end-point) at a 0.05 significance level. Owing to the slower than expected recruitment process, however, the decision was subsequently made to

reduce the power of the study to 80%; this required a total sample size of 150 participants.

An independent data monitoring committee reviewed the results and any SAEs every 12 months. They followed the highly conservative Haybittle-Peto approach which requires a significance level of less than 0.001 in the comparison between groups before making any recommendations to terminate the trial prematurely (Jennison & Turnbull 2000).

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5.3 Results

A total of 157 women were randomly assigned to the two study groups; 106 to the embolisation arm of the study and 51 to the surgical arm of the study. Of those women who underwent surgery, 43 underwent hysterectomy and 8 underwent myomectomy **(Figure 22)**.

Eight patients (5%) did not receive their allocated treatment (five in the embolisation group and three in the surgical group). There was one technical failure in the surgical arm with a myomectomy being converted to hysterectomy due to technical difficulties. All of the hysterectomies (43) and myomectomies (8) were performed through an abdominal incision. Three patients in the UAE arm had a technical failure due to difficulty identifying or catheterising one or both uterine arteries. The groups were well matched at baseline **(Table 5)**.

FIGURE 22

Trial profile at 12 months follow up

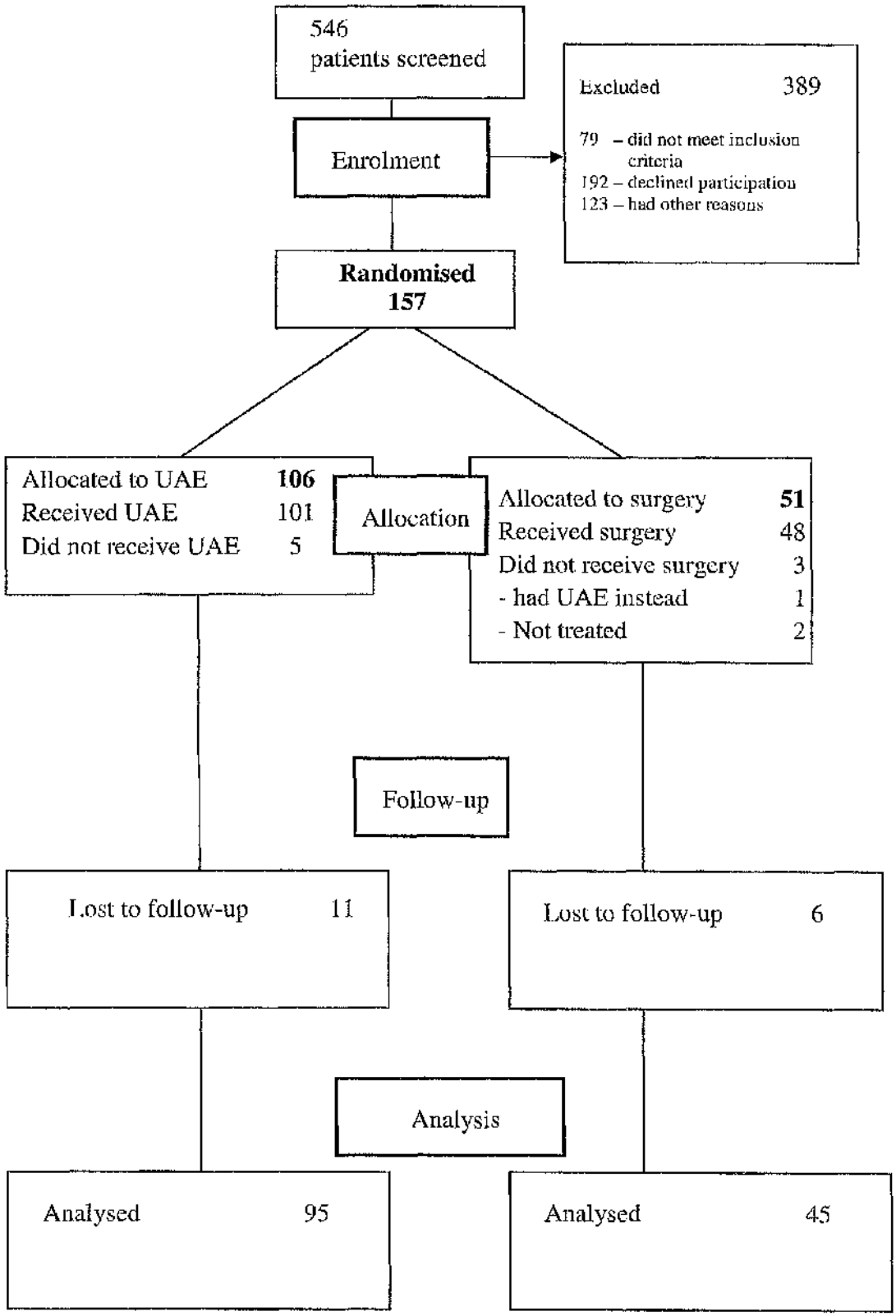


Table 5 Baseline characteristics of the patients

Characteristic	Embolisation (N=106) Mean	S.D.	Surgery (N=51) Mean	S.D.	P value
Age (years)	43.6	5.5	43.3	7.1	0.77
Largest fibroid diameter (cm)	7.5	3.0	8.5	3.9	0.12
Uterine volume (mls)	579	447	701	627	0.23
SF-36 Score					
physical function	82	19	77	20	0.16
role- physical	51	41	45	42	0.35
bodily pain	52	22	50	22	0.60
general health	61	19	60	23	0.92
vitality	41	22	42	23	0.93
social function	63	27	58	30	0.34
role- emotional	60	43	57	43	0.76
mental health	63	18	63	22	0.91
EuroQoL Score	70	16	63	20	0.04
Main presenting symptom:					
Number of patients (%)	102		50		0.92
Bleeding	56 (55)		29 (58)		
Pain	19 (19)		7 (14)		
Pressure	23 (23)		12 (24)		
Other	4 (4)		2 (4)		

SF-36 and EuroQol scores range from 0 (worst possible health status) to 100 (best possible health status).

S.D. = standard deviation

All study participants were pre-menopausal.

Primary Outcome

The primary outcome measure (12 month SF-36 score) was available for 140 (89%) of the 157 women. The results for the SF-36 and Euroqol at 1 and 12 months are given in **Table 6**. There was no statistically significant difference between UAE and surgery in any of the eight components of the SF-36 at 12 months, although at one month, the embolisation group had significantly greater improvement in scores than the surgery group for physical function, social function and role-physical components.

Secondary Outcomes

Women in the surgical arm of the trial had a significantly higher pain score at 24 hours (mean difference 1.6, 95% CI 0.8 to 2.3, $p < 0.001$). Symptom scores at one and 12 months after the procedure were significantly better in the surgical group. The percentage of women prepared to recommend their treatment to a friend at 12 months was high in both treatment groups (93% in the surgical group and 88% in the embolisation group) ($p = 0.32$) – **Table 7**. The median hospital stay for UAE was significantly shorter than after surgery (1 day versus 5 days, $p<0.001$). The median time until patients could resume all recorded usual activities was significantly lower in the UAE group – **Table 8**.

Table 6

The effect of uterine artery embolisation and surgery on quality of life measures (SF-36 & EuroQol scores)

	Embolisation (n=106)		**Surgery** (n=51)		**Absolute difference**	**P Value**
	mean	S.D.	mean	S.D.	(95% CI) *	
SF-36 score 1 month						
No.of patients	95		47			
physical function	85	16	57	25	-26 (-32 to -20)	<0.001
role- physical	37	44	11	24	-25 (-38 to -12)	<0.001
bodily pain	50	22	44	24	-6 (-14 to 2)	0.16
general health	70	19	74	17	4 (-1 to 10)	0.13
vitality	47	22	42	24	-6 (-13 to 1)	0.11
social function	64	27	44	29	-19 (-28 to -9)	<0.001
role- emotional	72	41	64	44	-7 (-22 to 7)	0.32
mental health	72	17	74	18	2 (-3 to 8)	0.39
12 months						
No.of patients	95		47			
physical function	92	14	89	20	0 (-6 to 5)	0.85
role- physical	76	40	81	34	7 (-7 to 20)	0.33
bodily pain	76	23	80	26	4 (-4 to 13)	0.28
general health	74	20	79	17	6 (0 to 12)	0.07
vitality	62	21	67	22	4 (-3 to 11)	0.26
social function	84	23	87	26	4 (-4 to 12)	0.35
role- emotional	81	35	87	30	7 (-4 to 18)	0.22
mental health	76	17	76	21	-1 (-7 to 5)	0.8
EuroQol at 1 mth						
No.of patients	92		47			
Score	74	17	67	19	-4 (-9 to 2)	0.24
EuroQol at 12 mth						
No.of patients	93		45			
Score	82	16	83	14	4 (-2 to 9)	0.18

S.D. = standard deviation

CI = confidence interval

For the differences in quality of life scores (SF 36 and EuroQol) between the surgical and embolisation arms, the analysis of covariance adjusted for baseline values. Thus, the differences between the two arms of the study are not the simple numerical differences. Negative values indicate higher scores in the embolisation arm whilst positive values indicate higher scores in the surgical arm.

Scores range from 0 (worst possible health status) to 100 (best possible health status)

Table 7

Post-embolisation and post-surgery symptom scores, pain scores and recommendation to a friend in the two treatment groups

	Embolisation			Surgery					
	N	Mean	SD	N	Mean	SD	Diff (E-S)	95% CI	P
24 hr pain score	**99**	3	2.1	**49**	4.6	2.3	1.6	0.8-2.3	<0.001
(0 [no pain] to 10[severe pain])									
Symptom Score									
1 month	**98**	1.5	2.4	**48**	2.8	2.6	1.3	0.4-2.2	0.004
12 months	**95**	3.6	2	**45**	4.3	1.7	0.7	0.1-1.4	0.03
(-5 [markedly worse] to +5 [markedly better])									
Recommend to a friend									
	YES			**YES**			**Diff (E-S)**		
1 month	74/97	76%		37/48	77%		1%	-14% to 15%	0.92
12 months	84/95	88%		42/45	93%		5%	-5 to 15%	0.32

Table 8

Length of hospital stay and the time taken to achieve lifestyle milestones in days

	Embolisation				Surgery				Diff in medians (E-S)	
Variable	**N**	**Median**	**Q1**	**Q3**	**N**	**Median**	**Q1**	**Q3**	**95% CI**	**P value**
Hospital stay	**100**	1	1	2	49	5	3	6	3 to 4	<0.001
Made cup of tea	86	2	1	3	42	6	4	11	3 to 5	<0.001
Made meal	83	6	3	9	41	17	9.5	23	6 to 14	<0.001
Drove	66	8	5	10	30	34	27	43	22 to 30	<0.001
Return to work	68	20	14	30	31	62	39	90	28 to 53	<0.001
Sexual intercourse	61	21	13	31	31	53	29	91	18 to 45	<0.001

Data are excluded for patients who did not have a response to a category (e.g. non-drivers).

Minor Complications

Minor complications were reported by 36 women (34%) in the embolisation group and 10 (20%) in the surgical group (P=0.06) – **Table 9**. Minor complications were most commonly associated with symptoms relating to the post-embolisation syndrome (52%) in the embolisation arm of the trial. In the surgical arm, minor complications were usually related to minor wound infections (25%).

Major Adverse Events

There were 16 major adverse events (15%) in the embolisation group and 10 (20%) in the surgical group during a median follow up of 32 months (interquartile range 23 – 41) – **Table 9**. When these events were categorized with respect to the timing of their occurrence (i.e. during the hospital stay, during the first year of follow up or after the first year of follow up), 8 out of the 10 major adverse events in the surgical group occurred during the hospital stay whilst 15 out of the 16 events in the embolisation group occurred after discharge from hospital.

Treatment Failures

Twenty-one patients (20%) in the embolisation group required an additional invasive procedure (hysterectomy or repeated embolisation) for continued or recurrent symptoms. Ten of these procedures took place during the first 12 months of follow up (2 of which were due to technical failures at the first attempt of embolisation) and 11 of these took place after the first year of follow up. In the surgical group, there was one conversion of myomectomy to hysterectomy at the time of the primary procedure – **Table 9**.

Table 9

Minor Complications within first year

Major Adverse Events and Interventions for Treatment Failure – occurring during median follow up of 32 mths

[for Major Adverse Events after 1 year, only those requiring hospitalization are reported.]

[P=0.047 for the comparison between the embolisation group and the surgical group for Minor Complications]

[P=0.22 for the comparison between the two study groups during the first year]

[the interquartile range for Major Adverse Events and Reinterventions for Treatment Failures was 23 – 41 mths]

Variable	Embolisation Group (N=106)	Surgical Group (N=51)
Minor Complications at 1 yr		
Patients reporting any minor complication – no. (%)	36 (34)	10 (20)
Total number of complications reported	50	16
Type of complication	postembolisation syndrome (26 patients)	infection (4 patients)
	vaginal discharge (9)	haemorrhage (3)
	sepsis (6)	other (9)
	other (9)	
MajorAdverse Events	**Embolisation Group**	**Surgical Group**
Patients reporting any adverse event – no. (%)	16 (15)	10 (20)
During hospital stay		
Total number	1	8
Type of event	severe vasovagal event requiring atropine (1 patient)	operative haemorrhage (2 patients)
		anaesthetic complication (2)
		wound infection (2)
		wound haematoma (1)
		urinary retention (1)
During first year of follow up		
Total number	12	2
Type of event	breast cancer (2 patients) - both diagnosed 2 mths after treatment	wound exploration under GA (1)
	pain and pelvic infection requiring readmission at 1 and 4 weeks (2)	wound infection at 3 weeks (1)
	severe pain and fibroid expulsion at 3, 4 and 6 weeks (3)	
	haematometra at 6 months - not treated (1)	
	pelvic abscess requiring hysterectomy at 10 mths (1)	
	persistent severe pain requiring hysterectomy at 8 mths (1)	
	temporary amenorrhoea for 5 and 9 mths (2)	
After first year of follow up		
Total number	3	0
Type of event	persistent severe pain requiring hysterectomy at 15 mths (1)	
	death from adrenal cancer (1) - diagnosis at 12 mths; death at 13 mths	
	severe pain and fibroid expulsion at 13 mths (1)	

Table 9 continued.

Interventions for Treatment Failure	Embolisation Group	Surgical Group
Patients reporting any intervention - no. (%)	21 (20)	1 (2)
During hospital stay		
Total number	2	1
Type of event	technical failure of procedure requiring hysterectomy (2 patients)	operative complication requiring conversion of myomectomy to hysterectomy (1 patient)
During first year of follow up		
Total number	8	0
Type of event	hysterectomy (4 patients) repeated embolisation (4)	
After first year of follow up		
Total number	11	0
Type of event	hysterectomy (8 patients) repeated embolisation (3)	

Other Outcomes

Up until September 2005, eight pregnancies have occurred in five women (seven in the embolisation group and one in the myomectomy group). Four of these pregnancies have miscarried, three have resulted in successful live births (two caesarean sections and one spontaneous vertex delivery) and one has culminated in an intrauterine death at 33 weeks gestation (no abnormalities were found on post-mortem).

5.4 Discussion

In this randomised trial comparing UAE with standard surgical treatment for women with symptomatic fibroids, there were no significant differences between the trial arms in terms of quality of life at 12 months, although in both arms, there were substantial improvements in each component of the SF-36 score relative to baseline. In contrast, the adverse event profiles were very different. Surgery was associated with the expected acute morbidity, but only one serious adverse event was recorded after the initial hospital stay. UAE was associated with a significantly faster recovery, including a shorter time to resumption of normal activities.

Rates of minor complications or major adverse events did not differ significantly between the study groups although the nature and timing of these events varied between both arms of the trial. Major adverse events in the surgical group typically occurred during the hospital stay, whereas in the embolisation group, such events occurred more often than not, after discharge from hospital. Of note, three of the major adverse events in the embolisation group were cancers, all of which were highly unlikely to be related to treatment.

At one year, however, 10 of the 106 women in the embolisation arm required a secondary procedure to treat persistent or recurrent symptoms. Beyond one year of follow up, 11 additional women were re-admitted to hospital for the same reasons. These findings are in keeping with data from previous uncontrolled published case series which indicate complications and treatment failures up to 48 months after embolisation (Marret et al. 2004a;Marret et al. 2005).

This trial compared two very different methods of treating women with symptomatic fibroids, namely surgery and UAE, where the particular surgical intervention and technical aspects of both procedures were not dictated by protocol. Thus both hysterectomy and myomectomy were included in the surgical

arm although in fact only 8 women underwent myomectomy. The primary outcome measure of this trial, the SF 36, has been shown to be sensitive to changes in quality of life that result from successful treatment of menstrual symptoms (Jenkinson, Peto, & Coulter 1996). This is an important feature of the questionnaire, bearing in mind the cyclical nature of symptoms experienced by many women with symptomatic fibroids. However, the SF 36 does not take specific fibroid-related symptoms into account, unlike the disease-specific UFS-QOL questionnaire (Spies et al. 2002a) which was not available at the commencement of the REST trial. In this study, we did not collect data on objective menstrual blood loss (MBL) measurements. With only eight women in the surgical arm of the trial undergoing myomectomy, it would not have been possible to present a meaningful comparison of MBL measurements between the surgical and UAE groups.

As mentioned previously, two other randomised controlled trials have been published, comparing hysterectomy with UAE for the treatment of symptomatic fibroids (Hehenkamp et al. 2005;Pinto et al. 2003). The first study used a rather controversial randomized consent methodology (Zelen 1979) where women assigned to the hysterectomy arm of the study were neither informed of the study nor told about the alternative treatment of UAE. This particular study was small, with recruitment of a total of 57 women. Length of hospital stay was the primary outcome measure and this was found to be shorter, in the embolisation arm of the study. Complication rates were similar in both study groups (Pinto et al. 2003). The second trial enrolled 177 women, randomising them to either hysterectomy or UAE. Six weeks following treatment, women in the embolisation group were found to have a significantly shorter mean hospital stay, but there was also a higher minor complication rate and re-admission rate associated with this group (Hehenkamp et al. 2005).

There are a number of limitations to the REST trial which must be acknowledged. The original target sample size of 200 was reduced to 150 owing to difficulties in recruitment. Whilst the 95% confidence intervals for the differences between the surgical and embolisation arms of the trial indicate that plausible results include as much as a 10-point difference between groups in some components of the SF 36, there is no suggestion of clinically important differences. In addition, the recruitment of only a small number of women into the myomectomy sub-arm of the trial made it difficult to compare this form of uterine-sparing surgery to embolisation. However, it was not our aim to make meaningful comparisons between UAE and myomectomy. The inclusion of myomectomy as a sub-arm of the trial was instead a pilot, to see if enough women could be randomised into a RCT in the future. Our experience with recruitment highlights how difficult it would be to compare UAE and myomectomy unless recruitment involved a very large population.

The use of time to resumption of usual activities as a secondary outcome measure can also be criticised as such an interval could be biased by the women's expectations (with or without caregivers' guidance) regarding time to recovery.

The REST trial shows very clearly that the choice between surgery and UAE for the treatment of symptomatic fibroids involves tradeoffs. The advantages of embolisation which include a significant reduction in length of hospital stay and 24-hour pain levels as well as more rapid return to usual activities, must be weighed against the risk of treatment failure necessitating a second intervention and the rare, but nonetheless possible risk of major late adverse events. Longer-term follow up is still required, with particular attention to the need for repeated intervention for treatment failure or late complications. This will allow women to be provided with better information to facilitate more informed decision making prior to choosing treatment for their symptomatic fibroids.

5.5 Conclusion

The results from the REST trial provide justification for the continued use of UAE in treating women with symptomatic fibroids. Advantages and disadvantages compared with surgery, however, are now defined with more clarity. Further long term follow up of women undergoing UAE is required to evaluate the long term outcome and durability of the procedure.

Chapter 6

Late complication of uterine artery embolisation – a case report

HM, a 51 year old para 3 + 0 presented to our gynaecology clinic with a pelvic mass extending to her umbilicus. She gave a three month history of lower abdominal discomfort associated with both backache and fatigue. Her periods were irregular, but normal in flow. HM had just stopped a sequential combined preparation of hormone replacement therapy, commenced five years earlier, for peri-menopausal symptoms. Abdominal examination revealed a central non-tender pelvic mass equivalent to a 16 week sized pregnant uterus; bimanual vaginal examination findings were consistent with the latter and confirmed that the mass was mobile. Subsequent pelvic ultrasound revealed a 14 x 12 centimetre (cm) solid mass within the fundus of the uterus, in keeping with a uterine fibroid. All other uterine findings were normal.

After a long discussion regarding the various treatment options for symptomatic fibroids, HM agreed to consent to entry into the REST trial. She was subsequently randomised into the embolisation arm of the trial and after review once again by a gynaecologist and thereafter, by an interventional radiologist, she underwent MRI of her pelvis as per protocol of the trial. This demonstrated a heterogenous uterine intramural mass 10.8 x 9.4 x 8.8cm consistent in appearance with a fibroid, and an overall uterine volume of 447cc. Gadolinium enhancement was not performed as HM was recruited in the early stages of the trial when this was not part of the standard protocol. White cell count, CRP and Hb levels were all within normal reference ranges prior to treatment. Outwith the REST trial, HM agreed to objective menstrual blood loss assessment using the alkaline haematin method. Her blood loss from one menses was calculated to be 16mls.

UAE was then performed using the standard technique described in chapter 1, section 1.8, page 36). Post-procedure, HM experienced only mild lower abdominal cramps. Pain was managed using a combination of intravenous opiates

and oral non-steroidal anti inflammatory drugs over the first twenty four hours and she was discharged from hospital the following day.

One month following treatment, HM was reviewed by a research nurse as per the REST trial follow-up protocol. Whilst she felt that her symptoms had slightly improved, she did admit to an increase in menstrual flow since treatment, in association with moderate dysmenorrhoea and lower backache.

Three months post-UAE, HM reported further improvement of symptoms to her gynaecologist. In particular, she felt that her abdominal mass had decreased in size, resulting in less abdominal and pelvic discomfort. She also remarked at this point that she would recommend UAE to other women.

At her six month review, HM continued to report improvement in abdomino-pelvic symptoms. However, she also reported fatigue, dysmenorrhoea and an increase in menstrual flow associated with the passage of clots, flooding and the requirement of additional sanitary protection. At this stage, the research nurse recommended the use of an oral anti-fibrinolytic, tranexamic acid, in order to reduce menstrual blood loss. Shortly after review, a six month post-treatment MRI was performed as per trial protocol. Whilst the fibroid mass was found to have decreased in size (8.9 x 6.0 x 7.5cms), it was noted to be surrounded by some fluid. Thus, combining fibroid and fluid volume demonstrated that uterine volume had decreased to 424cc, a very minimal overall reduction, when compared to pre-treatment values.

Eleven months after treatment, HM presented to her general practitioner with left lower abdominal pain and distension during the first few days of her cycle. After clinical examination, subacute bowel obstruction was suspected and thus HM was referred and admitted to hospital as an emergency. On admission, HM also presented with nausea and vomiting, subcostal pain radiating to both shoulder tips and heavy menstrual bleeding. On examination, she had a pyrexia of 38.5^0 C and

a soft, generally tender abdomen associated with a pelvic mass extending to her umbilicus. WCC was minimally elevated (13.8 x 10^{-9}/ L), CRP was increased at 145 mg/dL and Hb was within the normal reference range. Pelvic ultrasound performed at this time revealed a uterine cavity thought to contain an 8.0 x 7.4 x 7.7cms blood clot. Blood cultures were negative and after a single dose of intravenous antibiotics (augmentin), HM was apyrexial. Intramuscular opiate analgesia was administered and HM was discharged form hospital 3 days later when her abdominal pain resolved, with a combination of oral antibiotics and analgesia.

Unfortunately, HM's symptoms persisted over the next few weeks and after discussion with her consultant gynaecologist, the decision was made to proceed with total abdominal hysterectomy and bilateral salpingoophorectomy (TAH and BSO); this was scheduled for six weeks later.

Another MRI was performed in the interim, at twelve months post-UAE. The fibroid was measured to be 10cms in diameter and the uterine volume was 500cc. More specifically, the fibroid mass was noted to have a central oval solid component measuring 8.0 x 5.0 x 8.0cms within a larger outer cystic component measuring 10 x 10 x 10cms. These appearances were thought to be to be suggestive of the fibroid undergoing partial cystic necrosis, leaving a residual central component. Gadolinium enhancement was performed with this MRI, to assess uterine vascularity. This demonstrated poor enhancement of the remaining solid component, suggesting that the fibroid was relatively hypovascular. At this point, HM, influenced by a combination of her MRI findings and improvement of her clinical symptoms, decided to cancel her hysterectomy. Whilst she did have concerns regarding deterioration of her clinical symptoms, she was keen to avoid major surgery and instead, have her symptoms monitored over the course of the next few weeks. Objective menstrual blood loss measurement performed at twelve

months revealed a blood loss of 161mls, a volume much in excess of HM's pre-UAE measurement.

One month later (13 months post-UAE), HM was admitted to hospital as an emergency with lower abdominal pain, backache and heavy vaginal bleeding. A borderline pyrexia was noted. Pelvic ultrasound confirmed the presence of an echogenic fluid collection around the fibroid. HM was still keen to avoid surgery at this point and thus the decision was made to perform ultrasound-guided aspiration of peri-fibroid fluid; this yielded 80 mls of thick green fluid. Microscopy of the latter revealed a moderate amount of white blood cells in association with scanty gram negative cocci. Culture, however, yielded no growth. HM was discharged on the following day and was requested to complete a course of oral antibiotics in the form of augmentin and metronidazole. She was also given oral antiemetics and analgesia to self-administer as required.

Two weeks later, HM was reviewed by her consultant gynaecologist once again. Her symptoms of grumbling lower abdominal and pelvic pain persisted and she now was experiencing heavy vaginal bleeding. Taking her symptoms and imaging into consideration, she decided to go ahead with a hysterectomy in the near future. Surgery was scheduled for the following month and just prior to her operation, HM underwent a further pelvic ultrasound. This demonstrated a posterior uterine wall cystic mass ~ 13cms diameter. This was associated with free fluid in the pouch of Douglas. Within the cystic mass, locules were seen; these were thought to possibly represent an abscess.

Surgery in the form of TAH and BSO went ahead as planned, 15 months post-embolisation. Intraoperatively, a large fibroid uterus extending to the level of the umbilicus was noted. This was associated with dense bowel and omental adhesions in the pouch of Douglas which required mobilisation in order to facilitate hysterectomy. A myomectomy screw was inserted into the uterus for

elevation. This caused a purulent exudate to ooze from within the uterine cavity (**Figure 23**). Both tubes and ovaries were normal. Blood loss during surgery was estimated at 400mls. A drain was inserted into the abdominal cavity at the end of the procedure. Intraoperative and post-operative intravenous antibiotics were administered (metronidazole and gentamicin) owing to the presence of pus. Pre-operative Hb was 10.8 g/dL. This dropped to 9.3 g/dL immediately after surgery. On day one following hysterectomy, HM collapsed in the shower and was noted to have 400ml of blood in her pelvic drain. Hb was noted to have dropped further, to 6.3 g/dL. Two units of packed red cells were subsequently transfused which increased the Hb level to 7.7 g/dL. Oral iron supplements were administered, thereafter. HM subsequently made an excellent post-operative recovery and was discharged on day 6 post-surgery, on oral analgesia and hormone replacement therapy in the form of oral oestrogen.

Following hysterectomy, the fresh uterine specimen was opened; this revealed a large necrotic fibroid within the uterine cavity (**Figure 24**). Pathological analysis of the specimen revealed a large discoloured fibroid or leiomyoma, 10 x 7 x 7cms in size. This was almost 100% infarcted, presumably as a result of embolisation. The adjacent surrounding myometrial tissue appeared necrotic on macroscopic examination and microscopy showed extensive areas of acute suppuration with abscess formation accompanied by neutrophil polymorphs and histiocytes. There were also foci of tissue necrosis. Endometrium was proliferative and otherwise, pathology was unremarkable. Uterine swabs taken at the time of surgery were negative and revealed only scanty white blood cells.

HM was reviewed 6 weeks after her surgery and was found to be clinically well and asymptomatic. Despite her post-embolisation complications, she stated that she would still recommend UAE to women who have symptomatic fibroids.

Figure 23

Gross specimen of uterus demonstrating ooze of purulent exudate from site of myomectomy screw insertion

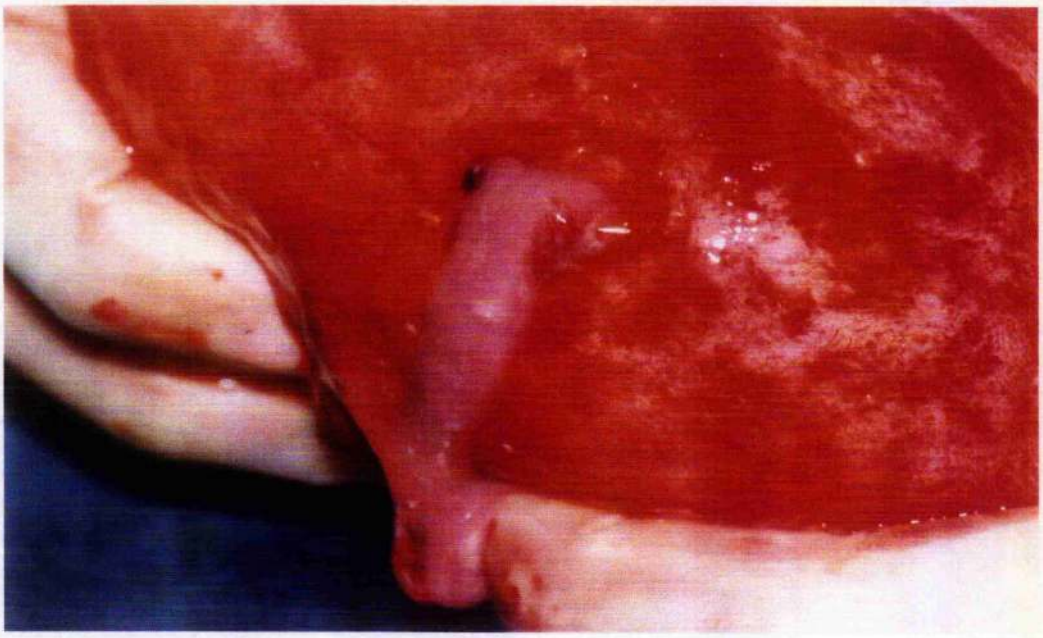
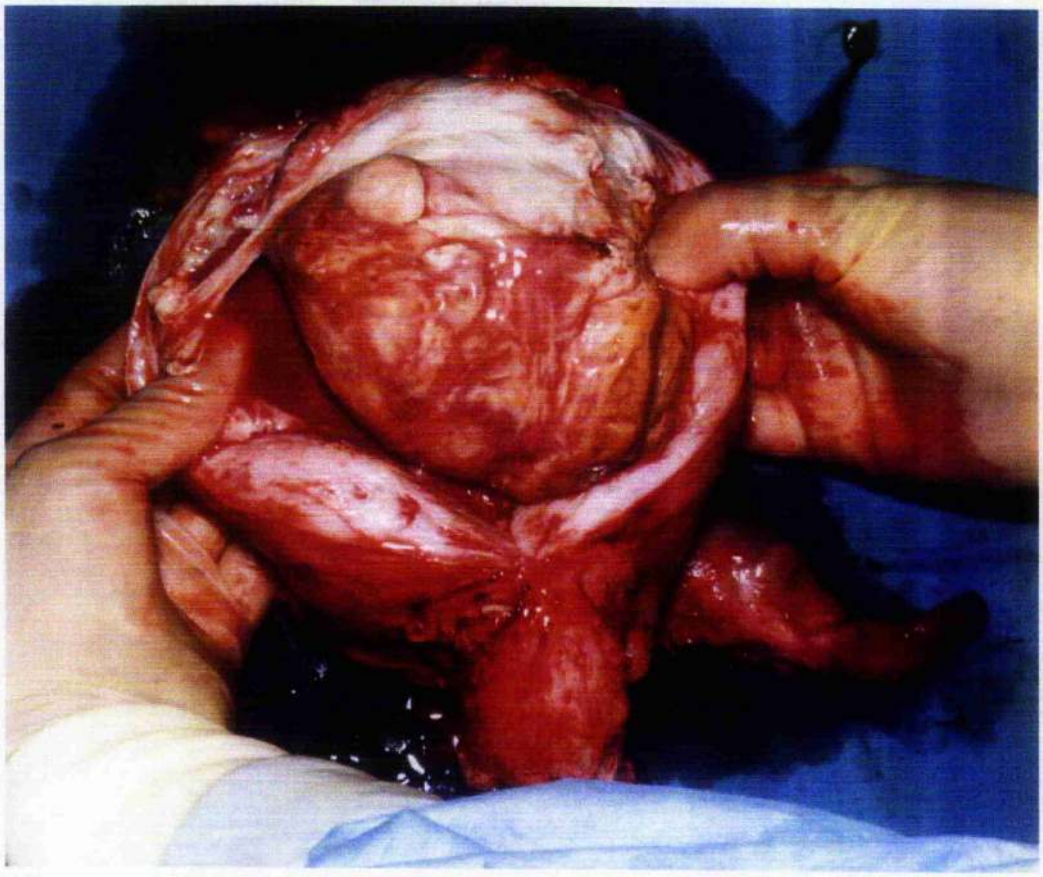


Figure 24

Large necrotic fibroid within uterine cavity



Discussion

Uterine artery embolisation appears to be comparable to hysterectomy in terms of safety, efficacy and patient satisfaction. However, as previous studies have demonstrated, the short hospital time and recovery period associated with the procedure must be balanced against the risk of complications and need for re-hospitalization and / or further treatment, in a minority of patients. We are aware of the minor complications associated with UAE such as pelvic pain, the post-embolisation syndrome and persistent vaginal discharge. Most of these problems are self-limiting and can be managed with conservative measures. Major complications such as pelvic sepsis necessitating hysterectomy and death have been reported, but these tend to occur rarely, within the first few weeks or months of treatment.

The case of HM is unusual in terms of the timing of presentation of symptoms after UAE and their course prior to hysterectomy. Late complications after UAE appear to be reported very rarely in the literature. Marret et al have published a case involving a woman who underwent successful UAE for a 13 x 12cm intra-mural fibroid (Marret et al. 2004a). Fibroid reduction was achieved and symptoms were successfully controlled until 44 months post-treatment, when the woman developed a persistent purulent vaginal discharge. Subsequent hysteroscopy revealed a 7cm posterior wall necrotic fibroid, partially submucosal and protruding into the uterine cavity. The woman declined hysterectomy and went on to spontaneously expel her fibroid per vaginum via the cervix, 6 months later, with no ill effects thereafter.

HM initially experienced improvement of symptoms at three months post-UAE. Six months after embolisation, however, she experienced deterioration in symptoms, with episodes of partial remission followed by further deterioration up to 13 months after treatment. Perhaps a number of women with her symptoms of

abdominal and pelvic pain associated with increasingly heavy periods would have opted for hysterectomy at an earlier stage. Certainly, HM's symptoms which warranted emergency admissions 11 and 13 months post-UAE, in combination with imaging performed on these separate occasions, hinted at an infective cause for her ongoing problems. It is nonetheless unusual for a patient to present with infective complications following UAE after such a lapse of time. This case report and the one published by Marret et al, emphasise the need for long term follow up of women undergoing embolisation. In addition, clinicians and patients should be made aware of the potential for delayed presentation of major complications after treatment. Our case also highlights the need for us to have a better understanding of the predictive factors (e.g. fibroid size) for the success of UAE for symptomatic fibroids.

Chapter 7A

Vasculature of human myometrium and leiomyomata

7A.1 Introduction

The human uterine vasculature is highly structured, exhibiting circumferential and radial branching. This structured architecture appears to be absent in uterine fibroids. Angiography used to study normal uterine vascular morphology has also been used to analyse fibroid vasculature and this shows substantial differences between normal and diseased tissue (Farrer-Brown, Beiby and Rowles 1970a;Farrer-Brown, Beilby & Tarbit 1970b;Farrer-Brown, Beilby & Tarbit 1970c;Farrer-Brown, Beilby & Tarbit 1970d). Morphometric analysis in tissue sections suggest that fibroids lack the well developed vascular pattern seen in normal myometrium (Casey, Rogers, & Vollenhoven 2000;Gargett et al. 2002;Hague et al. 2000;Poncelet et al. 2002;Weston et al. 2003). Although existing evidence indicates a vascular gradient within the myometrium, this has not been quantified stereologically. In addition, the apparent lack of a structured vascular gradient within fibroids has not been described in comparison with normal uterine tissue.

Based on our previous knowledge of the anatomical distribution of uterine vessels, it is postulated firstly, that a spatial gradient of vessel wall density and vessel size exists within the normal myometrium and secondly, that there is a precise relationship between vessel calibre and tortuosity. Finally, we postulate that this difference between inner and outer myometrium is disrupted in fibroid growth. It is hoped that the comparison between vessel muscularization in fibroid and normal myometrial tissue may determine potential reasons for fibroid growth and provide some explanation for the anatomical rationale for uterine artery embolisation.

7A.2 Materials and methods

7A.2.1 Tissue specimens

Archival samples of 12 non-fibroid and 19 fibroid uteri were obtained from Pathology Departments of North Glasgow Acute NHS Trust Hospitals for analysis. Eleven biopsies were also obtained on a prospective basis (eight fibroid and three non-fibroid) from women undergoing hysterectomy for menorrhagia. Negative controls included slides incubated without the primary antibody and sections with a mouse monoclonal antibody against immumoglobulin G (IgG). Placental tissue was used as a positive control for the primary antibody (mouse monoclonal antibody) used. The mean ages of patients in the non-fibroid and fibroid groups were 39 years (range 29-48) and 41.25 years (range 33-54), respectively.

Five of the patients in the fibroid group had undergone previous treatment with a gonadotrophin-releasing hormone analogue, but all had at least a 3-month drug-free period prior to hysterectomy. No hormonal treatment had been administered to any of the women without fibroids before surgery. Of the 15 non-fibroid uteri, the endometrium was proliferative, secretory and menstrual in 5, 9 and one specimen, respectively. In the fibroid group, endometrium was secretory, proliferative, inactive and severely autolysed in 15, 8, 3 and one case, respectively. Three specimens in each group were found to have other pathology; specifically, four cases of endometriosis, one case of adenomyosis and one endometrial polyp. None of these pathologies had any effect on the acquisition of numerical data. Two fibroid specimens were excluded before analysis owing to distortion secondary to necrobiosis, making it impossible to analyse the tissue.

The mean cross-sectional diameter of fibroid analysed was 25.25mm (range 5-40mm). Six uteri contained a single fibroid whilst 21 uteri contained multiple

lesions. Of the fibroids that were not analysed, the largest was 200mm in diameter. Twenty two of the 27 fibroid specimens contained intramural fibroids. The remainder were submucosal in origin.

Ethics approval and patient consent were obtained for this study.

7A.2.2 Stereological analysis of the myometrial vascular gradient

Formaldehyde fixed and wax embedded tissue sections (5µm) orientated along the endometrial-serosal axis were used for analysis. The vascular endothelium was visualised by immunocytochemical detection of CD-31 (Dako) after antigen retrieval. Endogenous peroxidase activity was inactivated by immersing the slides in 0.5% hydrogen peroxide in methanol. The sections were then microwaved in 0.01mol/l citrate buffer pH 6 for 8 minutes. Antibody-binding sites were blocked with 20% horse serum in phosphate buffer solution (PBS) for 30 minutes at room temperature. Antibody binding was detected with an avidin-biotin-peroxidase kit (Vector laboratories, Peterborough, UK) and diaminobenzine. The sections were then counterstained with haematoxylin. The distribution of vascular smooth muscle was estimated using the pattern of haematoxylin stained nuclei, as it is difficult to distinguish antigenically between myometrial smooth muscle and vascular smooth muscle in wax embedded tissue sections.

Images of whole sections were obtained by transmitted light scanning with a 35mm film scanner (Nikon Coolscan III). A uniform 3x3mm sampling grid, randomised by starting point and position, was applied to these map images with the grid function of Adobe Photoshop (Russ and Dehoff, 2000). The tissue was thus divided into six zones, zone 1 being nearest to the myometrial-serosal boundary (outer myometrium) and zone 6 being nearest to the endometrial-myometrial boundary (inner myometrium) – **Figure 25A**. Sampling points on the map image were then matched with the microscopic field of view (2x objective,

Olympus BX-50 microscope). Digital images of microscope fields centred on the grid points were captured with a 40x objective using a 3-CCD colour video camera (KY55, JVC). Stereological analysis of the sample fields was then carried out using the grid function of an image analysis programme (Image Pro Plus 4.5, Media Cybernetics). A 48 point orthogonal grid with a spacing of 100x100 pixels was applied in order to make estimates of muscularized vessel wall and lumen, and capillaries within each tissue section (fractional volume estimates). A total of 720 fields were counted in normal myometrial sections and 1248 fields in fibroid specimens (**Table 10**).

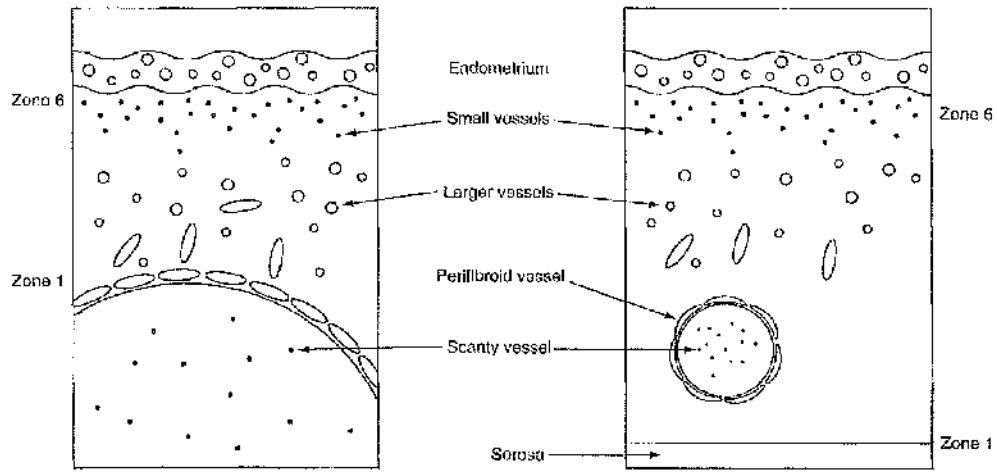
Table 10

The number of fields used within each zone for the stereological analysis of vessel volume fraction

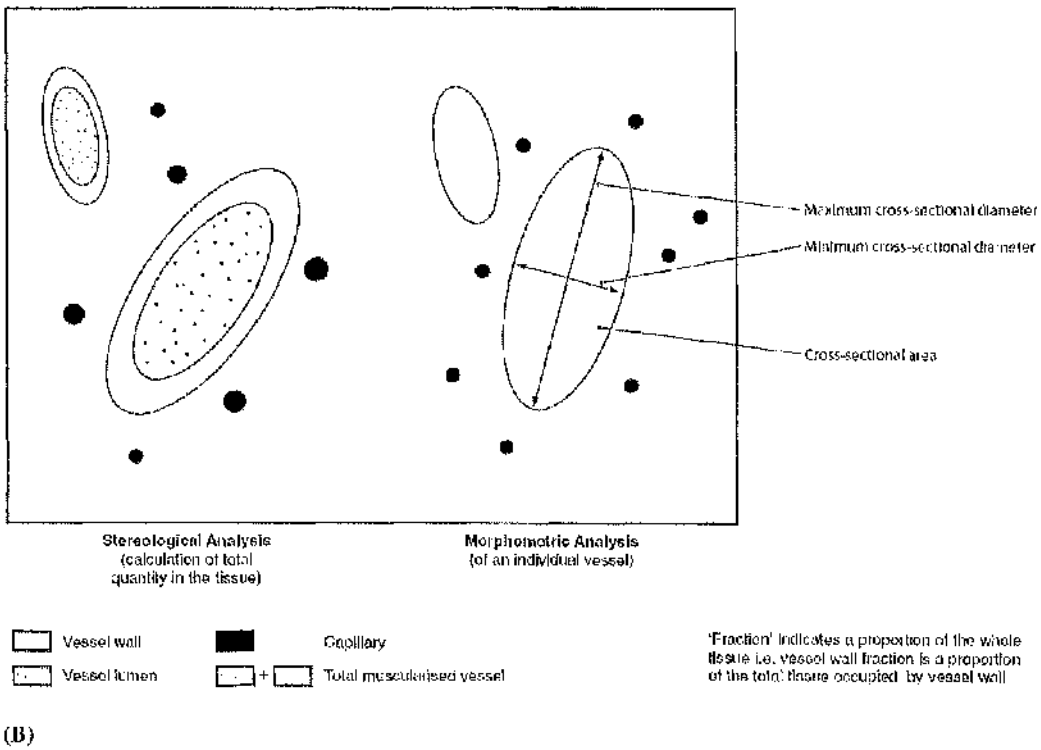
Zone	Number of fields analysed			
	Normal Myometrium	Fibroid		
		Perifibroid	Fibroid	Myometrium Around Fibroid
1	124	7	110	87
2	120	29	102	99
3	136	44	86	82
4	118	50	94	122
5	124	42	72	110
6	99	31	76	55

Figure 25

Diagrammatic representation of the tissue cross sections being analysed.



(A)



(B)

(A) Stereological analysis of tissue in zones applied to two different fibroids, varying in size and position along the endometrial–serosal axis.

(B) Illustrations of the measurements undertaken for stereological analysis of vessel volume fraction (left) and morphometric analysis (right). Volume fraction was determined by counting the number of grid points intersecting with each phase of interest. Simple linear measurements of vessel cross section were made for morphometric analysis.

7A.2.3 Morphometric analysis of vessel size

Unlike stereological analysis, morphometric analysis enables the characteristics of a single vessel to be described. Morphometric analysis was undertaken to determine whether or not there was a gradient in vessel size within the myometrium. Cross-sectional area (luminal area) and maximum and minimum diameters of vessel lumen were measured on 960 and 315 vessel cross sections in normal myometrium and fibroid tissue, respectively.

In order to study the spatial gradient of vessel size, sampling lines orientated along the endometrial-serosal axis were generated on the scanned map images. Sampling points spaced at the equivalent of 1mm intervals on the tissue were then used to locate microscope fields. Each of these sampling points was viewed using a 40x objective, and a digital image of the vessel nearest the sampling point was captured. Where the entire vessel could not be visualised in one image, the slide was rotated to capture it. Care was taken to ensure the same vessel was not re-sampled. Manual image analysis tools (Image Pro Plus 4.5, Media Cybernetics) were used to make measurements of cross-sectional luminal area by manually outlining with the mouse. The maximum and minimum diameters, represented by Feret diameter (or calliper width), were computed automatically by the programme from the outline **(Figure 25B)**.

Orientation of the muscularized vessel cross-sections was also measured manually, from the low magnification (2x) images, using a protractor. Profiles with a circular or near-circular plane of section were excluded. Only fibroid specimens in which the endometrium was clearly visualised and orientation was clear were used for this analysis.

In order to represent the large variation in vessel size, histograms with discontinuous axes were created to encompass the range of values. Composite scattergrams were required to show the spatial distribution of vessel size within

fibroid containing specimens. This was due to the fact that the specimens varied in width and the fibroids varied in cross-sectional diameter and position within the muscle. The data were broken into 4 subsets and aligned on the histogram using 4 spatial boundaries (endometrial-myometrial boundary, edge of fibroid nearest endometrium, edge of fibroid nearest serosa and the myometrial-serosal boundary). Data are presented as mean +/- the standard error of the mean (S.E.M.).

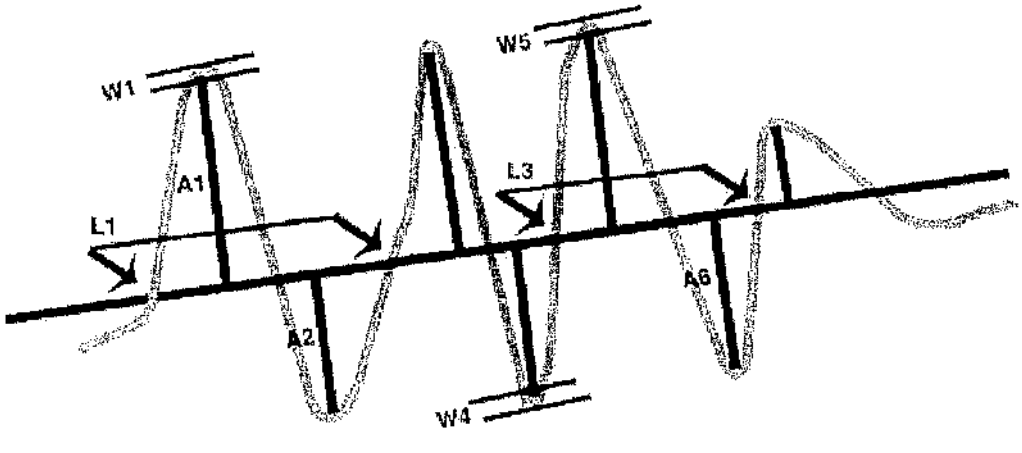
7A.2.4 Image analysis of vascular tortuosity

Initial attempts to produce high resolution 3-dimensional images of the uterine vasculature on isolated hysterectomy specimens with a small bore 7-Tesla magnetic resonance imager produced images that were not suitable for analysis. Although the tortuosity of the larger vessels could be directly observed, artefacts within the preparation produced an artificially dilated appearance of the lumen. However, as the detection of small vessels was limited by resolution, manual image analysis was carried out on the published uterine arteriograms of Farrer-Brown et al (1970a). The projections of the vessels seen in these images were scanned at 600 dots per inch (dpi), and linear measurements were then made on 38 arbitrary vessel segments using the measure tool of Adobe Photoshop. Only tortuous vessels segments were used, as straight portions in the plane of view would have had zero values for the parameters measured. Luminal diameter was measured at the beginning and end of a segment and at each projected turning point. In order that tortuosity could be measured at a consistent luminal diameter, measurements were restricted to segments where there was less than 10% end-to-end variation in vessel width. Projected amplitude was measured orthogonally from an arbitrary line connecting both ends of the segment. Wavelength was

calculated as the number of bending cycles divided by the projected straight linear distance (**Figure 26**).

Figure 26 Diagrammatic representation of an arterially filled vessel segment from Farrer-Brown *et al.* (1970d).

Manual analysis of the projected image was undertaken with the linear measurement tool. Luminal width (*W*) was measured at turning points and at the beginning and end of each measured segment. Tortuosity of the vessels was assessed by measuring wavelength (*L*) and amplitude (*A*). 2D error bars are included because of the variation in assessment of luminal diameter over short vessel segments. The error bars represent the SEM.



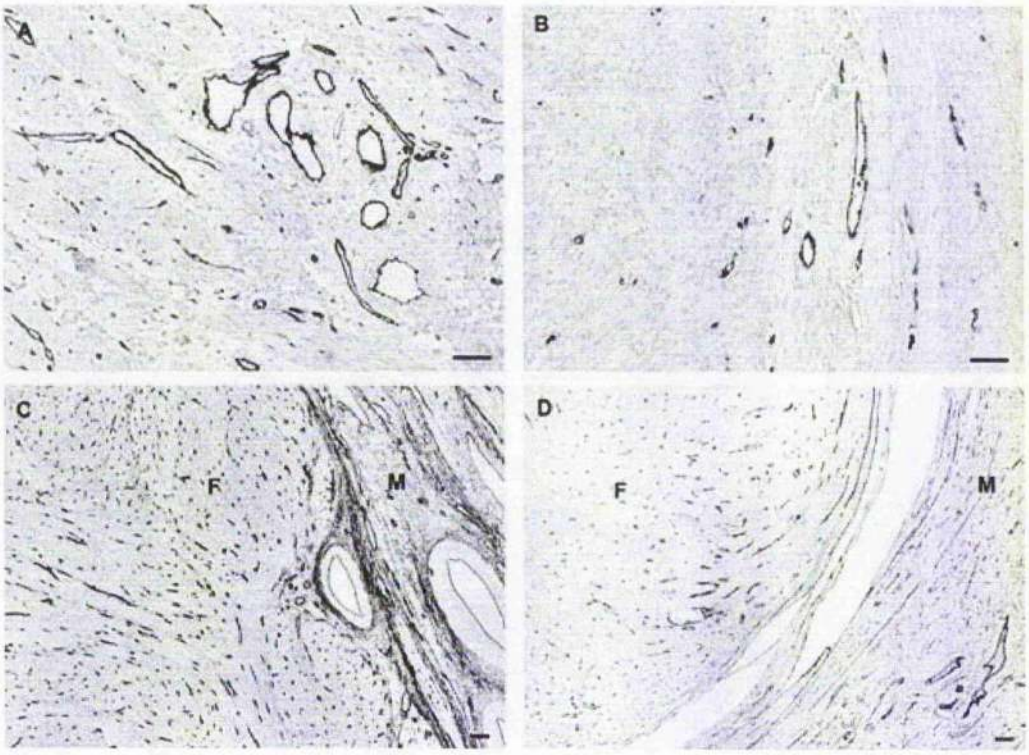
7A.3 Results

Immunolocalisation of CD-31 allowed identification of blood vessels within myometrium and fibroids **(Figure 27A-D)**.

Figure 27

Immunocytochemical localization of vascular endothelium with anti-CD-31 antibodies.

(**A**) Normal myometrial tissue showing a range of muscularized vessels. (**B**) Fibroid tissue with an absence of muscularized vessels and a lower density of vascularization. (**C**) Lower magnification images showing fibroid (F) and myometrial tissue (M). Large muscularized vessels are present close to the fibroid boundary. (**D**) Perifibroid tissue containing large thin-walled circumferential vessels, fibroid (F) and myometrium (M). Note the lower area fraction of vessel in the fibroid shown in **D** compared with that shown in **C**. Analysis was carried out at higher objective lens magnifications than illustrated. Scale bars = 100 μm.



Large thick-walled vessels were present within the myometrium but were not detected in small fibroids. The perifibroid tissue, near the fibroid, contained large

thin walled, circumferential vessels and was distinct from both normal and fibroid tissue in this respect **(Fig.27D)**. Stereological analysis of normal myometrium specimens (n=15) confirmed the existence of an anatomical gradient of vessel muscularity within the myometrial vascular system **(Figure 28)**. This gradient is unaffected by the stage of the menstrual cycle.

A two-way analysis of variance demonstrated a significant variation along the endometrial-serosal axis (P=0.04) and no effect of menstrual cycle stage (P=0.71). There is a greater volume fraction of vessel wall in the outer myometrium than in the inner myometrium **(Figure 28A)**. This spatial gradient could not be detected within fibroids (n=27), which had a negligible volume fraction of muscularized vessel wall, or within the perifibroid tissue. Myometrial tissue outside the perifibroid zone of large thin-walled vessels also appeared affected by the presence of the fibroid since the gradient of average vessel fraction and the ratio of vessel lumen to wall seen in normal tissue was disrupted **(Figure 28A and B)**.

The ratio of vessel lumen to wall had a similar distribution to vessel volume fraction, confirming that vessels were less muscularized within the inner myometrium **(Figure 28B, Table 11).** The lumen to wall ratio could not be reliably calculated in fibroid tissue as muscularized vessels were observed very infrequently **(Figure 28B)**.

Figure 28 Stereological analysis of vessel wall distribution within the muscularized vessels. (**A**) There is a continuous decrease in the vessel wall fraction from the serosa to the endometrium in normal tissue ($n = 15$) (continuous line connecting diamonds). By contrast, the fibroid has very little or no vessel wall ($n = 27$) (dotted line connecting triangles). Large thin-walled vessels are seen in the perifibroid area (dots and dashes connecting crosses). The perifibroid region has a consistently higher volume fraction throughout the width of the myometrium. The myometrium in fibroid specimens outwith the area of large vessels does not demonstrate a continuous trend in vessel size (dashes connecting boxes). (**B**) The ratio of vessel wall:lumen (continuous line connecting diamonds) shows a similar continuous trend as average vessel fraction. Tissues out with the fibroid do not show this continuous trend (dashes connecting boxes and dots and dashes connecting triangles). Error bars represent the standard error of themean (SEM).

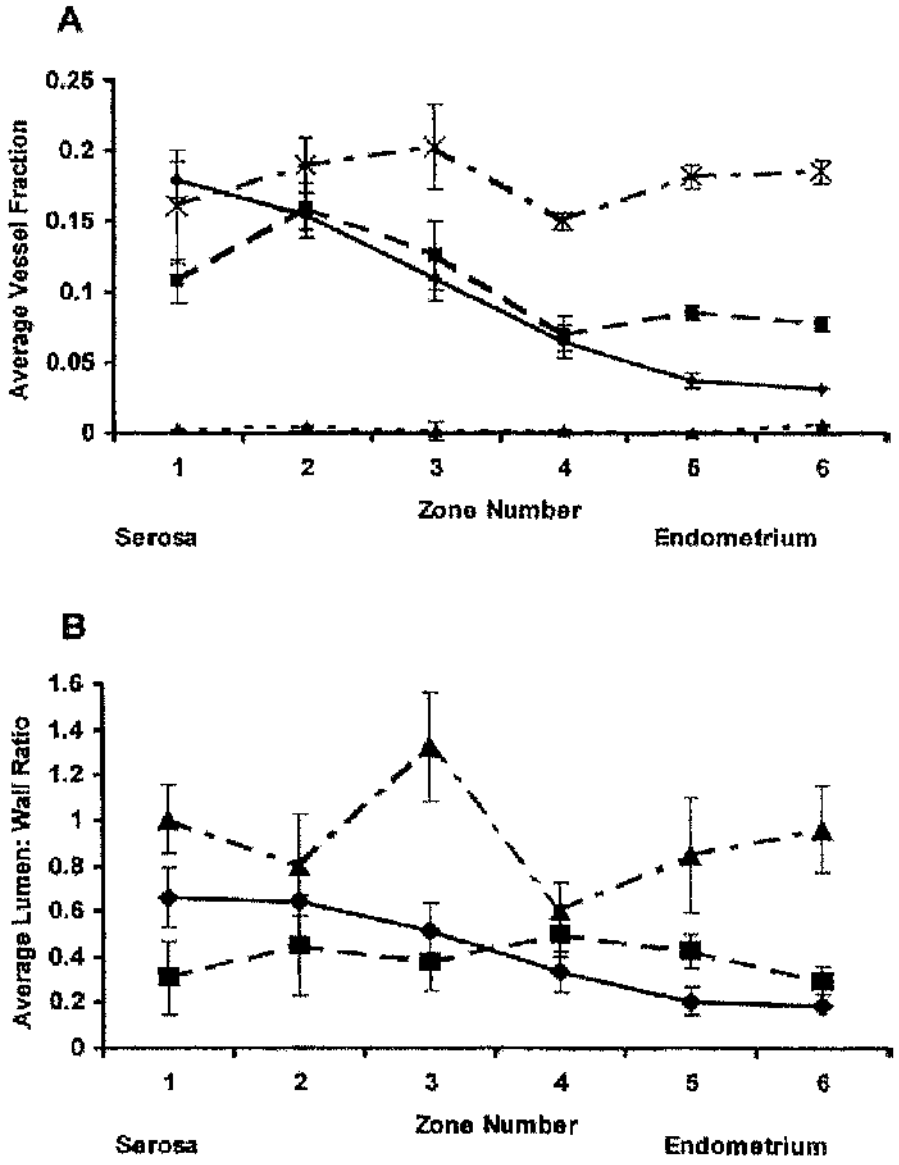


Table 11

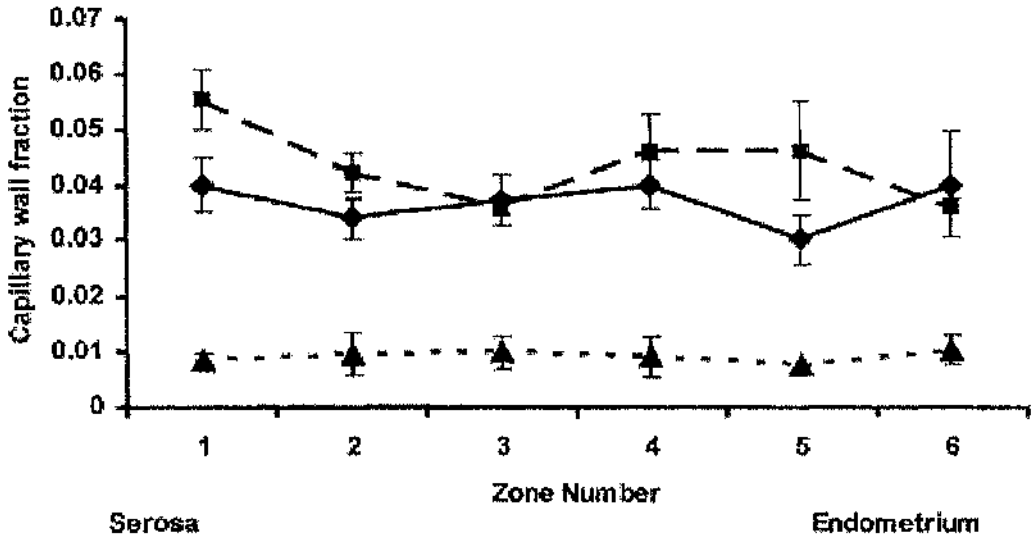
Average muscularized vessel volume fraction comparison by endometrial stage

Phase of the menstrual cycle did not influence myometrial vascularity as judged by the muscularized vessel volume fraction within nonfibroid tissue		
Average muscularized vessel volume fraction comparison by endometrial stage		
Zone	Proliferative	Secretory
1	0.18	0.175
2	0.16	0.17
3	0.1	0.11
4	0.06	0.06
5	0.05	0.055
6	0.05	0.05

Analysis of the volume fraction of capillary walls within the normal tissue suggested that there was no spatial difference in microvascularity across the myometrium **(Figure 29)**. The same was true of fibroids. It was also apparent that the fibroids had a smaller fraction of capillaries as determined by the capillary wall fraction **(Figure 29)**.

Figure 29

Stereological analysis of the capillary wall fraction – shows no obvious spatial gradients, within normal tissue (n = 15) (continuous line connecting diamonds), within the fibroid (n = 27) (dotted lines connecting triangles) or in the tissue surrounding the large perifibroid vessels (dashes connecting squares).



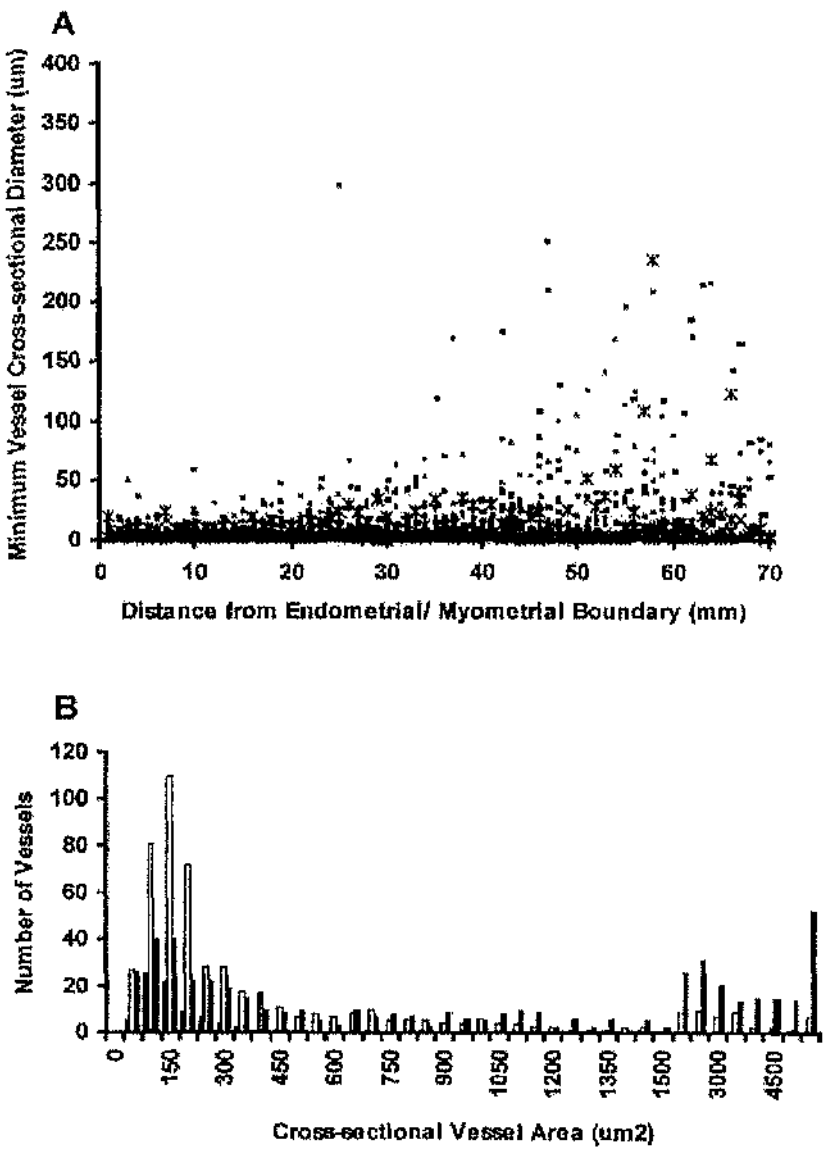
Morphometric analysis demonstrated a difference in the distribution of vessel size that was consistent with the stereological data. The inner myometrium close to the endometrium contained vessels of small minimum cross-sectional diameter. With increasing distance from the endometrial-myometrial boundary, vessels with larger cross-sectional areas were also present **(Figure 30A)**. There appeared to be a bimodal distribution of vessel cross-sectional area within the inner and outer myometrium. The smaller peak corresponded to capillaries and the larger one, to muscularized vessels. The larger muscularized second peak of the distribution was more obvious in the outer myometrium where larger vessels were present **(Figure 30B)**. Vessels within fibroids were smaller and did not have a bimodal distribution since the larger muscularized vessels were almost totally absent. Analysis of the minimum vessel cross-sectional diameter demonstrated that the

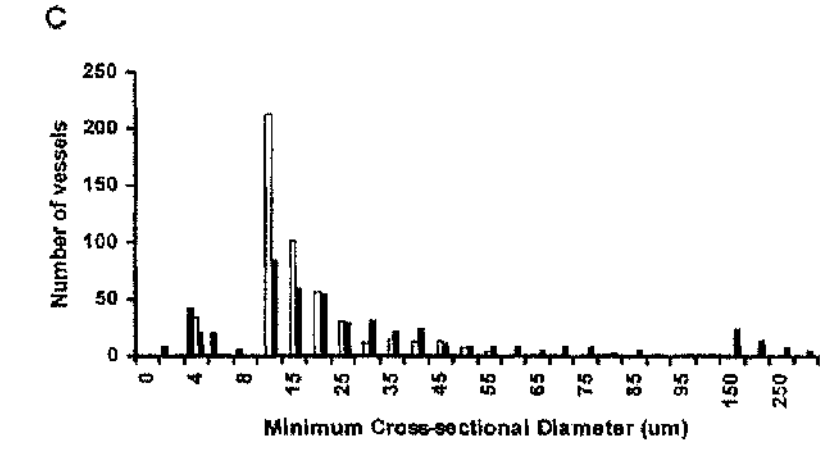
distribution of vessel size within the fibroid had a smaller modal value and a very narrow range (**Figure 30C**), indicating that the capillaries within the fibroid were smaller and more uniform in size than capillaries in the normal myometrium. Within the perifibroid tissue there were vessels of large cross-sectional area (**Figure 30D**).

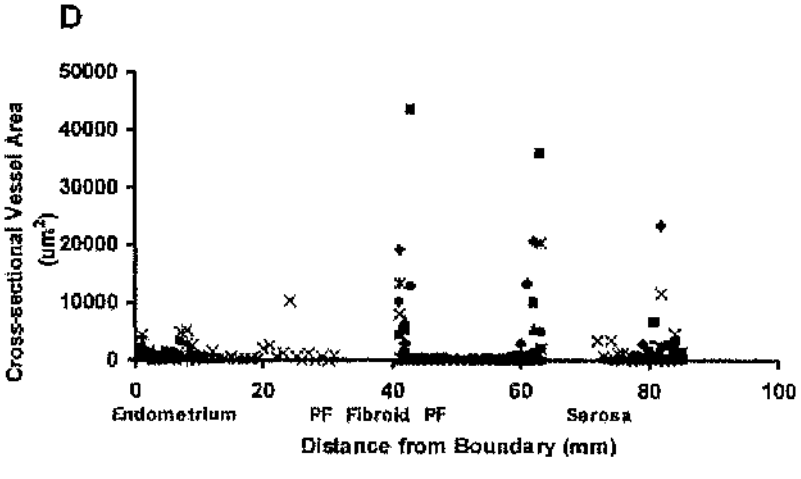
Figure 30

Morphometric analysis of cross-sectional vessel area and minimum diameter.

(**A**) The scattergram of minimum cross-sectional vessel diameter in normal tissue demonstrates that there is a change in the distribution in vascular size between inner and outer myometrium. Each point represents one vessel cross section, whereas each symbol type corresponds to one patient. (**B** and **C**) Discontinuous histograms of cross-sectional vessel area (**B**) and minimum vessel diameter (**C**) in inner and outer myometrium and in fibroid tissue. Hatched bars show the outer half of the normal myometrium, open bars represent inner myometrium and black bars show fibroid tissue. A bimodal distribution of cross-sectional vessel area is particularly obvious in the outer myometrium (**B**). The vessels within the fibroid have a smaller modal value and range than the normal myometrium (**C**). (**D**) Composite scatterplot of cross-sectional vessel area in fibroid specimens demonstrating large vessels in the perifibroid region. The scale of the horizontal axis is split and aligned by four spatial boundaries (endometrial–myometrial boundary, fibroid edge nearest endometrium, fibroid edge nearest serosa and serosal boundary). Each point represents one vessel cross section, whereas each symbol represents one patient.





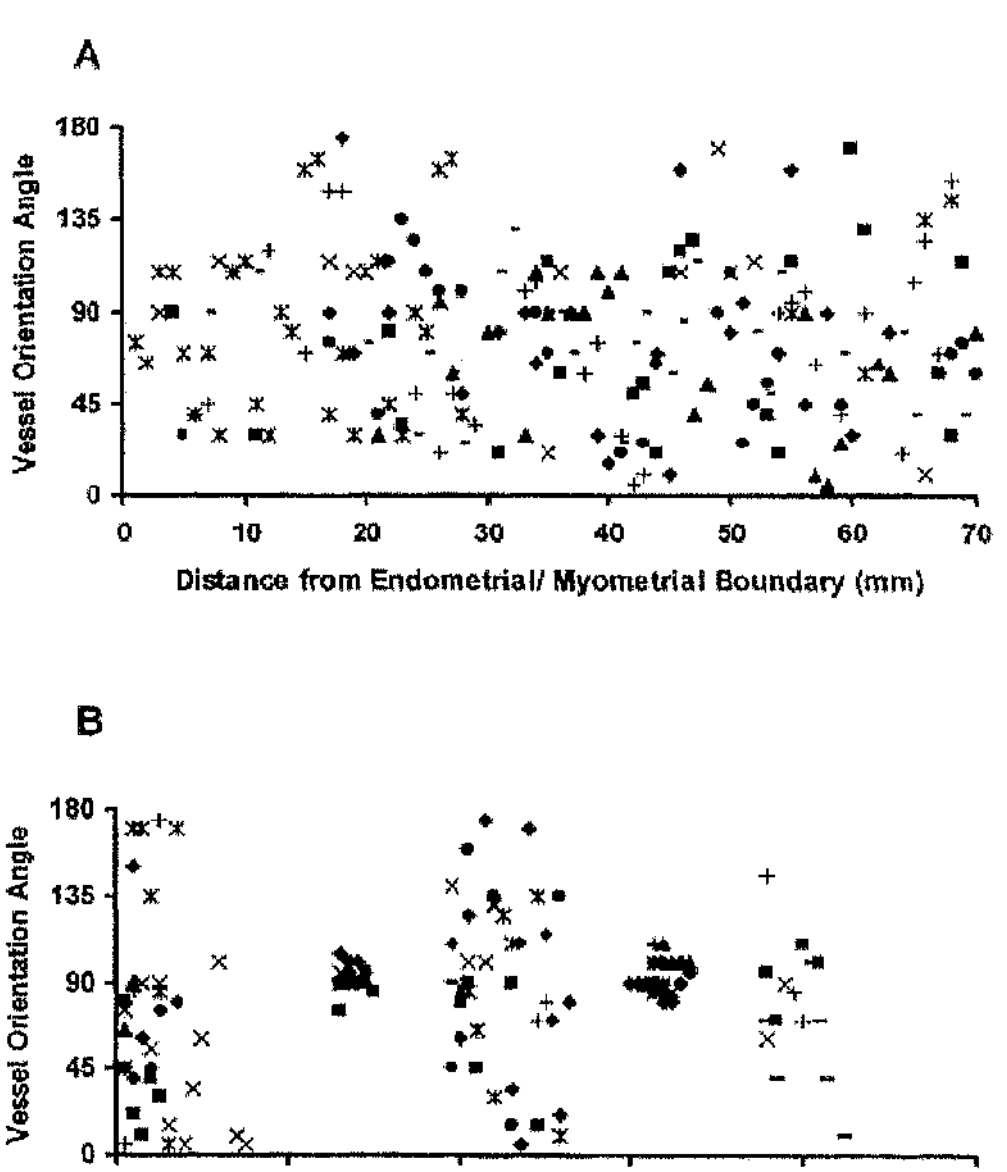


There was no obvious orientation of vessel cross-sections within the normal myometrium (**Figure 31A**). The circumferential perifibroid vessels appeared concave in cross-section. A line joining the end points of the cross-sections tended to be orientated perpendicularly to the endometrial-serosal axis **(Figure 31B)**. This orientation resulted from the plane of section.

Figure 31

Composite scatterplots of vessel cross-section orientation.

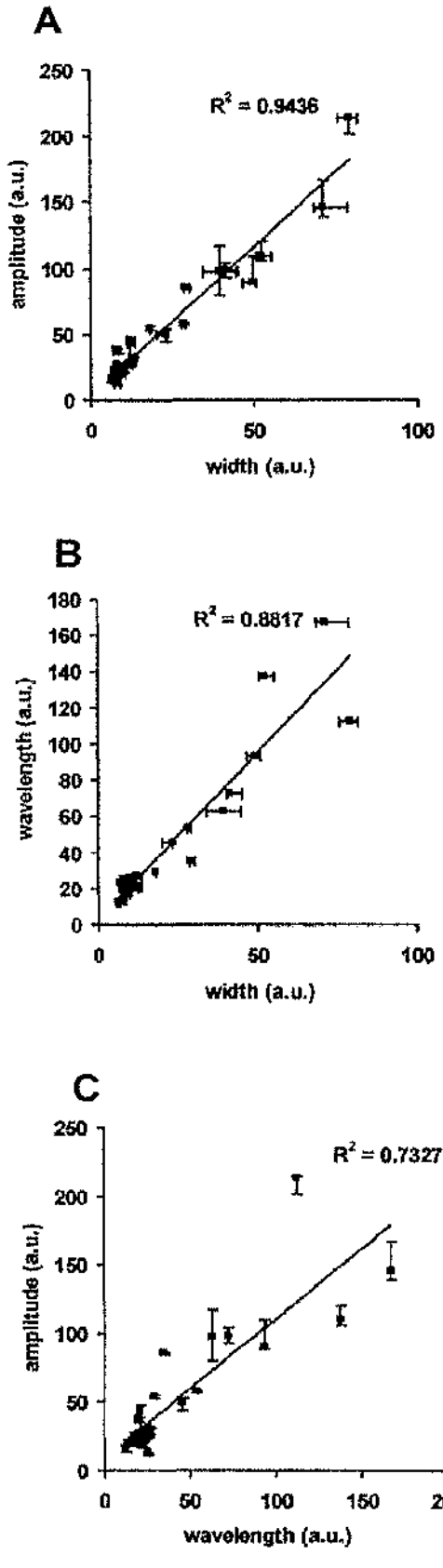
The scale of the horizontal axis is split and aligned by four spatial boundaries (endometrial–myometrial boundary, fibroid edge nearest endometrium, fibroid edge nearest serosa and serosal boundary). **(A)** There is a lack of vessel orientation along the endometrial–serosal axis in normal myometrium. **(B)** Within fibroid-containing specimens, the perifibroid region has large thin-walled circumferential vessels. The ends of their concave cross sections have a similar position along the endometrial–serosal axis, which produces a clustering in the orientation scattergram.



Manual analysis of the arterial angiographic projection images of Farrer-Brown and colleagues (1970a) demonstrated a linear relationship between luminal size and tortuosity. Larger vessels exhibited a higher amplitude and lower frequency of bending than smaller vessels **(Figure 32).**

Figure 32

Manual image analysis of the angiograms of Farrer-Brown *t al.* shows a clear correlation between (A) luminal width and amplitude, (B) luminal width and wavelength and thus (C) a co-correlation between amplitude and wavelength.



7A.4 Discussion

Stereological and morphometric analysis in the present study demonstrated a decreasing gradient of vascular smooth muscle abundance from the outer to inner myometrium, but a uniform capillary distribution. These observations were complemented by our analysis of the uterine arteriograms previously published by Farrer-Brown *et al.* In contrast, an absence of vascular smooth muscle and large diameter vessels was observed in small fibroids. These results supplement existing research in the field, allowing clarification of the precise anatomy of uterine and fibroid vasculature. They also provide further insight into fibroid vascular development and permit speculation regarding the anatomical rationale for uterine artery embolisation. Furthermore, this study uses robust methodological techniques to draw a comparison between fibroid vasculature and that of normal myometrium.

The present findings agree with Casey *et al.* (Casey, Rogers, & Vollenhoven 2000) who selected areas of high and low vascular density and used image analysis to conclude that small fibroids have a smaller vascular diameter than normal myometrium. However, the measurement of vascular luminal diameter in Casey *et al.* was dependent on the endothelial marker used. Paradoxically, CD-31 did not highlight any differences in luminal diameter of vessels within fibroids. The present study also varied methodologically from that of Casey *et al.* because we made no attempt to select areas based on vascular density. A deliberate selection of vessel types could exaggerate the difference between normal tissue and fibroids since it is not possible to select large vessels within fibroids.

Other studies of fibroid vasculature have used the 'hot spot' counting method, which erroneously counts vessel profiles and biases the analysis in favour of highly vascularized areas (Poncelet et al., 2002). Although there is no quantitative justification for the use of a non-randomized sampling design as practiced by the

'hot spot' method, it might be that tumour growth and the risk of dissemination is related to peaks of angiogenesis in particular types of tumours. Correlations between 'hot spot' counts and patient survival in malignant conditions are likely to be numerically associated as only the upper tail of the distribution is being selected for counting (Tas et al., 2000; Skoldenberg et al., 2001). In the case of non-malignant solid tumours such as fibroids, 'hot spots' of vascularization could be related to local growth. 'Hot spot' counting is intrinsically unsuited to the measurement of average values, contravenes modern design-based sampling approaches and should therefore not have a place in the assessment of normal tissue (Howard & Reed 1998).

Another important methodological consideration is the nature of the counting (stereological) probes used in vascular analysis. Some authors now seem to use the Chalkley counting grid in a way different to that originally contemplated by its designer (Hague et al. 2000). Chalkley originally designed the probe to be applied in a random position and orientation so that unbiased estimates could be produced (Chalkley 1943). Recent studies of the fibroid vasculature have deviated from the principle of random grid orientation. Use of the Chalkley method is also non-standard when applied to 'hot spots'.

The decreasing gradient of vascular smooth muscle abundance within the myometrium (from outer to inner aspects) demonstrated in the current study is consistent with previous angiographic studies outlining the distribution of arteries and veins within the myometrium (Farrer-Brown, Beilby and Tarbit 1970b; Farrer-Brown, Beilby and Tarbit 1970c). The anatomical explanation for these findings is that the larger arteries and veins present in the serosal aspect of the myometrium then branch and taper to form narrower radial vessels penetrating deep into the myometrium (Ramsey 1994). By contrast, there was a uniform distribution of capillaries throughout the myometrium as determined by the tissue

fraction occupied by capillary wall. The even density of capillary wall suggests that there is uniformity of perfusion in inner and outer myometrium.

Orientation of vessel cross sections would be expected to be influenced by the tortuosity of vessels running radially within the myometrium. The present findings suggest that tortuosity does indeed randomize these orientations. Paradoxically, the orientation in perifibroid tissue deviates from the random pattern seen in healthy tissue. It might have been expected that the course of radial vessels would be distorted by the presence of small fibroids, and they would thus appear to be orientated along the endometrial–serosal axis as they deviated around the fibroid. However, our findings show that the concave vertically sampled vessel cross sections were orientated in a plane parallel to the endometrial–myometrial junction. This illustrates their circumferential nature around the fibroid rather than orientation.

Analysis of the published arterial angiographs of Farrer- Brown *et al* (Farrer-Brown, Beilby and Tarbit 1970b) demonstrated a linear relationship between luminal width and measures of arterial tortuosity, providing a link between vessel size and the pattern of bending. It would seem reasonable to assume that tortuosity is related to wall thickness and consequently physical stiffness of the vessel. However, even highly flexible large diameter structures could not bend to the same extent as small diameter vessels purely for reasons of size. Arteries within the myometrium assume a tortuous course, because the length of the vessel is greater than that of the equivalent linear dimensions of the surrounding tissue (Jackson, Gotlieb, & Langille 2002). It seems likely that similar reasons also exist for the spiralling of vessels in the endometrium. Examination of uterine vessel cross sections shows that the vessels are not spiralling around cores of the interweaving smooth muscle fibres. The coils of larger vessels would instead seem to be contained within a matrix that is rich in elastin (Metaxa-Mariatou et al.

2002). It has been postulated that the highly coiled nature of the myometrial arteries is designed to permit expansion of the myometrial smooth muscle as it hypertrophies during pregnancy (Farrer-Brown, Beilby and Tarbit 1970b;Farrer-Brown, Beilby and Tarbit 1970d). During pregnancy-induced hypertrophy of rat uterine arcuate arteries, there is an increase in arcuate vascular diameter that is reversed in the post-partum period (St Louis et al. 1997). The vascular pattern seen in these hysterectomy specimens, taken from largely multiparous women, would be expected to be a product of several remodelling cycles. It would seem likely that a balanced system of continued remodelling and maturation is required for vessel development within the myometrium. Smooth coils without points of inflection or straight segments would be expected to develop at points in the system where there is a balance between radial and axial growth and local remodelling of vascular muscle orientation. Angiogenesis is controlled by the equilibrium that exists between pro- and anti-angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and tumour necrosis factor α (TNFα) (Bikfalvi et al. 1997). Existing vessels must then mature under the control of other signaling molecules such as angiopoetins, platelet-derived growth factor (PDGF), integrins and matrix metalloproteinases, permitting pericyte recruitment, basement membrane formation and extracellular matrix deposition to increase vessel diameter (Thurston et al. 1999). This maturation phase encompasses the recruitment of cells to form vessel walls and may contribute to the gradient of vessel wall density seen in normal myometrium. During the course of fibroid development, in which there is abnormal increase in muscle growth, there is accompanying dysregulation of the normal vascular pattern. The small fibroids appear to lack well-developed muscularized vessels and show no evidence of spiralling. Although fibroid growth must be accompanied by angiogenesis, the present data show that there is a lack of

vascular smooth muscle cells. This apparent lack of vascular maturation can be explained by either the failure of myoblastic precursor cells to migrate into the tumour or the suppressive arteriogenic environment of the tumour. In the present work, only small fibroids were studied, and it is possible that larger, muscularized vessels may develop in larger fibroids with time. Nonetheless, the striking difference in vascularization seen in small fibroids makes it likely that the angiogenic stimulus or anti-angiogenic profile is altered within fibroids relative to that occurring in normal tissue development with corresponding lack of elongation, muscularization and spiralization (Hague et al. 2000;Poncelet et al. 2002). Furthermore, there would appear to be alterations in the distribution of vessels in apparently normal myometrium around the fibroid (Hamid, Daly, & Campbell 2003).

This study has also shown that the gradient of vessel size seen in non-fibroid myometrium was lost in the myometrium around the fibroid. These results are consistent with those of Hague *et al.* (Hague et al. 2000) who showed increased expression of hypoxia-induced secretory factors, adrenomedullin and VEGF in the myometrium of leiomyoma-bearing uteri compared with controls. Perfusion in myometrial vessels around the fibroid has also been shown to be reduced by Hickey *et al.* (Hickey & Fraser 2000) who also postulate that a dysregulation of angiogenic factors in the myometrium is responsible for these findings. Further work in this area is required to determine the initial stimulus for fibroid generation.

Stereological examination showing small fibroids to be relatively avascular, with highly vascular pseudocapsules, may provide an explanation for the rationale for uterine artery embolisation. Contrary to previous reports on embolisation, which discuss the 'hypervascular' nature of fibroids, the present stereological analysis suggests that although the perifibroid region has a high volume fraction of vessel

lumen, the fibroid itself is relatively avascular. The perifibroid region is abnormal in vascular organization; therefore, the term 'hypervascularity' should not be taken to imply adequate perfusion of this zone. The vascular perifibroid regions are likely to correspond to the peripheral rim of well vascularized vessels seen on ultrasound and used as the plane of tissue dissection during myomectomy (Fleischer 1919). The other alternative is that these abnormal vessels are on the venous side of the vascular tree and correspond to the venular ectasia first described by Sampson in 1912 and referred to, thereafter, by Stewart and Nowak (Stewart & Nowak 1996).

Previous clinical studies have shown that embolisation has little effect on the normal myometrium (Siskin et al. 2000). From this study, many reasons can be postulated as to why the fibroid may be targeted by embolisation. One may be that the relatively avascular nature of the fibroid makes it more prone to tissue hypoperfusion than the normal tissue. Another may be that the polyvinyl alcohol particles used may selectively occlude the large perifibroid vessels and hence the blood supply to the fibroid. Following histopathological examination of uteri that had previously undergone UAE, it was found that the majority of particles collected in the perifibroid region post-embolisation (Weichert et al. 2005). It may also be that the lack of large vessels within the fibroid makes it more vulnerable to ischaemic insult due to lack of collateral flow.

7A.5 Conclusion

In conclusion, the results of this study suggest a gradient of vessel size, wall thickness and tortuosity between inner and outer myometrium. This appears to be lost in fibroids, leading us to postulate that vessel wall maturation stimuli or the precursor mural cells are lacking in fibroids. This is likely to have clinical implications, particularly in the field of uterine artery embolisation as a treatment for symptomatic fibroids.

Chapter 7B

Vasculature of human myometrium and leiomyomata

7B.1 Introduction

The three-dimensional nature of the microvascular network on cut surfaces of fresh human uterus, has already been demonstrated recently with the technique of diffusion labelling (Hamid, Daly, & Campbell 2003). This highlighted the vascular differences between normal myometrium and fibroid tissue. In an attempt to take things one step further, we aimed to carry out a study using the method of perfusion labelling via the uterine arteries of fresh human uterus ex-vivo. This technique of systemic labelling was carried out just prior to capturing images of the fresh uterine specimens with the aid of MRI. This was in the hope of obtaining greater detail of the microvascular network of the human uterus and being able to demonstrate the differences between myometrial and fibroid tissue with more clarity. Using such new methods of visualisation to demonstrate uterine vascular networks ex vivo may further assist in our understanding of the success and failure of uterine artery embolisation.

7B.2 Materials and methods

Ethics approval was obtained prior to carrying out this prospective study. Eight uteri (four fibroid and four normal) were obtained from women undergoing elective hysterectomy for benign disease, in South and North Glasgow University Hospitals NHS Trusts. All women undergoing surgery had menorrhagia and had not received hormonal treatment for at least six months prior to surgery. Following hysterectomy, right and left uterine arteries were immediately cannulated in turn, using paediatric umbilical catheters (Vygon, 3.5 French Gauge). The catheters were carefully secured with a non-absorbable suture and the uterine arterial system flushed on each side with 20ml heparinised saline (**Figure 33**). This was done in the hope of removing as much blood clot from the arterial microvasculature as possible. The uterine specimen was then immersed in

a bucket of normal saline and transferred promptly to the North Glasgow radiology department. Within three hours of hysterectomy, the specimen was removed from the saline bath and each of the uterine arteries confirmed patent with a further injection of 10ml heparinised saline on each side. Thereafter, the specimen was placed within a MRI machine (1.5 Tesla) used for standard clinical practice, for images to be captured by an experienced radiographer. Once completed, 10ml of a radiopaque contrast dye in the form of gadolinium (Omniscan, 0.5 millimoles/ml, Amersham Health) was injected into each of the uterine arteries and MR-angiography was then performed.

In an attempt to obtain better resolution of uterine vessels, we then went on to perform higher resolution magnetic resonance imaging of fresh uterine specimens (n=3). Using the technique described above, each of three uterine specimens was placed in turn, within a 7-Tesla magnetic resonance imager belonging to Glasgow Vetinary School research department **(Figures 34 and 35)**. The magnet's designation as 7-Tesla indicates its field strength and is used for research purposes only. The increased magnetic field strength of the 7-Tesla machine facilitates the production of highly detailed images and it was anticipated that we would obtain a greater level of detail from this machine, when compared to images obtained from standard MRI used in routine clinical practice. In addition to gadolinium, a fluorescently conjugated plant lectin called Ulex Europaeus Agglutinin I [UEA-1]), a known marker of the endothelium, was used to achieve systemic endothelial labelling in two of the fresh uterine specimens (Hormia, Lehto, & Virtanen 1983). Thus, 5ml (5 micrograms/ml) of UEA-1 tetramethylrhodamine isothiocyanate (TRITC) (Vector Ltd., Peterborough, UK) was injected into each of the uterine arteries after an initial flush with saline. Once again, T1 and T2-weighted images were captured; these were then image processed to create high intensity projections.

Following MRI, randomly biopsied tissue (approximately 3-4mm in depth) from inner to outer myometrium, were taken from the two uterine specimens injected with the fluorescent lectin. Each piece or block of uterine tissue was then mounted on to a microscope slide after washing for five minutes in phosphate buffered saline (PBS). By binding to vascular endothelial cells, the fluorescent lectin facilitated visualisation of the vascular network on the cut surface of the uterus (**Figure 36**). UAE-1 absorbs blue light and thus using blue light with a microscope equipped with epi-illumination, images were captured with the aid of a digital camera (Nikon). These images were then processed post-capture, using Adobe Photoshop 7.

Figure 33

Specimen of fresh human uterus – *both uterine arteries cannulated in preparation for gadolinium enhanced MRI*

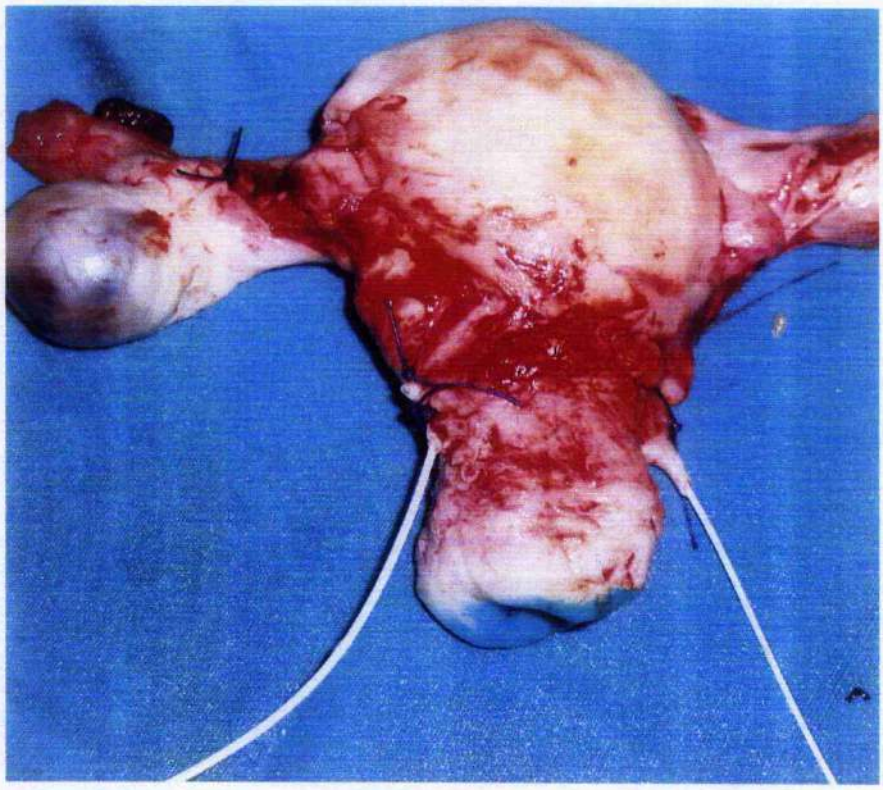


Figure 34

Specimen of fresh human uterus placed in an open plastic bottle – *awaiting insertion into 7 Tesla MRI*

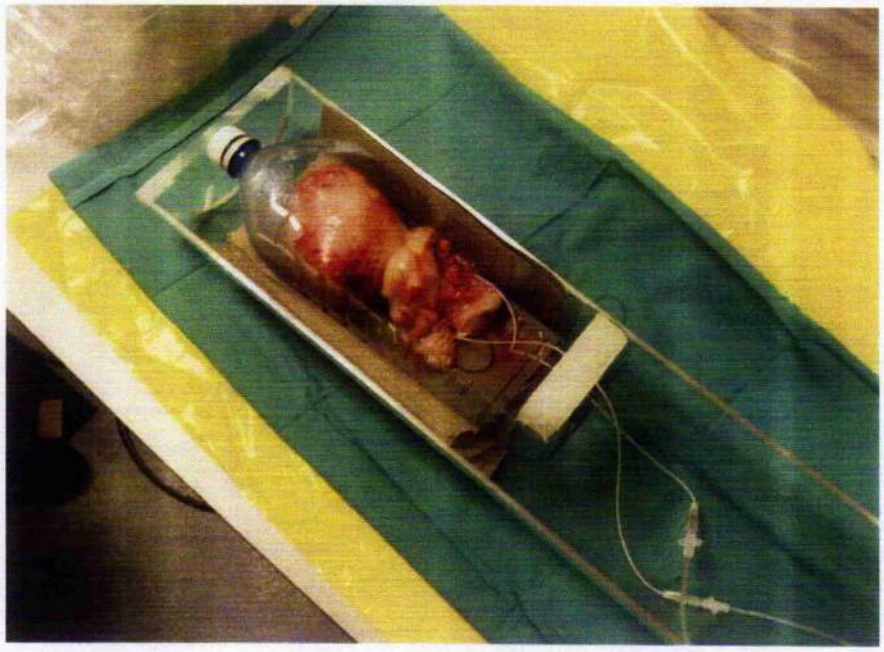


Figure 35

7 Tesla MRI

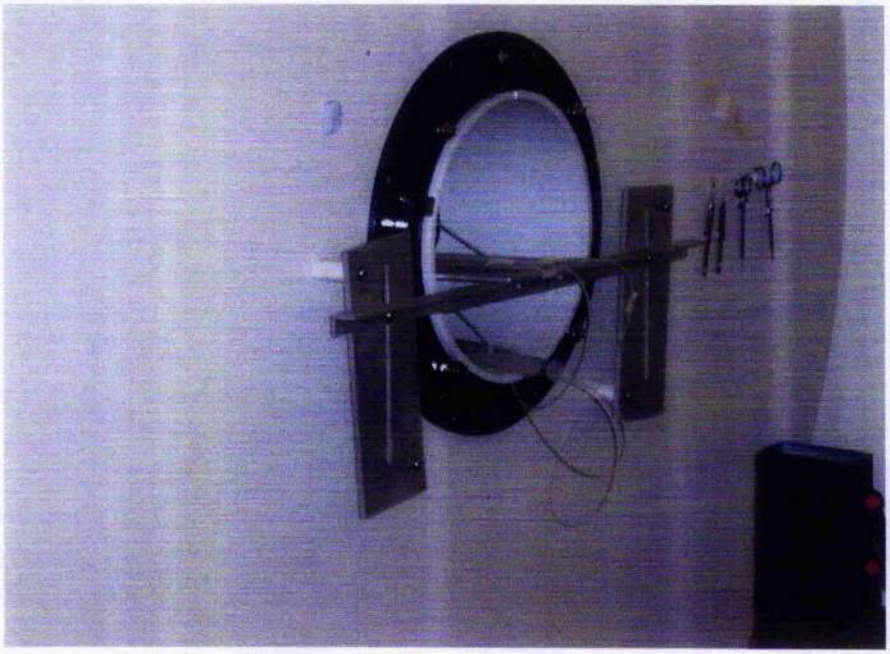
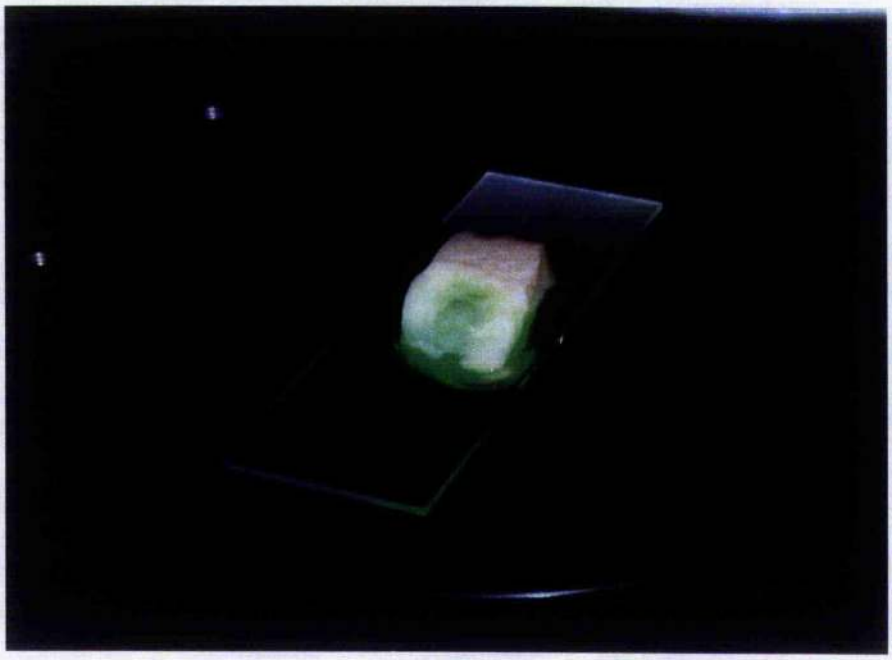


Figure 36

Cut surface of fresh human uterus perfused with a fluorescent lectin



7B.3 Results

Images obtained after standard MR-angiography of fresh uterine specimens perfused with gadolinium were very variable in quality. Unfortunately, in four out of the eight specimens, we failed to demonstrate the vascular tree of the uterus. However, it was possible to demonstrate the tortuous vasculature of the uterus in some cases (**Figure 37**). Whilst these images confirmed that it was possible to display the vascular network of the uterus ex-vivo using standard MR-angiography, we were unable to show a level of detail that had previously been anticipated. In addition, it was not possible to distinguish between fibroid and normal myometrial vasculature.

Higher resolution MR imaging of human uterus using the 7-Tesla machine was also disappointing as we did not obtain better resolution of uterine microvasculature. Instead of the thin spirals that we expected, vessels were seen as "fat wiggly sausages" (**figure 38**). We did, however, obtain better resolution of the non-vascular components of fibroids. The heterogeneity of these lesions could be clearly demonstrated as well as the lack of presence of large vessels (**figure 39**).

Images obtained from microscopy of cut surfaces of fresh uterus perfused with the fluorescent lectin demonstrated the myometrial vascular network in 3D (**figures 40A and B**).

Figure 37

MR-angiography of fresh human uterus perfused with gadolinium

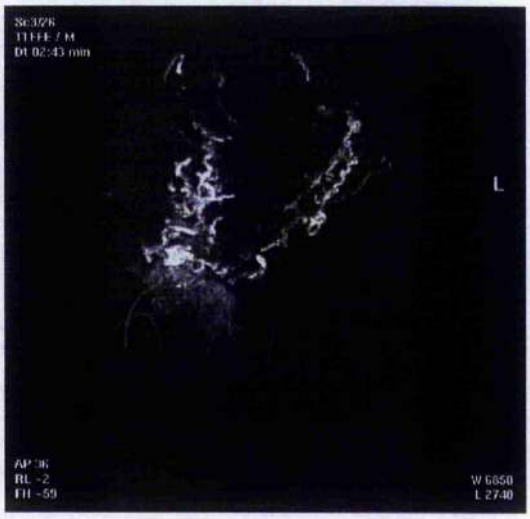
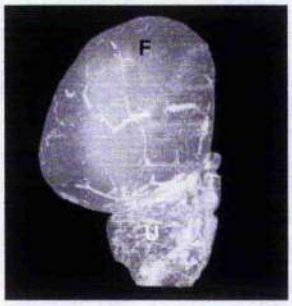
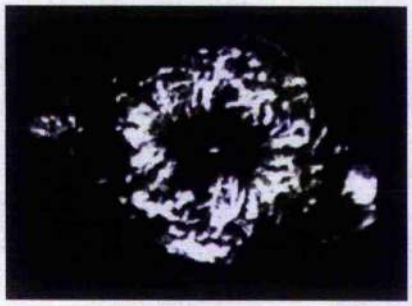


Figure 38

High resolution MR images after rendering of T1-weighted minimum intensity projections. **A**. *Large surface vessels of a pedunculated fibroid (F) and distorted uterine body (U).* **B**. *Distribution of large vessels in mid-slice of myometrium*



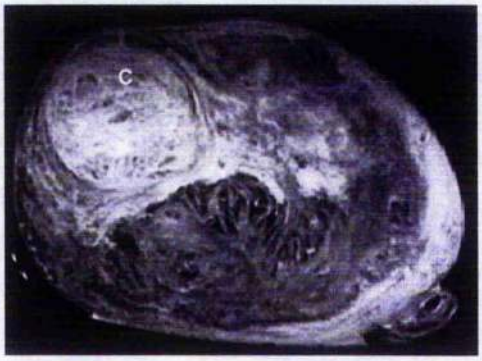
A



B

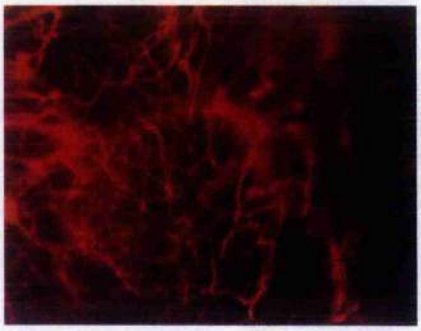
Figure 39

High resolution MR image of a T2-weighted cross-section of a large pedunculated fibroid – *(C) is a highly cellular area. Note the heterogeneity of non-vascular components of the fibroid and the lack of large vessels seen in the tumour*



Figures 40A and B

Perfusion labelling of human uterus demonstrating human myometrial microvascular pattern in 3D



A

B

7B.4 Discussion

Experimental work using perfusion methods in combination with standard and high resolution MRI, has confirmed that it is possible to visualise uterine vascular networks ex-vivo. However, we were unable to obtain better resolution of uterine microvasculature, as had been anticipated. One of the reasons for this is likely to be artefact, perhaps caused by air embolism, blood clot or a combination of both. In addition, it was not always possible to perfuse the uterus beyond the level of the main uterine artery. Interestingly, the pathologist examining the uterine specimens which had undergone uterine arterial flushing followed by perfusion with gadolinium plus or minus the fluorescent lectin, found the uteri to be particularly white and avascular in appearance. Both standard and high resolution MR-angiography appear to have their limitations in terms of demonstration of uterine and fibroid vasculature although high resolution MRI demonstrated non-vascular components of the uterus in great detail. The images of uterine microvasculature that we obtained are still not equivalent to the traditional angiography techniques that Farrer-Brown and colleagues performed on thin uterine slices. Nonetheless, our new methods of visualisation have facilitated a better understanding of tissue and fibroid structure as well as the vascular patterns of both myometrium and fibroids.

7B.5 Conclusion

It is possible to demonstrate the vascular network of the uterus ex-vivo, using perfusion and systemic labelling techniques in conjunction with both standard and high resolution MRI. In addition, high resolution MRI facilitates demonstration of non-vascular components of the uterus and fibroids with a high level of detail. This study demonstrates the vascular patterns seen in human myometrial and

fibroid tissue, and to some extent, the differences between normal and diseased uterine tissue.

Chapter 8

Final Conclusions

8.1 Summary

We have shown that UAE is effective in reducing objective MBL and uterine volume up to 48 months and 6 months after treatment, respectively (chapter 2). This is the only published study to date, which evaluates the effect of UAE on MBL objectively. Whilst this study is small and does not compare UAE to other uterine-sparing modalities, it is useful to have objective measures of outcome when evaluating a new technique in the early stages of its introduction to clinical practice. In addition, having objective evidence to support the use of a relatively new treatment can be very useful when counselling women about the therapeutic options available to them.

We have also demonstrated that UAE is associated with a significant improvement in health status (as assessed by the SF 36) which is maintained up to 60 months post-embolisation (chapter 3). This is in keeping with other recent studies evaluating the effect of UAE on HRQoL (Scheurig et al. 2006). Using a tool such as the SF 36 allows us to demonstrate that overall health, and not just menstrual symptoms, improves after embolisation.

Data from observational studies is useful, but one needs to compare a new therapy to the gold standard treatment before introducing it into routine clinical practice. The REST trial addresses this issue (chapter 5). This study concluded that there is no significant difference between surgery and embolisation in quality of life at one year, in women with symptomatic fibroids. Embolisation was associated with a significantly shorter hospital stay and recovery time whilst surgery was associated with significantly better symptom scores at one year. The trial reported similar complication rates after both interventions and in particular, highlighted the need to monitor women post-embolisation for complications which do not always present immediately after the embolisation procedure. It also reported a treatment failure rate of 9% at one year in the embolisation arm of the trial and

commented that secondary procedures for persistent or recurrent symptoms were required in some cases, after one year.

It was difficult to extrapolate meaningful conclusions from the comparison of UAE to myomectomy in the REST trial, as only a small number of women underwent myomectomy. Other studies comparing the clinical results of UAE and myomectomy in women planning future pregnancy have not been prospective (Broder et al. 2002;Goldberg et al. 2004;Razavi et al. 2003) whilst one prospective randomised controlled trial comparing the two therapies was unable to evaluate the reproductive outcomes of both treatments although it did suggest that embolisation and myomectomy are clinically effective in most cases and not associated with a significant number of serious complications (Mara et al. 2006). Thus longer term data from prospective comparative trials involving larger numbers of women is required in order to evaluate the effects of embolisation versus myomectomy on reproductive function and perinatal outcomes in women with symptomatic fibroids. Until this data is available, it remains uncertain as to whether UAE or myomectomy is the best option for women who wish to retain their fertility.

We experienced successful pregnancy outcomes after UAE although it remains unclear whether or not pregnancy outcomes are affected by the procedure. Less than 200 pregnancies have been reported in the literature to date. Whilst there have been good pregnancy outcomes reported after embolisation, the rates of miscarriage, pre-term delivery, caesarean section and post-partum haemorrhage appear to be increased, when compared to the general obstetric population (Huang et al. 2006;Pron et al. 2005;Walker & McDowell 2006). Demographics such as increased maternal age of the women in the study population may partly explain these findings; advanced age not only reduces the chances of successful conception, but also increases the chances of adverse obstetric outcomes. Thus

further data on pregnancy outcomes is still required in order for us to be able to counsel women appropriately on this point prior to undergoing UAE.

The risk of premature ovarian failure after embolisation remains an important issue for women considering UAE prior to completion of their family. The rate of ovarian failure was relatively high in our group of predominantly peri-menopausal women (chapter 2), but in general, permanent amenorrhoea or the menopause occurs in up to 15% of women above the age of 45 years and in less than 1% of younger women. Several studies have assessed the impact of embolisation on ovarian function, all of which have measured follicle-stimulating hormone (FSH) before and after UAE (Ahmad et al. 2002;Healey et al. 2004;Spies et al. 2001). These studies did not show a significant difference in baseline and post-embolisation FSH values. It should, however, be borne in mind that amenorrhoea or the menopause is a measure only of severe ovarian dysfunction and the number of women with milder forms of ovarian damage is likely to be substantially higher than we estimate. Women, therefore, should be thoroughly counselled about the important potential side effect of ovarian failure and its consequences, prior to undergoing embolisation.

We are well acquainted with the option of UAE as a treatment of symptomatic fibroids in pre-menopausal women. However, it has been thought to be of little benefit in postmenopausal women, based on the theory that fibroid tumour shrinkage occurs at this time in a woman's life, as a result of the absence of ovarian hormonal stimuli. Thus all women in our studies were pre-menopausal. Recently, however, a retrospective study has been carried out evaluating the effect of UAE in postmenopausal women with fibroid-related bulk symptoms (Chrisman et al. 2007). This study reported a significant reduction in fibroid volume and improvement in bulk-related symptoms up to a period of 24 months after treatment. The authors concluded that UAE is a viable treatment option in

carefully selected post-menopausal women with known fibroids associated with bulk-related symptoms. It is therefore worth considering embolisation as a treatment option in women with symptomatic fibroids, both pre- and postmenopausal, after careful evaluation of their clinical symptoms.

Studies have been carried out recently to determine the particular baseline fibroid tumour characteristics which predict outcome for UAE. Chapter 2 concluded that UAE is associated with a significant reduction in uterine volume (and thus fibroid shrinkage) at 6 months post-treatment (median reduction – 41%). However, there was no correlation between the degree of fibroid shrinkage and change in objective menstrual blood loss measurement for individual patients at that time. Spies et al reported that a greater baseline fibroid volume results in less shrinkage after UAE and overall, the larger the dominant fibroid prior to embolisation, the less volume reduction after UAE and the greater likelihood of patient dissatisfaction with the outcome of embolisation (Spies et al. 2002b). Other studies report an association between fibroid size, location and MR signal intensity with outcome (Burn et al. 2000;McLucas, Adler, & Perrella 1999). A submucosal location appears to be associated with a better outcome in the short term. In addition, a high fibroid signal intensity on T1-weighted MR images (thought to result from haemorrhagic necrosis and the presence of blood breakdown products) was strongly predictive of a poor response to UAE whilst a high signal intensity on T2-weighted images (thought to be due to increased fibroid cellularity and/or vascularity) was predictive of a good response to UAE. Interestingly, the degree of gadolinium enhancement did not correlate with fibroid volume reduction. It should be emphasised that these studies have their limitations and there continues to be a need for further research into the effect of baseline uterine/fibroid size and location on clinical outcomes as well as the impact of

fibroid volume reduction and degree of devascularization on both short and long term outcomes of UAE.

The treatment failure rate after UAE was 9% at one year in the REST trial with a further 10% requiring a secondary intervention after one year's follow up. These findings not only highlight the need for longer term follow up of women post-embolisation, but also encourage us to think about the reasons for treatment failures and the options for secondary procedures. The latter may be required for women who have no improvement of symptoms after UAE or for those women who have a recurrence of symptoms, usually due to increasing dominant fibroid size or increasing number of fibroids. Within the setting of the REST trial, most secondary procedures were hysterectomies. However, in the future, we perhaps need to consider repeat UAE in more women. Yuosefi et al carried out a study of 24 women who underwent repeat embolisation for recurrent or persistent fibroid-associated symptoms (Yousefi et al. 2006). MR-angiography was performed prior to the repeat procedure. Whilst this study was small and short term, the authors reported that repeat UAE is effective in controlling symptoms in the majority of patients, although embolisation of the ovarian arteries is often required. Complications were infrequent, but they concluded that further studies are required in order to establish the safety and efficacy of repeat embolisation.

The risks of major complications such as pelvic sepsis and emergency hysterectomy were low in our group of women undergoing UAE and these rates are comparable to those quoted in other published literature. Chapter 6, however, highlights the need for us to be aware of the potential for late presentation of infective complications after UAE.

We found that uncomplicated UAE is associated with a significant rise in WCC, CRP and IL-6, with peaks occurring three days post-embolisation followed by a reduction and normalisation of levels at one month after UAE (chapter 4). No

correlation was found between pain scores and individual inflammatory marker levels at that time. With the exception of one individual, inflammatory mediator levels did not rise during the time period in which a proportion of our group of women complained of clinical symptoms in keeping with the post-embolisation syndrome. The results of this study are not what we expected. An alternative explanation therefore must exist for the pathophysiology of the post-embolisation syndrome, a syndrome which must be more complex than we previously thought. The differences that have been observed between normal and diseased (fibroid) uterine tissue have been exploited clinically by uterine artery embolisation for the treatment of symptomatic fibroids. Examination of the uterine vascular network in greater detail has confirmed that such differences exist (chapter 7A and B). We have shown that a gradient of vascularity exists within the myometrium and also, that there are no mature vessels within fibroids. With increasing knowledge and understanding of uterine and fibroid microvasculature, we may, in the future, be able to predict with more accuracy, cases that are likely to be successful and those that are likely to fail, after UAE.

In final conclusion, UAE appears to be a safe and efficacious method of treatment for women with symptomatic fibroids, in the short and mid-term. More evidence, however, is required on long term outcomes following UAE, with particular attention to complications, treatment failures and fibroid growth / recurrence after embolisation. Further long term data is also needed regarding the effects of UAE on fertility and pregnancy. In addition, more prospective comparative studies are required to compare embolisation to the various techniques of hysterectomy. We also need to compare and evaluate the outcomes of embolisation and other uterine-sparing modalities, in particular, myomectomy. The inflammatory response to UAE and its molecular basis requires further evaluation and we still need a better understanding of fibroid pathogenesis and uterine vasculature, in

order to further develop therapeutic options for symptomatic fibroids, and understand the exact underlying mechanisms of treatments such as UAE.

8.2 Future work possibilities

1. Long term follow up of patients recruited into the REST trial with particular attention to relapse and recurrence of symptoms, long term complications, treatment reintervention, fertility and pregnancy outcomes.

2. Further investigation of the inflammatory response to UAE and the aetiology of the post-embolisation syndrome, using alternative biomarkers to those used in our study

3. Further analysis of uterine and fibroid microvasculature with more research into the detection and use of biological markers which distinguish arteries form veins.

4. Research into the use of animal models for uterine fibroid and UAE studies

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Appendix 1

Short Form 36

The following questions ask for your views regarding your health and how you feel about life in general. Answer all the questions, if you are unsure then think about your overall health and give the best answer you can.

1/ In general, would you say your health is ?
(circle one)

excellent	1
very good	2
good	3
fair	4
poor	5

2/ Compared to four months ago, how would you rate your general health now ?

much better	1
somewhat better	2
about the same	3
somewhatworse	4
much worse	5

3/ The following questions are about activities you might do during a typical day. Does your health limit you in these activities ? If so, how much ?
(circle one number for each question)

ACTIVITIES	YES, LIMITED A LOT	YES, LIMITED A LITTLE	NO, NOT LIMITED AT ALL
a/ vigorous activities, such as running, lifting heavy objects or strenuous sports ?	1	2	3
b/ moderate activities such as moving a table, hoovering ,bowling or golf ?	1	2	3
c/ lifting or carrying groceries ?	1	2	3
d/ climbing several flights of stairs ?	1	2	3

e/ climbing one flight of stairs ?	1	2	3
f/ bending, kneeling or stooping ?	1	2	3
g/ walking more than a mile ?	1	2	3
h/ walking half a mile ?	1	2	3
I/ walking 100 yards	1	2	3
j/ bathing or dressing yourself	1	2	3

4/ During the past 4 weeks , have you had any of the following problems with your work or other regular daily activities as a result of your physical health ?

	YES	NO
a/ cut down on the amount of time you spent on work or other activities ?	1	2
b/ accomplished less than you would have liked ?	1	2
c/ were limited in the kind of work or activities ?	1	2
d/ had difficulty performing the work or other activities (e.g. it took extra effort)?	1	2

5/ During the past 4 weeks , have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	YES	NO
a/ cut down on the amount of time you spent on work or other activities ?	1	2
b/ accomplished less than you would have liked ?	1	2
c/ didn't do work or other activities as carefully as usual ?	1	2

6/ During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with friends, family or groups ?

	(circle one)
not at all	1
slightly	2
moderately	3
quite a bit	4
extremely	5

7/ How much bodily pain have you had in the past 4 weeks ?(circle one)

none	1
very mild	2
mild	3
moderate	4
severe	5
very severe	6

8/ During the past 4 weeks, how much did pain interfere with your normal work
(including both work outside the home and housework) ?

not at all	1
a little bit	2
moderately	3
quite a bit	4
extremely	5

9/ These questions are about how you feel and how things have been with you during the past 4 weeks.
For each question, please give the one answer that comes closest to the way you have been feeling. :- .....
(circle one number for each question)

HOW MUCH TIME IN THE LAST 4 WEEKS	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a/ did you feel full of life ?	1	2	3	4	5	6
b/ have you been nervous ?	1	2	3	4	5	6
c/ have you felt so down in the dumps that nothing could cheer you up ?	1	2	3	4	5	6
d/ have you felt calm and peaceful ?	1	2	3	4	5	6
e/ did you have a lot of energy ?	1	2	3	4	5	6
f/ have you felt downhearted and low ?	1	2	3	4	5	6
g/ did you feel worn out ?	1	2	3	4	5	6
h/ have you been	1	2	3	4	5	6

happy ?

I/ did you feel tired ? 1 2 3 4 5 6

10/ During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.) ?

all of the time 1

most of the time. 2

some of the time 3

a little of the time 4

none of the time.... 5

11/ How TRUE or FALSE is each of the following statements for you (circle one number for each question)

	DEFINITELY TRUE	MOSTLY TRUE	DON'T KNOW	MOSTLY FALSE	DEFINITELY FALSE
a/ I seem to get ill more easily than other people	1	2	3	4	5
b/ I am as healthy as anybody I know	1	2	3	4	5
c/ I expect my health to get worse	1	2	3	4	5
d/ My health is excellent	1	2	3	4	5

Appendix 2

Publications

Fibroid Embolisation
"Progress in Obstetrics & Gynaecology"– volume 17
Curchill Livingston, Ed. Studd, Tan & Chervenak
2006
Chapter 22, p333-43
***Khaund A**, Lumsden MA*

The normal human myometrium has a vascular spatial gradient absent in small fibroids
Human Reproduction
October 2006;21(10):2669-78
*Aitken E, **Khaund A**, Hamid S, Millan D, Lumsden MA, Campbell S*

Evaluation of the effect of uterine artery embolisation on menstrual blood loss and uterine volume
BJOG - an International Journal of Obstetrics and Gynaecology
July 2004;111:700-705
***Khaund A**, Moss J, McMillan N, Lumsden MA*

All about hysterectomy
The Menopause Exchange
Spring 2004; Issue 20
***Khaund A**, Lumsden MA*

Uterine fibroids – do size and location determine menstrual blood loss?
European Journal of Obstetrics & Gynaecology and Reproductive Biology
July 2004;1115, issue 1:85-89
*Sulaiman S, **Khaund A**, Moss J, McMillan N, Lumsden MA*

Oral Presentations

Uterine artery embolisation for fibroids
Gynaecological Visiting Society
Oct. 2006 (Glasgow)

Fibroids – current management options
Joint RCOG & BSGE meeting - "Recent Advances in Gynaecological Surgery"
Nov. 2005 (RCOG – London)

Effect of uterine artery embolisation on menstrual blood loss and uterine volume – 48 month review
Cardiovascular & Interventional Radiology Society of Europe (CIRSE)
Sept. 2004 (Barcelona, Spain)

The short form 36 and health-related quality of life following uterine artery embolisation – 5 year review
Cardiovascular & Interventional Radiology Society of Europe (CIRSE)
Sept. 2004 (Barcelona, Spain)

Effect of uterine artery embolisation on menstrual blood loss and uterine volume – 48 month review
30th British Congress of Obstetrics &Gynaecology (BCOG)
July 2004 (SECC – Glasgow)

The short form 36 and health-related quality of life following uterine artery embolisation
29th Annual Scientific Meeting for the Society of Interventional Radiology (SIR)
March 2004 (Phoenix, Arizona)

Health-related quality of life following uterine artery embolisation
British Society of Interventional Radiologists (BSIR)
November 2003 (Bournemouth)

The effect of uterine artery embolisation on menstrual blood loss and uterine volume
Joint Blair Bell/Munro Kerr Society Research Meeting
June 2003 (Glasgow)

The effect of uterine artery embolisation on menstrual blood loss - 36 month review
28th Annual Scientific Meeting for the Society of Interventional Radiology (SIR)
March 2003 (Salt Lake City, Utah)

The effect of uterine artery embolisation on menstrual blood loss and uterine volume
Scottish SpROGs Conference
March 2003 (Dalmahoy, Edinburgh)

The effect of uterine artery embolisation on menstrual blood loss – 18 month review.
Glasgow Obstetrical & GynaecologySociety – Trainees Night
January 2002 (RCOG - Glasgow)

Poster Presentation

Visualisation and analysis of uterine vascular networks ex vivo in healthy and fibroid tissue
Simpson Symposium
July 2005 (Edinburgh)
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