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Uterine Fibroids

Response to novel treatment modalities

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I declare that work contained herein is my own and that work of others is appropriately acknowledged.

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Abstract

Introduction

Magnetic resonance-guided focused ultrasound (MRgFUS) is a non-invasive, thermal ablation treatment for uterine fibroids. There is currently limited data regarding the long-term efficacy of this method and the effect of this treatment on circulating cytokine and growth factors.

In this thesis I have:

1. Presented the most accurate method of measuring fibroid volumes, and propose a new classification system for describing fibroid uteri.
2. Reviewed the characteristics of a cohort of uterine fibroid subjects and perform a longitudinal analysis of MRgFUS results.
3. Assessed the fibroid volume treated, pain scores and cytokine levels and growth factor levels following MRgFUS and UAE.

Results

The Parallel Plannimetric method is an accurate and reliable method of measuring uterine, fibroid and non-perfused volumes. Classifying fibroid uteri by numbers of fibroids and the presence of dominant fibroids is useful for distinguishing between those cases to be treated by MRgFUS or UAE. Since the introduction of MRgFUS to our unit the percentage non-perfused volumes (NPV) achieved have increased from 41.22 to 50.49 ($p=0.038$), however the re-intervention rate at 5 years remains high at 50%. MRgFUS has an excellent safety record, and the introduction of the new ExAblate 2100 system also appears to be safe and well tolerated, with encouraging initial NPVs achieved. Following both MRgFUS and UAE circulating interleukin-6 (IL-6) is significantly raised, although this is not affected by the degree of pain experienced or the volume of fibroid treated. Following UAE there is rise in circulating vascular endothelial growth factor (VEGF) seen at one week, however no significant change in VEGF levels is seen following MRgFUS. These changes in VEGF are not related to fibroid volume.

Discussion

MRgFUS is a safe, well tolerated treatment for uterine fibroids, although re-intervention rate is high. Further developments in this treatment modality may continue to improve outcomes, however at present its routine use cannot be recommended.

Contents

Abbreviations

2D	Two Dimensional
3D	Three Dimensional
BMI	Body mass index
cm	Centimetre
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
ER β	Oestrogen receptor β
ERα	Oestrogen receptor α
ESGE	European Society for Gynaecological Endoscopy
FDA	Food and Drug Administration
G-6-PD	Glucose-6-phosphate dehydrogenase
GnRHa	Gonadotropin releasing hormone agonists
GP	General Practitioner
HiFU	High intensity Focused Ultrasound
IL-1	Interleukin-1
IL-6	Interleukin-6
IM	Intramural
IUS	Intrauterine system
IVF	in-vitro fertilization
LAVH	Laparoscopic assisted vaginal hysterectomy
MDT	Multidisciplinary team meeting
mm	Millimetre
mL	Millilitre
MRgFUS	Magnetic resonance guided focused ultrasound
MRI	Magnetic resonance imaging
mRNA	Messenger Ribonucleic acid
MiRNA	Micro-Ribonucleic acid
NMR	Nuclear magnetic resonance
NPV	Non-perfused volume
NSAIDS	Non-steroidal anti-inflammatory drugs
PAI	Plasminogen activator inhibitor

PCA	Patient controlled analgesia
PDGF	Platelet derived growth factor
PPH	Postpartum haemorrhage
PVA	Polyvinyl alcohol
RCT	Randomised control trial
SD	Standard deviation
SERM	Selective oestrogen-receptor modulator
SM	Sub-mucous
SPRMs	Selective progesterone receptor modulators
SS	Sub-serosal
TCRF	Trans-Cervical Resection of Fibroid
TCRF	Trans-cervical resection of fibroid
UF	Uterine fibroids
UAE	Uterine Fibroid Embolism
UFS -QOL	Uterine Fibroid Symptom and health-related Quality of Life questionnaire score
US	Ultrasound
VEGF	Vascular endothelial growth factor
WMD	Weighted mean difference
MR	Magnetic resonance
CI	Confidence intervals
SD	Standard deviation
UAE	Uterine artery embolization
Rpm	Revolutions per minute
CRP	C-Reactive protein
TNF-α	Tumour necrosis factor-α
ECM	Extracellular matrix

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1. Introduction and literature review

Leiomyomas of the uterus, myomas, or uterine fibroids are the most common benign tumour found in women. Epidemiological studies indicate that up to 74 percent (%) of pre-menopausal women will have evidence of uterine fibroids on histological examination (Cramer and Patel, 1990). The prevalence of fibroids increases with age up until the menopause (Lurie et al., 2005), and due in part to an increase in the average age of conception (McDonald et al., 2011), fibroids are increasingly resulting in fertility problems. Despite the high prevalence of uterine fibroids, funding into research of these tumours has been relatively poor compared with other non-malignant disorders, possibly due to the large proportion of asymptomatic women, and due to the resulting morbidity rather than mortality associated with this condition (Walker and Stewart, 2005). While some women with uterine fibroids may be asymptomatic, many will experience significant effects on their fertility and quality of life. These wide variety of symptoms can range from menstrual (menorrhagia, dysmenorrhoea), to pressure effects (bladder and bowel symptoms) and effects on every stage of the reproductive process, from conception to post-partum haemorrhage. In addition to the clinical burden these symptoms will have on quality of life, the economic burden of uterine fibroids is substantial, with up to 50% of women requiring some form of treatment for their symptoms (Mauskopf et al., 2005). One in five gynaecology outpatient attendances will be due to fibroid-related symptoms and the indirect costs of managing uterine fibroids are considerable (Lee et al., 2007). This introduction will review the impact of uterine fibroids on women and describe how the management of these tumours has developed in line with the changing needs and expectations of women in the 21st century.

Prevalence

The reported prevalence of uterine fibroids varies dramatically in the literature. As some women with fibroids may not have symptoms related to their tumours, many remain undiagnosed. It has been reported that around 50% of women with uterine fibroids will be asymptomatic, however some suggest that this may be an underestimate, possibly as a result of the under-investigation of women with heavy menstrual loss (Divakar, 2008). Uterine fibroids smaller than one centimetre (cm) in diameter may be difficult to detect by radiological imaging (Griffin et al., 2010), and the gold standard for diagnosis remains histological examination. In a fine-slice histological analysis of 100 hysterectomy specimens, 77% of premenopausal women were found to have some fibroid tissue, in some cases as small as two millimetres (mm) in diameter (Cramer and Patel, 1990). This study found an average of 7.6 uterine fibroids in pre-menopausal women undergoing hysterectomy and 4.2 uterine fibroids in post-menopausal women undergoing hysterectomy (Cramer and Patel, 1990). An obvious bias of such a study is that women with fibroids are more likely to have symptoms requiring treatment by hysterectomy. Previous studies suggest between 20-40% of women over 30 years of age (Day Baird et al., 2003) have uterine fibroids. However more recent cross-sectional studies

suggest that the percentage is much higher. A cross-sectional study examining 1364 randomly selected pre-menopausal women found evidence of uterine fibroids on ultrasound imaging in 80% of black women by 50 years of age, and 70% of white women by this same age (Day Baird et al., 2003). These cross-sectional studies contain less selection bias compared with earlier studies and appear to give a more accurate reflection of the true prevalence of fibroids in the general population. The prevalence of uterine fibroids increases up to the menopause, after which the development of new uterine fibroids is rare (Marshall et al., 1997).

Subgroups of uterine fibroids and classification

Uterine fibroids are commonly described by their position in relation to the surrounding myometrium. Uterine fibroids are usually described by the subgroups; intramural (IM), sub-mucous (SM), sub-serosal (SS) and cervical (Figure 1). Many women will have fibroids in a number of different positions.

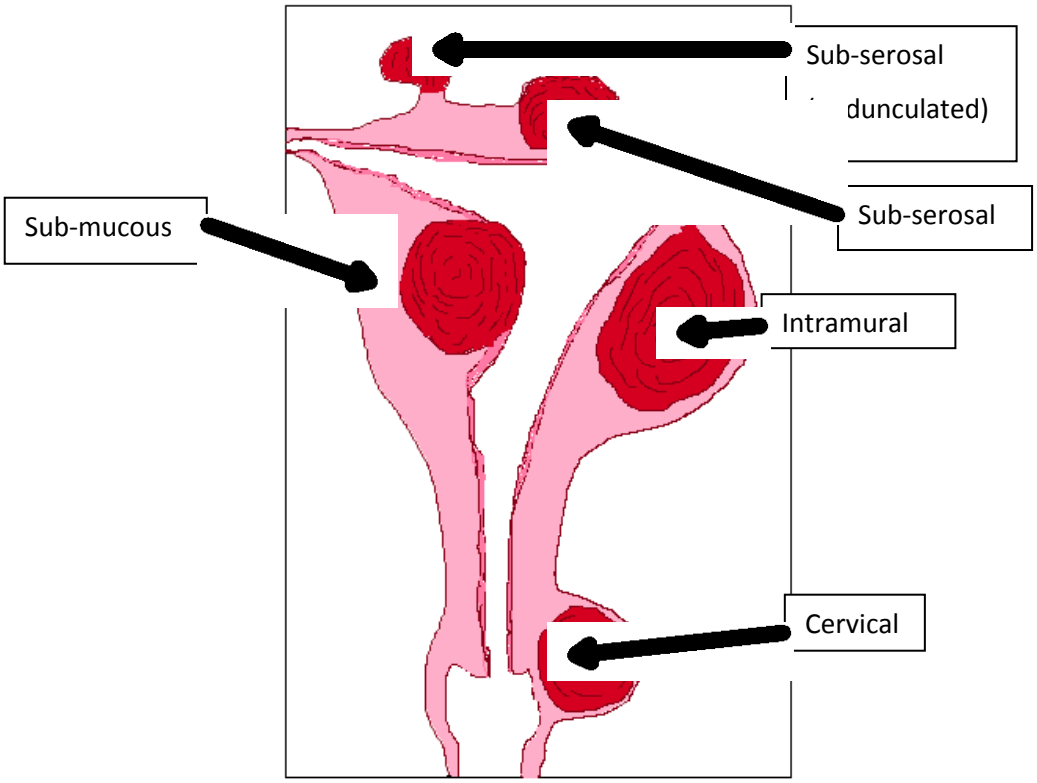


Figure 1: Subgroups of fibroids

Sub-mucous (SM) fibroids

Sub-mucous fibroids account for around 5% of all fibroids (Shaw et al., 2003), although this may be an underestimate due to inadequate imaging. These are defined as fibroids that project in to the uterine cavity and have an endometrial covering. This group includes pedunculated SM fibroids which project in to the uterine cavity on a stalk, which can on occasion prolapse through the cervix.

An additional classification system was proposed by Wamsteker for describing SM fibroids (Wamsteker et al., 1993) which was subsequently adopted by the European Society for Gynaecological Endoscopy (ESGE). This classification describes the fibroid in relation to the intramural component. Pedunculated fibroids with no intramural extension are referred to as type 0 fibroids. SM fibroids with no peduncle or stalk, and an intramural component of less than 50% are referred to as type I. An intramural component of greater than 50% is referred to as type II (see figure 2). Although often smaller in size than SS or IM fibroids, SM fibroids can be the most troublesome in terms of symptoms and fertility effects. Effects on menstrual loss and

fertility may be mediated by distortion of the endometrial cavity (Stovall, 2001). SM fibroids with more than 50% of the fibroid tissue projects into the endometrial cavity may be suitable for trans-cervical resection of fibroid (TCRF). Pedunculated fibroids may be particularly problematic due to their possible prolapse through the cervix and resultant ulceration and infection.

	Features
Type 0	Pedunculated Intra-cavity
Type I	<50% Intramural
Type II	≥50% Intramural

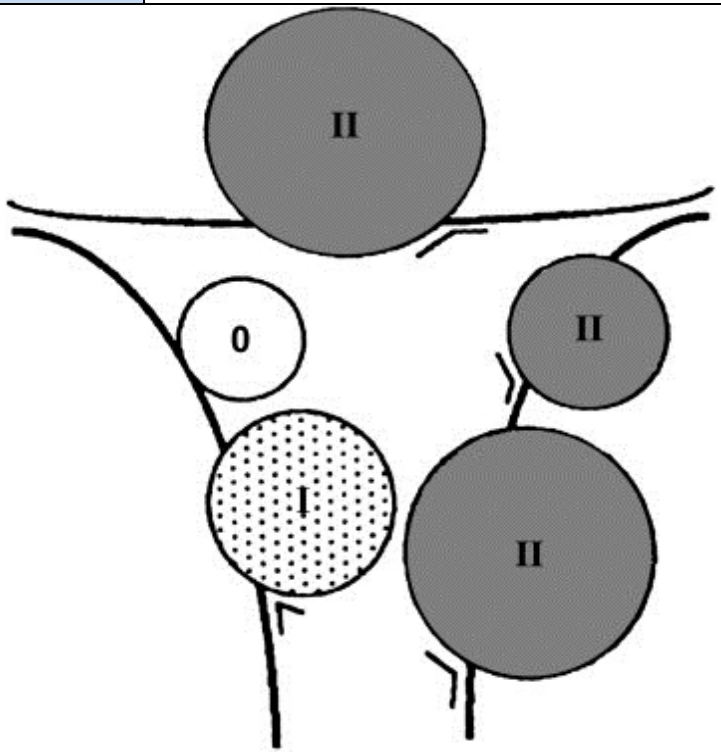


Figure 2: Classification of the three types of sub-mucous fibroid as first described by the European Society of Hysteroscopy.

Following the ESGE classification of SM fibroids, the International Federation of Gynaecology and Obstetrics (FIGO) developed an additional classification system for the causes of abnormal uterine bleeding which included a continuation of this system, including intramural and sub-serosal uterine fibroids (Munro et al., 2011) (Figure 3).

0	Pedunculated Intracavitary
1	<50% Intramural
2	≥50% Intramural
3	Contacts endometrium; 100% Intramural
4	Intramural (not contacting endometrium)
5	Subserosal ≥50% Intramural
6	Subserosal <50% Intramural
7	Subserosal Pedunculated
8	Other (specify e.g cervical, parasitic)

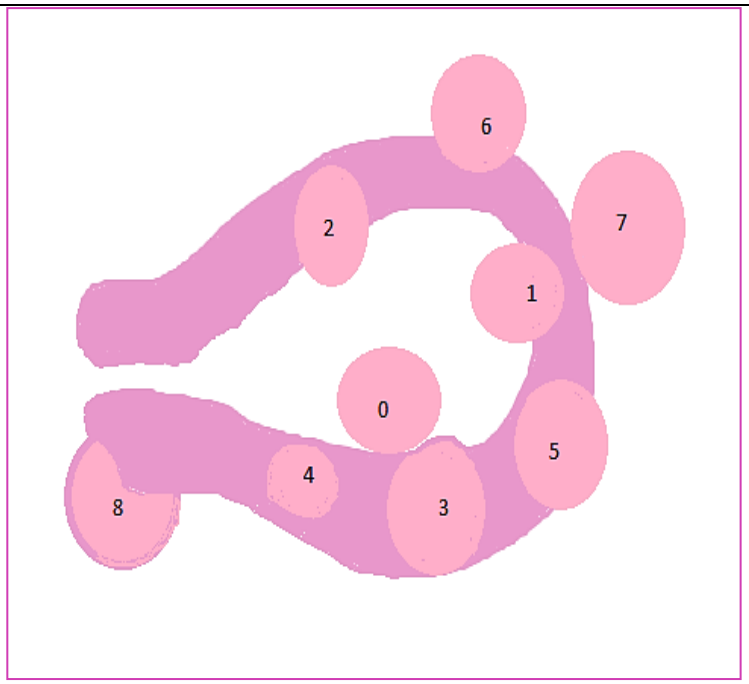


Figure 3: FIGO classification of uterine fibroids

Intramural (IM) fibroids

IM fibroids lie within the wall of the uterus, separated from the surrounding myometrium by a layer of connective tissue known as the pseudo-capsule, or false capsule (Shaw et al., 2003). These are the most commonly encountered subgroup of uterine fibroids, and can be the most difficult to treat surgically due to their position deep within the uterine tissue. Depending on the size of IM uterine fibroids they may lead to distortion of the endometrial cavity, leading to worsening menstrual symptoms and possible effects on fertility, in common with SM uterine fibroids (Oliveira et al., 2004).

Sub-serosal (SS) Fibroids

These fibroids lie just below the serosal surface of the uterus, but superior to the myometrial wall of the uterus and project out in to the abdominal cavity. This group of uterine fibroids may have a narrowed connection with the uterine body (or "stalk"), which can undergo torsion, a rare but serious complication (Gupta and Manyonda, 2009). Sub-serosal fibroids with a very narrow stalk may detach and lead to complications. The detachment of a SS uterine fibroid leads to the phenomenon known as parasitic myomas (Cucinella et al., 2011). This detachment can be as a result of torsion of the fibroid pedicle and development of an alternative blood supply from another source, such as the omental or mesenteric vessels. A proposed alternative mechanism is that these lesions may develop from metaplasia of the peritoneum (Al-Talib and Tulandi, 2010). Reports of parasitic fibroid following laparoscopic myomectomy using a morcellator have been described (Epstein et al., 2009, Moon et al., 2008).

Broad ligament fibroids arise from the smooth muscle fibres within the broad-ligament. These may be confused with lateral wall SS fibroids, and may displace the ureters, or sigmoid mesocolon (Shaw et al., 2003).

Cervical fibroids

These fibroids arise from the wall of the cervix and are relatively uncommon. They present a greater challenge to the surgeon due to their relative inaccessibility and close proximity to the bladder and the ureters. Enlargement of these fibroids can lead to upward displacement of the uterus, and impaction of the fibroid, with associated urinary retention or ureteric obstruction (Shaw et al., 2011). The presence of cervical fibroids is thought to reduce fertility by blockage of the cervical canal, and where pregnancies do occur, complications include haemorrhage, obstruction of the birth canal during delivery, infection, pain and urinary stasis (Straub et al., 2010).

Vaginal fibroids

Vaginal fibroids occur rarely, with only around 300 described in the literature (Chakrabarti et al., 2011). Vaginal fibroids have almost always been described in white women (as opposed to uterine fibroids which appear more commonly in black women) (Sangwan et al., 1996). Treatment has been described as careful surgical excision by the vaginal route, with particular care to remove the entire fibroid mass, due to concerns regarding the risk of recurrence (Gowri et al., 2003).

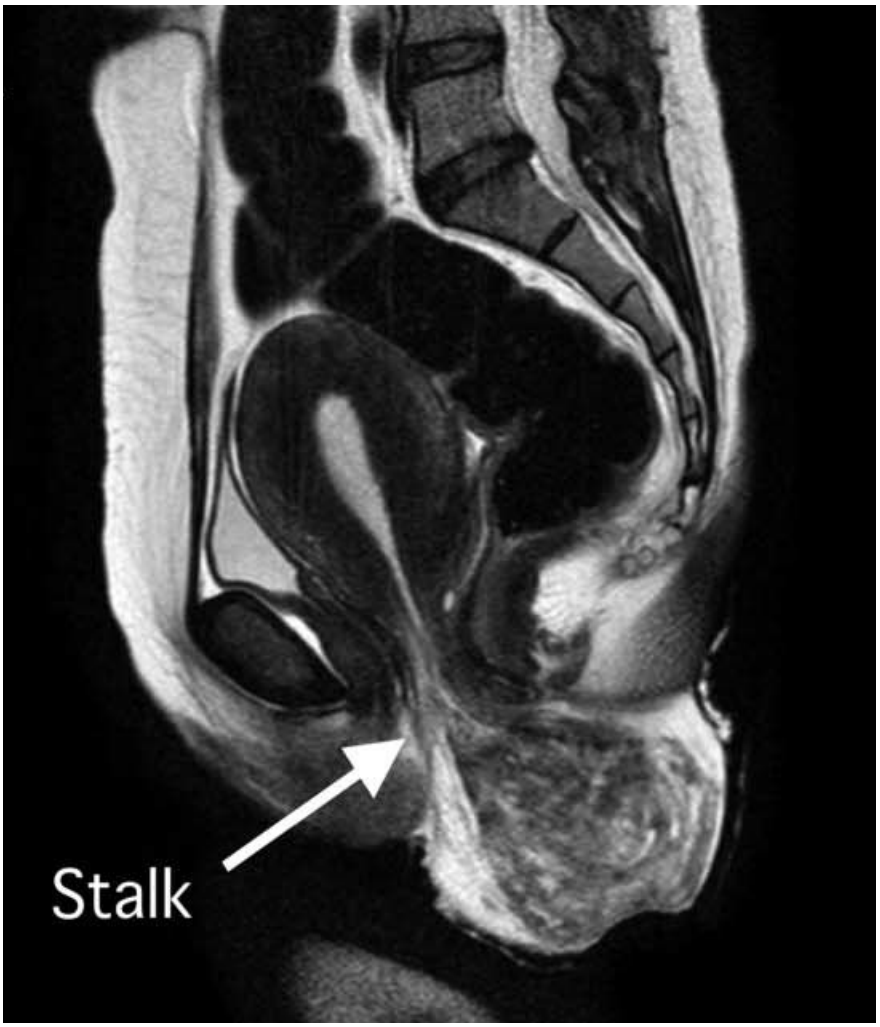


Figure 4: MRI appearances of a vaginal fibroid (Imai et al., 2008)

Fibroid symptoms

The symptoms experienced by women with uterine fibroids vary significantly. Although fibroids themselves are very common, only around half of women with uterine fibroids will experience significant symptoms requiring medical intervention (Novak et al., 2002). The symptoms experienced are highly dependent on the location of the fibroids. Sub-mucosal uterine fibroids are more likely to be symptomatic than sub-serosal fibroids, especially in relation to menstrual disorders, due to the effect on the endometrium. A pedunculated sub-serosal fibroid is likely to remain asymptomatic unless it undergoes torsion, or becomes large enough to cause pressure effects on the surrounding organs, such as the bladder or bowel (Divakar, 2008). It is unclear

at what size uterine fibroids start to cause symptoms. Some women may have fibroid uteri palpable above the umbilicus found as an incidental finding, often after presenting with a non-related medical problem (Divakar, 2008).

There are a variety of tools for measuring symptom severity. Menstrual diaries and urinary symptom questionnaires can help quantify how these symptoms are affecting the quality of life of the women concerned, as well as providing a measurable, reproducible method of assessing response to treatment. A uterine fibroid-specific quality of life questionnaire has been introduced (Spies et al., 2002a) that allows measurement of different aspects of quality of life, including symptom severity, concern, sexual function, activities, energy/mood, experiences of control and self-consciousness (see Appendix A).

Menstrual symptoms

Fibroids distorting the uterine cavity may lead to symptoms of menorrhagia, with associated flooding and passage of blood clots during menstruation. Uterine fibroids are also associated with the phenomenon of metrorrhagia, or prolonged vaginal bleeding. Both of these bleeding patterns may result in anaemia. Clinically significant pain experienced during the menstrual period that interferes with activities of daily living (dysmenorrhoea) is a common finding, and often associated with menorrhagia. The mechanisms by which fibroids cause increased menstrual blood loss are not fully understood, however a number of theories have been developed (Stovall, 2001, Lockwood, 2011). One explanation is the effect of uterine fibroids on the surface area of the endometrium. One study found that compared with asymptomatic women, premenopausal women with abnormal uterine bleeding had a higher prevalence of sub-mucous fibroids (21 versus 1%), and intramural fibroids (58 versus 13%) (Murase et al., 1999). Sub-mucous fibroids may be associated with ulceration of the overlying endometrium (Murase et al., 1999). Uterine fibroids may also cause increased blood loss and pain as a result of interference of the intramural fibroids on myometrial contractility. One theory suggested as early as 1912 (Stewart and Nowak, 1996) suggests that local dysregulation of the vasculature of the uterus is responsible for this bleeding. This theory has been updated to incorporate evidence of abnormal levels of angiogenic growth factors in the fibroids, including basic fibroblast growth factor, heparin-binding epidermal growth factor (HBEGF), vascular endothelial growth Factor (VEGF), transforming growth factor-beta, platelet-derived growth factor, parathyroid hormone related protein and prolactin (Stewart and Nowak, 1996). The over-production of these factors results in dilatation of blood vessels within the endometrium and potential increase in blood loss. Although fibroid growth is effected by steroid hormones, fibroids do not appear to have any effect on the function and sex steroid production of ovaries, and therefore do not cause abnormal uterine bleeding through any effect on ovarian function (Stovall, 2001).

Pressure symptoms

Many women will present to their clinician with symptoms related to the size and location of their uterine fibroids, rather than menstrual problems. In cases of very large fibroids, abdominal distension associated with “looking pregnant” can be very distressing, especially for women with associated fertility problems. Women may complain of low abdominal discomfort, find it difficult to fit into clothes, and may experience discomfort or pain during intercourse (dyspareunia). The mass-effect of uterine fibroids may result in bladder and bowel symptoms. The bladder symptoms described by women with uterine fibroids vary from increased urinary frequency and urgency to urinary stress incontinence. The size of the fibroid uterus may affect the volume of urine at which the woman first notices the desire to pass urine and the maximum capacity of the bladder may be reduced, resulting in increased urinary urgency. Uterine fibroids may also result in changes in the urethrovesical junction, leading to incomplete emptying of the bladder or difficulty in initiating voiding (Stovall, 2001). When very large the fibroid uterus may obstruct one or both of the ureters, which may affect renal function. Large posterior wall fibroids may press on the rectosigmoid colon leading to constipation and bloating (Bukulmez and Doody, 2006). It is important that women presenting with uterine fibroids and gastrointestinal symptoms be fully investigated to exclude other bowel pathology as evidence for improvement of bowel symptoms following removal of fibroids is scant (Gupta et al., 2008).

Pain

Women with fibroids may experience pelvic pain or discomfort, although they are only slightly more likely to experience pain overall than women without fibroids (Parker, 2007). A cross-sectional study of non-treatment seeking women found that women with uterine fibroids were more likely to experience dyspareunia and pain not related to their periods (OR 2.8, 95% confidence interval [CI] = 0.9-8.3), which the authors describe as significant despite the 95% CI containing 1 (Lippman et al., 2003). This study also found that moderate to severe dysmenorrhea was not higher in those women with fibroids (adjusted OR = 1.1, 95% CI = 0.5-2.6) and that the number and total volume of the uterine fibroids was not related to pain scores described.

Very large uterine fibroids may outgrow their blood supply with resulting ischemia and necrosis (Stovall, 2001). There is often associated systemic symptoms such as low grade fever and leucocytosis (Phelan, 1995).

Acute pain may also occur as a result of torsion of a pedunculated sub-serosal fibroid, or the prolapse of a pedunculated sub-mucosal fibroid through the cervical canal. Chronic pain is usually less severe and related to the mass effects of the fibroids. Posterior fibroids may cause lower back pain, and lateral wall or broad ligament fibroids may cause unilateral back pain or sciatic nerve pain (Stovall, 2001). During pregnancy uterine fibroids may undergo "red-degeneration" resulting in severe pain, most commonly in fibroids greater than 5cm in diameter, during the second and third trimesters (Lee et al., 2010). There are a number of theories to explain this; one is that oestrogen-related rapid growth of the fibroid during pregnancy results in the fibroid tissue outgrowing it's blood supply, with resulting tissue ischemia and necrosis (Lee et al., 2010). Alternatively, the growth of the surrounding myometrium during pregnancy may result in a disruption or kinking of the vessels supplying the uterine fibroids, again leading to ischemia (Parker, 2007). Pain is not a common presenting symptom of uterine fibroids, and other causes should be excluded prior to attributing pain solely to fibroids (Gupta et al., 2008).

Sub-Fertility

Uterine fibroids can complicate all aspects of fertility from problems with conception through to post-partum complications. Uterine fibroids are thought to occur more frequently in women with a history of infertility (Khaund and Lumsden, 2008), however the incidence of fibroids in older women undergoing treatment for infertility is reported as 12-25%, lower than many other cross-sectional studies reported above (Klatsky et al., 2008, Lurie et al., 2005). Once other causes for infertility have been excluded, uterine fibroids are thought to account for only 2-3% of infertility cases (Khaund and Lumsden, 2008). These figures are based on a case series from 1981 (Buttram and Reiter, 1981) involving women undergoing investigation for infertility and is therefore subject to selection bias, and further investigation is on-going. The precise mechanism by which fibroids cause infertility is not fully understood, however as with menorrhagia a number of theories have been suggested. Changes to the structure of the endometrium caused by the presence of underlying uterine fibroids may affect implantation of the embryo, including ulceration of the endometrium, endometrial atrophy, and distortion of the endometrial glandular tissue (Richards et al., 1998). Fibroids appear to have an effect on the overall receptivity of the endometrium for implantation (Cakmak and Taylor, 2011). During the implantation period there is an overall decrease in endometrial expression of HOXA10, HOXA11 (proteins involved in implantation) and BTEB1 (Basic transcription element binding protein 1- a target transcription factor of HOXA10 and HOXA11) in uteri with sub-mucosal fibroids when compared with normal controls (Matsuzaki et al., 2009, Rackow and Taylor, 2010).

The position of the fibroids within the myometrium may affect uterine contractility, thought to contribute to sperm, ovum and embryonic transport. Uterine fibroids distorting the endometrial cavity in particular seem

to be linked with infertility, as they are likely to result in impaired implantation. Studies investigating the role of trans-cervical resection of fibroid tissue (TCRF) distorting the endometrial cavity suggest that pregnancy rates improve following TCRF (Bernard et al., 2000, Ubaldi et al., 1995). A systematic review of randomised controlled studies found an increased pregnancy rate following removal of sub-mucosal fibroids hysteroscopically, however the authors were critical of the available evidence, suggesting more randomised control trial (RCT) evidence was required (Bosteels et al., 2010). The RCT that was examined by this review lacked information about whether the treatment groups were comparable, contained no blinding or allocation concealment, and used ill-defined terms ("fibroid knot") (Casini et al., 2006).

It is possible that uterine fibroids may disrupt the blood supply to the endometrium thereby affecting the process of implantation. Uterine fibroids may also result in a hostile environment for sperm within the endometrium, as a result of local inflammation secondary to their presence (Oliveira et al., 2004).

There have been a number of studies investigating the effects of intramural and sub-serosal fibroids on in-vitro fertilization (IVF) outcomes. These IVF studies are useful as they are able to exclude other infertility factors such as abnormal sperm function, abnormal anatomy and ovulatory disorders (Rackow and Arici, 2005). A small prospective study of 61 women with small intramural fibroids (<5cm) not affecting the endometrial cavity was compared with 61 matched controls. They found women with small intramural fibroids tended towards a lower implantation and pregnancy rates, lower live birth rates and higher miscarriage rates (Check et al., 2002), however none of these results reached statistical significance. In addition this study failed to adequately describe the study groups in terms of other potentially confounding features that might have affected fertility outcomes such as BMI, smoking, co-existing medical pathologies and years of infertility.

A case-control study of 112 women with intramural fibroids and 322 controls found that intramural fibroids appear to half the successful IVF pregnancy rates (Hart et al., 2001). This study was again limited by the authors poorly describing the demographics of the study groups, and the control group having a higher percentage of male-factor infertility compared with the fibroid group. Although the initial comparison found a significant reduction in positive pregnancy tests (OR 0.51 95% CI 0.30–0.90) for women with intramural fibroids less than 5cm, once these figures were adjusted for age and embryos available the odds ratio (OR) was no longer significant (OR 0.58 95% CI 0.32–1.04).

A retrospective matched-control study of 490 women found that women with intramural fibroids less than 4cm in diameter, not encroaching on the uterine cavity had comparable outcomes from in-vitro fertilisation-intracytoplasmic sperm injection (IVF-ICSI) with those women with no fibroids (Oliveira et al., 2004). The authors suggested that women with uterine fibroids greater than 4cm should have treatment for their fibroids prior to commencing IVF-ICSI cycles. This study however fails to pay attention to potential

confounders, with no appropriate comparison of the demographics between groups. Although the authors found no significant difference between the women with intramural fibroids less than 4cm and the control group, no difference in the live birth rates for any of these groups were reported.

A systematic literature review and meta-analysis of existing controlled studies concluded that sub-mucosal fibroids reduce the success of IVF and that removal improved outcomes; intramural fibroids do appear to decrease fertility, however their removal does not significantly improve pregnancy rates; whereas sub-serosal fibroids do not affect fertility outcomes, and removal has no benefit (Pritts et al., 2009). A further systematic review and meta-analysis of 19 observational studies examined the association between non-cavity distorting intramural fibroids and IVF outcomes (Sunkara et al., 2010). When compared with women without fibroids, women with intramural fibroids not distorting the uterine cavity were found to have a significant decrease in their live birth rate (RR=0.79, 95%CI: 0.70-0.88, p<0.0001) and clinical pregnancy rates (RR=0.85, 95%CI: 0.77-0.94, p=0.002)

The relationship between uterine fibroids and IVF outcomes remains controversial mainly due to the poor quality of the studies that have been undertaken. However, current opinion favours the view that sub-mucosal and intramural fibroids which distort the endometrial cavity do negatively affect assisted reproductive outcomes (Khaund and Lumsden, 2008) and should therefore be removed. Further good quality research into this area is required.

Pregnancy Complications

During pregnancy the rise in circulating oestrogen and progesterone is thought to be a cause for potentially rapid growth of uterine fibroids (Stovall, 2001). However, ultrasound studies of uterine fibroid growth in pregnancy indicate that only 20-30% of fibroids increase in size during pregnancy, and that those that do increase in size do so by no more than 25% of their original volume (Aharoni et al., 1988). Those fibroids that do increase in size may be at risk of undergoing red-degeneration as described earlier. The resultant pain from red-degeneration during pregnancy can be severe and require in-patient opioid analgesia.

There has been one case-series of 13 women undergoing myomectomy for severe pain before 26 weeks gestation (Exacoustòs and Rosati, 1993). The authors found that 8 of these 13 pregnancies delivered at term, and five delivered preterm, but after the 32nd week of their pregnancy, and there were no neonatal deaths.

However myomectomy during pregnancy is generally not current practice due to concerns regarding blood loss and risks to the fetus.

Spontaneous miscarriage rates in both the first and second trimester of pregnancy do appear to be higher in women with intramural and sub-mucosal fibroids (Kroon et al., 2011). However many of the studies suggesting increased miscarriage rates with intramural fibroids have been criticized for not adequately assessing the effect of these fibroids on the shape of the endometrial cavity (Kroon et al., 2011). It therefore remains unclear whether intramural fibroids that do not distort the uterine cavity result in higher miscarriage rates.

One mechanism for the effects of fibroids that has been suggested is that abnormal uterine contractility results in pregnancy loss (Khaund and Lumsden, 2008). Muscles cells of fibroid uteri have been found to be structurally abnormal compared with those of normal uteri. Myometrial cells in fibroid uteri contain higher intracellular calcium, and this increased intracellular calcium concentration may result in myometrial irritability and hyperactivity (Richards et al., 1998). Further growth and degeneration of fibroids during early pregnancy has also been suggested as cause of spontaneous miscarriage (Khaund and Lumsden, 2008). It could be that the initial growth of the fibroid in early pregnancy may alter blood flow within the uterus and have an effect on placental development. Additionally, degeneration of a fibroid is likely to result in the release of cytokines and other inflammatory mediators with the potential to affect the placental bed.

One case report suggested that fibroids may result in fetal growth restriction (Aziz et al., 2005). It was suggested that the uterine fibroids could contribute to abnormal placental development, although due to the high prevalence of fibroids in pregnant women we would expect this effect to be more frequently observed, therefore this mechanism seems unlikely. There have been no other studies to support this suggestion.

Large uterine fibroids may affect the position of the term fetus and increase the incidence of abnormal lie, and a literature review found an odds ratio (OR) of 2.9 for mal-presentation at term for women with uterine fibroids, an increased risk of caesarean section (OR 3.7), and pre-term delivery (1.5) (Klatsky et al., 2008). The methods of this literature review where unfortunately unclear from the paper, and it is unclear whether this study was a systematic review of all the literature on this topic, and no test for heterogeneity of these studies could be identified in this paper.

Previous surgery for fibroids increases the likelihood of caesarean section (as do fibroids in general), especially if myomectomy was from the upper segment of the uterus. Any procedure that interferes with the ability to form decidua may result in abnormal placentation, and this may occur over the site of a myomectomy scar with resultant placenta, increta, or percreta (O'Brien et al., 1996).

Following vaginal delivery, women with fibroids are at an increased risk of postpartum haemorrhage and retained placenta (Olive and Pritts, 2010).This is thought to be caused by the fibroids affecting the

contraction of myometrial muscle fibres following delivery, with a resultant increase in the likelihood of uterine atony.

Psychological aspects of a diagnosis of uterine fibroids

Although the majority of women may be reassured that their uterine fibroids are benign tumours, the distress caused by the diagnosis should not be underestimated. Women may be very anxious about their fibroids, in particular the possibility of malignancy, need for surgical intervention, effects on fertility, and potential growth of the fibroids (Divakar, 2008). Once the diagnosis has been made by imaging and clinical assessment, careful counselling of the woman, including a clear explanation of what fibroids are, their benign nature, and the treatment options available, including conservative options, is essential. Following initial diagnosis, 50% of American women sought a second opinion and the most common question asked was regarding the need for hysterectomy (Evans and Brunsell, 2007).

Questionnaire surveys of 200 women attending a central London gynaecology outpatient clinic reported that 35% of women scored in the borderline or clinical range for depression and 61% in the borderline or clinical range for anxiety, and those with fibroids were among the most distressed (Glover et al., 2002). A qualitative study of the experiences of women with uterine fibroids identified a number of themes, with two overall (high order) themes of “managing uncertainty” and “struggling between defeat and optimism” (Nicholls et al., 2004). With regards to “managing uncertainty” most of the women in the study expressed concerns regarding the effects of fibroids, and most had little prior knowledge about uterine fibroids. As women often have no frame of reference for what to expect following diagnosis, they are prone to uncertainty and anxiety. This is where patient information and education is essential in increasing awareness and education and reducing anxiety about fibroids and the possible treatments necessary. “Struggling between defeat and optimism” refers to women’s thoughts about their fibroids, concerns about not being able to lead their lives in the ways that they had hoped (with regards to fertility and lifestyle), the effects on the woman and the perceptions of others, and finally the idea of gaining control of the uterine fibroids. The impact of a women’s social background should also be taken into account, as different cultural groups will experience difficulty and shame in discussing their diagnosis within their community (Nicholls et al., 2004). In particular, women from African communities may find difficulty discussing their condition within their peer-group, and may be particularly worried about the implications of possibly requiring a hysterectomy, or undergoing a treatment that may carry the potential risk of a hysterectomy (Augustus, 2002). These factors may result in a delay in seeking treatment for some women.

Fibroid Aetiology

The mechanisms by which uterine fibroids arise and develop remains only partially understood, however there are a number of identifiable risk factors. These include high body mass index (BMI), race and a positive family history (Flake et al., 2003). A summary of the identified factors associated with uterine fibroids is given in Figure 5.

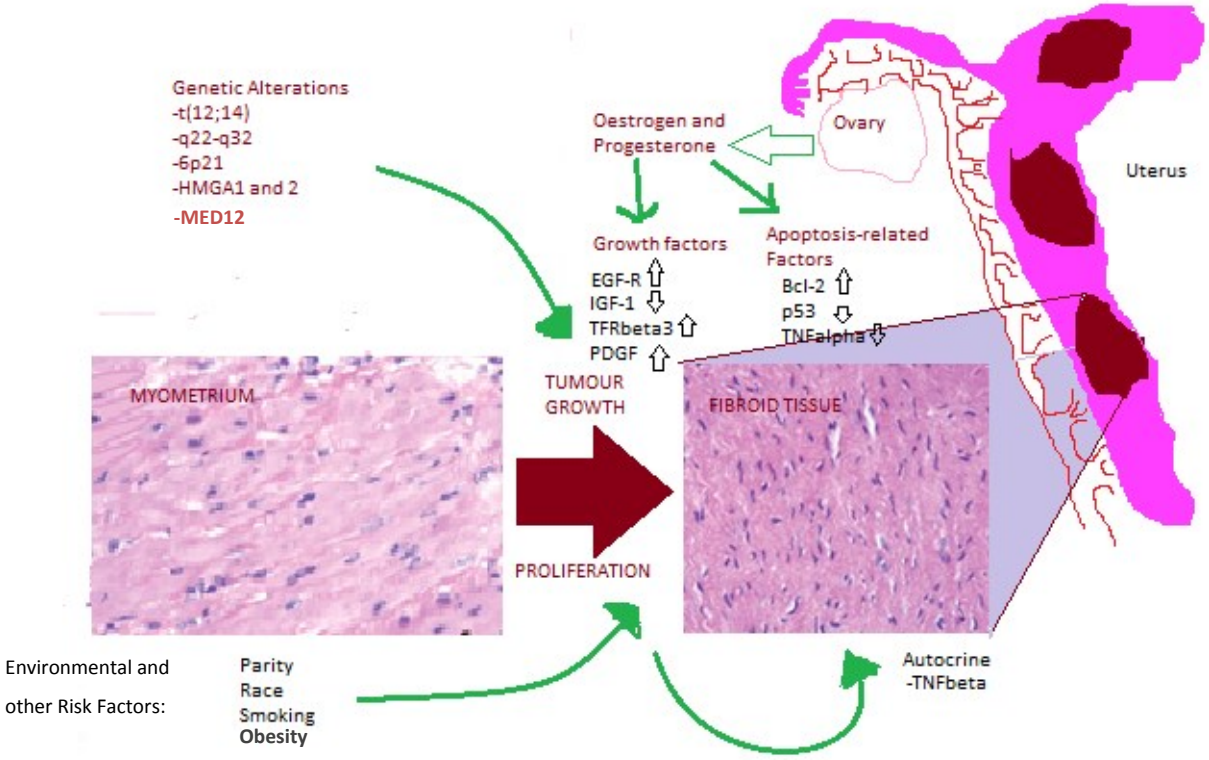


Figure 5: Factors affecting Fibroid Growth ***(Adapted from Uterine fibroids: The Elephant in the Room, Cheryl Lyn Walker and Elizabeth A. Stewart. Science 308, 1589 (2005)***

Obesity

A clear link between increased body mass index (BMI) and fibroid growth has been found. A prospective study found an approximately 21% increase of fibroid incidence for every 10kg-increase in body weight (Ross et al., 1986). A more recent study found a 7.56g increase in uterine weight for every 1 point increase in BMI (Dandolu et al., 2010). Increased adiposity can potentially increase peripheral oestradiol production, especially in those with android adiposity (Tankó et al., 2004). This has been noted in women whose fibroids are diagnosed post-menopausally, when the sole source of their circulating oestrogen is from adipose cells

(Tankó et al., 2004). The effects of post-menopausal oestrogen and progesterone hormone replacement therapy on fibroid growth only appear in those women with a low BMI, and hence lower circulating oestradiol levels (Reed et al., 2004). In additional to oestrogen production, obesity is also associated with a decrease in the hepatic production of sex-hormone binding globulin, leading to a larger amount of unbound physiologically active oestrogen (Flake et al., 2003).

Race

The prevalence of uterine fibroids is greater in black women than white women. Black women are more likely to have larger fibroids, a greater number of individual fibroids within the uterus, and are also more likely to experience anaemia and severe pelvic pain (Kjerulff et al., 1996). A report of 95,061 pre-menopausal nurses with no background history of uterine fibroids found an incidence of fibroids in black women to be three times higher than white women (Marshall et al., 1997). In diet-controlled studies black women had significantly higher circulating oestrogen, oestrone, and free oestradiol when compared with white women (Woods et al., 1996), a possible explanation for the increased incidence and overall size in this group. Polymorphisms in the oestrogen receptor alpha (ERα) genotype (PP genotype) are more commonly found in black women compared with white or Hispanic women (Al-Hendy and Salama, 2006). The ERα PP genotype is associated with a higher risk of uterine fibroids in black and white women, and this genotype is also associated with a greater volume of fibroid tissue than other fibroid genotypes. Higher aromatase mRNA levels have been found in black compared with white women (Ishikawa et al., 2009). As aromatase is an important enzyme for the production of oestrogen, it is possible that this may also contribute to the increased incidence and overall fibroid growth seen in black women.

Parity

Parity appears to be inversely related to the risk of developing uterine fibroids, with one study finding women with five term pregnancies having a quarter of the risk of nulliparous women (Ross et al., 1986). It has been suggested that this is related to a reduced duration of exposure to unopposed oestrogen in parous women (Parazzini et al., 1996).

Diet

The role of diet in the development of uterine fibroids has been investigated, however little conclusive evidence has been found. A moderate association with beef, other red meats and ham has been suggested,

while a high consumption of green vegetables may have a possible protective effect (Chiaffarino et al., 1999). A recent prospective study demonstrated a moderate protective effect of diary consumption in black women (Wise et al., 2010). There appears to be a reduced risk of fibroids among women with a greater dietary intake of fruit and preformed vitamin A (Wise et al., 2011). Foodstuffs containing phytoestrogens have also been implicated in fibroid growth. Phytoestrogens are diphenolic compounds that are converted to oestrogenic substances in the intestine (Ginsburg and Prelevic, 2000). These compounds were investigated following the observation of a significantly lower incidence of menopausal symptoms and diseases associated with low oestrogen levels (osteoporosis, cardiovascular disease, breast cancer) in countries with a high dietary consumption of phytoestrogen-containing foodstuffs such as soya. Phytoestrogens predominately act as weak oestrogens but may also produce anti-oestrogenic effects, depending on concentration (Ginsburg and Prelevic, 2000), and overall may act as a natural selective oestrogen-receptor modulators (SERM). Experimental animal models have demonstrated that genisten, a soy-derived phytoestrogen, has an inhibitory effect on peroxisome proliferator-activated receptor-gamma (PPARgamma), reducing proliferation of fibroid cells (Miyake et al., 2009).

Smoking and Alcohol

Smoking has been associated with lower serum oestrogen in some, but not all studies (Flake et al., 2003). The recent Black Women's Health Study found no positive correlation between smoking and the incidence of uterine fibroids (Wise et al., 2004), but it did find a positive associated with alcohol consumption, particularly beer. There is, however a higher consumption of beer as compared with wine and other forms of alcohol in black American women. As with smoking, there is conflicting evidence for the effect of alcohol on circulating oestrogen levels (Hankinson et al., 1995, Dorgan et al., 1994).

Hypertension

A prospective study of risk factors for women between 25-42 has demonstrated that hypertension has an independent, positive association for clinically detectable fibroids (Boynton-Jarrett et al., 2005). In a sample of 104,233, a 10mmHG rise in diastolic blood pressure was associated with an 8% rise in multivariate relative risk. The authors of this study have suggested a mechanism for fibroid development similar to that of atherosclerosis. Both uterine fibroids and atherosclerosis appear to have a monoclonal origin and both have can become fibrotic and calcified (Moss and Benditt, 1975). In atherosclerosis, hypertension results in increased haemodynamic stress and subsequent arterial smooth muscle injury and endothelial dysfunction,

increased permeability, migration of smooth muscle cells, and resultant plaque formation. Uterine smooth muscle cells may be injured by a similar mechanism, initiating fibroid formation. It has been suggested that hyper-insulinaemia, itself a risk factor for hypertension and atherosclerosis may increase the incidence of uterine fibroids due to increased oestrogen production (Poretsky and Kalin, 1987). However a recent study of circulating insulin concentrations and fibroid prevalence found an inverse relationship between the two in black women, and only a weak association amongst white women (Baird et al., 2009).

Mechanisms of fibroid growth

Studies have suggested a monoclonal origin for uterine fibroids (Townsend et al., 1970). What causes this initial cell proliferation and development is not known, however several theories have been suggested. Mayer suggested that fibroid cells originate from myoblasts from within the uterus (Mayer, 1930) and this theory is generally accepted (Fujii, April, 2004). One hypothesis is that cell injury related to menses may result in an inflammatory response (Islam et al., 2013). It is possible that the contraction of myometrial cells at the end of menstruation could lead to ischemia or an ischaemic-reperfusion injury to a uterine myometrial cell, leading got the transformation to a progenitor cell (Fujii, April, 2004). The cell proliferation-associated antigen Ki-67 is found on oestrogen and progesterone receptors more commonly in the luteal phase of the menstrual cycle compared with the follicular phase of the cycle (Kawaguchi et al., 1991). Each menstrual cycle, myometrial cells exhibit proliferative activity, in preparation for a potential pregnancy; however if this does not occur these cells are subject to a hypoxic state as a direct result of myometrial constriction. The majority of these hypoxic cells will be eliminated as apoptotic cells or cell cycle-arrested cells; however some cells may acquire a protective mechanism against oxidative stress and apoptosis. This repeated cycle of increased proliferation and hypoxia may result in the transformation of these cells to a progenitor fibroid cell (Fujii, April, 2004). This may partially explain the beneficial effect of increased parity for uterine fibroids, as those women will experience fewer menstrual cycles, thereby increasing the period of time the uterus is relatively free of these oxidative stresses (Zhou et al., 2011). The hypoxia-related gene products hypoxia-inducible factor (HIF)-1 alpha, HIF-2 alpha, glucose transporter (GLUT)-1 or carbonic anhydrase (CA) IX are not detected in uterine fibroid tissue, and this suggests that hypoxia contributes to the development of fibroids independent of the HIF-system (Zhou et al., 2011). While some authors have claimed that uterine fibroids have a protective mechanism against oxidative stress (Fujii, April, 2004), more recent investigation has found that uterine fibroid cells contain lower concentrations of antioxidant enzymes, superoxide dismutase and catalase than normal myometrial cells (Fletcher et al., 2013).

The role of Oestrogen

While the initial event leading to proliferation of a single smooth muscle clone is unknown, the continued growth of fibroids appears to be related to oestrogen and progesterone. Fibroid growth does not occur before the menarche, fibroids decrease in size after the menopause or a period of treatment with gonadotropin releasing hormone agonists (GnRHa), and fibroids increase in size during pregnancy. There is however no evidence of women with uterine fibroids having abnormal circulating oestradiol or progesterone concentrations.

Oestrogen exerts its physiological effects by binding to specific nuclear receptors on target cells, of which two subtypes are known, oestrogen receptor α (ERα) and β (ERβ) (Jensen, 1968). Both ERα and ERβ messenger ribonucleic acid (mRNA) levels are elevated in fibroid cells compared with myometrium (Kovács et al., 2001). Although both ERα and ERβ can stimulate the transcription of target genes in a similar manner, ERβ is activated to a lesser extent than ERα (Enmark and Gustafsson, 1999). When compared with autologous myometrium, fibroid cells have elevated expression of several genes known to affect oestrogen regulation, such as type I and III collagen, connexin 43 gap junction protein and insulin-like growth factor (IGF)-1 genes (Andersen et al., 1995). An overexpression of aromatase p450, an oestrogen synthase that catalyses the conversion of androgens to oestrogen, has been identified within uterine fibroids. It is suggested that this has a role in fibroid growth in an autocrine/paracrine fashion (Shozu et al., 2001). The expression of aromatase P450 in fibroid cells can be inhibited by Gonadotropin releasing hormone agonist (GnRHa) treatment. This may explain the mechanism for GnRHa-induced reduction in oestrogen and fibroid volume (Maruo et al., 2004). The tumour-suppressor gene p53 is the most frequently mutated gene in human tumours, and oestrogen is known to reduce the p53 protein concentration within fibroid tissue (Gao et al., 2002).

The continued growth of fibroid cells and induction of mitosis is also controlled by oestrogen. This is achieved by the protein tyrosine phosphorylation of such intracellular proteins as growth-associated protein (GAP), phosphatidylinositol 3-kinase (PI-3-K), and phospholipase C (PLCγ) and the activation of other secondary protein kinases. Protein tyrosine phosphorylation is activated by E2-induced platelet-derived growth factor (PDGF) secretion (Barbarisi et al., 2001). In addition to PDGF, epidermal growth factor (EGF) and insulin-like growth factor production are also controlled by oestrogen (Huet-Hudson et al., 1990), both of which are known to effect cell growth and proliferation within uterine fibroids.

Progesterone and fibroid growth

There is increasing evidence that progesterone also has an important role in controlling fibroid growth. Progesterone up-regulates the expression of proliferating cell nuclear antigen (PCNA) and EGF in fibroid cells (Maruo et al., 2000). Again, as with the oestrogen receptors, progesterone receptor mRNA and proteins have increased expression within fibroid cells, compared with the surrounding myometrium (Brandon et al., 1993). The mitotic activity of fibroid cells is higher during the secretory phase of the menstrual cycle, when progesterone levels are also at their highest (Kawaguchi et al., 1989). Conversely, when anti-progesterones (RU-486 mifepristone) are administered, there can be a significant reduction in the overall mitotic activity and volume of uterine Fibroids (Tiltman, 1985, Murphy et al., 1993). In addition to growth factors, progesterone also increases the expression of Bcl-2 protein, an apoptosis-inhibiting gene product of the oncogene bcl-2 (Matsuo et al., 1997). Progesterone also decreases the expression of tumour necrosis factor α (TNFα), one of the dominant cytokines involved in programmed cell death (apoptosis) (Kurachi et al., 2001). This overall decrease in apoptosis is thought to contribute to continued uterine fibroid cell proliferation. In addition to stimulating the growth of uterine fibroids, progesterone can also inhibit fibroid growth by down regulating IGF-I expression (Maruo et al., 2003).

Genetics of uterine fibroids

There is no single genetic defect responsible for the growth of uterine fibroids. Studies analysing multiple fibroids from the same uterus have demonstrated that these tumours can contain different chromosomal anomalies, in some cases over 40 within a single uterus, suggesting that each tumour has arisen independently (Ligon and Morton, 2000). The monoclonal origin of these tumours suggest mutation as the initiating event in their genesis (Markowski et al., 2012). Many specific cytogenetic translocations, duplications and deletions can be detected in up to half of all uterine fibroids (Nilbert et al., 1990). There are specific chromosomal regions (1p36, 6p21, 12q15 and 14q24) associated with uterine fibroids that are also associated with other lesions such as lipomas, pulmonary chondroid haemartomas, and endometrial polyps (Tallini et al., 2000). It has therefore been suggested that these benign mesenchymal tumour types may share a common pathway that may initiate or maintain tumour growth (van Rijk et al., 2009). As yet, no relationship between age, parity and the type of chromosomal anomaly has been found, however a positive correlation has been found between the type of chromosomal anomaly and anatomical location of the uterine fibroids (Brosens et al., 1998). It was discovered that sub-mucous fibroids had significantly fewer chromosomal anomalies (12%) than sub-serosal (29%) or intramural fibroids (35%). A relationship between fibroid size and karyotype has been demonstrated, with the largest fibroids carrying the t(12;14) mutation

(Rein et al., 1998), the most frequently encountered (approximately 20%) subgroup of karyotypically abnormal fibroids (Meloni et al., 1992). Conversely, deletion of bands q22-q32 of chromosome 7 occurs in 17% of karyotypically abnormal fibroids and is associated with generally smaller fibroids (Rein et al., 1998). Other cytogenetic changes include rearrangements of 6p21 (in <5%), rearrangements of either the long or short arm of the X chromosome, rearrangements of chromosomes 1 and 3, monosomy 10 and deletions of 10q (Sandberg, 2005). The proto-oncogenes c-fos and c-jun are involved in cell growth and differentiation, and mRNA of c-fos and c-jun is significantly reduced in fibroids compared with surrounding myometrium (Gustavsson et al., 2000).

High-mobility group I (HMGI) proteins are predominantly expressed during embryonic development and are usually inactive in mature tissues (Rogalla et al., 1996). Many benign tumours, including uterine fibroids will have damaged HMGA2 and HMGA1 genes which code for these proteins. When compared with surrounding myometrium, fibroid cells express high levels of HMGA1 and HMGA2 proteins (Klotzbucher et al., 1999). HMGA proteins are non-histone chromatin proteins involved in many cellular processes, including regulation of inducible gene transcription, replication, DNA repair and the metastatic progression of cancer cells. Damage to HMGA2 and HMGA2 results in the abnormal production of these proteins, contributing to tumour growth (Hodge et al., 2012).

Mutations of the Mediator complex subunit 12 gene (MED12) have been frequently been detected in fibroids (Mäkinen et al., 2011). MED12 is a part of the Mediator complex, a multi-protein complex that functions as a transcriptional co-activator and has a regulatory role in RNA polymerase II activity (Thompson et al., 1993). MED12 is part of the sub-units that form the Mediator complex, and specifically its CDK8 sub-module (Markowski et al., 2012). CDK8 is a positive co-regulator of p53 target genes, and this may provide a mechanism for MED 12 mutations resulting in tumorigenesis (Markowski et al., 2012). Those fibroids with MED 12 mutations are predominately found in those with a normal karyotype and in the absence of the 12q14-15 rearrangements (Mäkinen et al., 2011). Uterine fibroids with MED 12 mutations have high levels of WNT4 mRNA expression (Markowski et al., 2012). WNT4 (Wingless type MMTV integration site family, member 4) is a glycoprotein cell signalling molecule involved in embryogenesis and again, may contribute to tumorigenesis (Boyer et al., 2010).

Epigenetic factors

Epigenetics refers to all heritable alterations of gene expression that are not coded in the DNA sequence. It is now understood that tumorigenesis depends not just on genetic mutations but on epigenetic factors. These may include deoxyribonucleic acid (DNA) methylation, histone modifications and the dysregulation of microRNA. DNA methylation occurs at position 5 of the cytosine ring of most CpG dinucleotides, by the effect of DNA methyltransferase. DNA methylation is involved in a variety of processes such as regulation of gene expression, embryonic development and maintenance of epigenetic memory (Bird, 2002). DNA methyl acceptance assays have found that the incorporation ability of methyl donors is significantly higher in fibroid tissue when compared with corresponding myometrial tissue (Li et al., 2003). The oestrogen receptor (ER) – α gene has been found to be hypo-methylated in uterine fibroids with a corresponding higher expression of ER-α mRNA (Asada et al., 2008).

Another epigenetic factor that has a role in gene expression is histone modification. The expression of the enzyme histone deacetylase (HDAC) 6 is increased in uterine fibroids along with ER-α, and reduction of HDAC 6 is associated with a decrease in ER-αexpression (Wei et al., 2011).

MicroRNAs are small (approximately 23 nucleotide), non-protein coding RNAs that function via base-pairing with complementary sequences within mRNA. MicroRNA can effect transcriptional and post-transcriptional gene expression, often by down-regulation of gene expression by blocking mRNA translation, or by degrading the mRNA transcript (Bartel, 2009). MicroRNA microarray analysis of uterine fibroid tissue has found differences in expression of many microRNAs in fibroid tissue when compared with surrounding myometrium (Marsh et al., 2008). MicroRNA 21 (miRNA21) levels are 2-3-fold higher in fibroid tissue when compared with surrounding myometrium (Fitzgerald et al., 2012). An analysis of tissue from uterine fibroids and myometrium with miRNA21 knockdown found increased levels of programmed cell death 4 (PDCD4) , a protein with many important roles such as cell cycle regulation, neoplastic transformation and apoptotic regulation (Fitzgerald et al., 2012). In fibroid tissue with normal miRNA21 levels PDCD4 was still elevated compared with surrounding myometrium. This is different from other, malignant tumours where PDCD4 acts as a tumour suppressor gene and loss of PDCD4 is associated with tumour progression.

The Role of the Extracellular Matrix

The components of extracellular matrix (ECM) play a pivotal role in the growth and development of all tissue types. A characteristic feature of uterine fibroids is an excess of production and deposition of ECM proteins, including type I-III collagens, proteoglycans and matrix metalloproteinases (MMPs) (Malik et al., 2010). The

fibroid ECM contains growth factors, cytokines, chemokines, angiogenic and inflammatory mediators that have key roles in the regulation of cell growth and differentiation, and contribute to an autocrine/paracrine mechanism for fibroid growth (Moore et al., 2010). Uterine fibroid-derived fibroblasts stimulate the growth of uterine fibroid cells in addition to the production of collagen and growth factors (Moore et al., 2010). Changes in the ECM affects the mechanical stresses within fibroids, and this can activate internal signalling which may contribute to fibroid proliferation (Islam et al., 2013). Measurements of mechanical stress within fibroid tissue found increased loading stress when compared to the surrounding myometrial tissue (Rogers et al., 2008). This increased stress is also associated with increased levels of the Rho- guanine nucleotide-exchange factors (GEFs), A kinase anchor protein (AKAP13); one of the factors involved in activation of GTPase.

As important part of the structure of uterine fibroids are the glycosylated proteins, proteoglycans (Islam et al., 2013). One small study found higher amounts of glycoaminoglycans compared with the surrounding myometrium, and a different proteoglycan composition (Carrino et al., 2012). The authors found higher levels of versican (a proteoglycan involved in cell proliferation) and decreased levels of decorin (a proteoglycan that has a role in regulation of the cell cycle), and suggested that these differences may play a role in excess fibroid growth. The growth of uterine fibroids results in a reduction in the activity of endoglycosides responsible for degrading glycoaminoglycans (Wolańska et al., 2003). This difference in enzyme activity within uterine fibroids is likely to have an effect on tumour growth.

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptases that are involved in tissue remodelling, angiogenesis and tissue repair. The action of MMPs is controlled by tissue inhibitors of MMPs (TIMPs), and the expression of different forms of MMPs and TIMPs is different in fibroids compared with myometrial tissue (Wolańska et al., 2004, Dou et al., 1997).

Vasculature of the fibroid

The internal iliac artery supplies the walls of the pelvis and the pelvic organs. The internal iliac artery bifurcates into the anterior and posterior branches. The anterior branches of the internal iliac artery include the inferior gluteal, obturator, internal pudendal, vesical, middle haemorrhoidal and genital (uterine and vaginal) arteries (Pelage et al., 2005). The uterus is supplied by an anastomosis formed from the uterine, ovarian and vaginal arteries. Within the uterus the ascending uterine artery, radial, arcuate and peripheral arteries provide blood flow to the body of the uterus. The blood supply of fibroids arises from the uterine arteries, which become distorted and are commonly enlarged (Pelage et al., 2005). In addition the ovarian arteries may provide some of the blood supply and this occurs in around 10% of uterine fibroid cases (Barth and Spies, 2003). The arterial supply to the round ligament and the lumbar artery may also provide

additional supply occasionally (Razavi et al., 2002). Compared to arteries supplying normal myometrium, peri-fibroid arteries are larger in diameter (Pelage et al., 2003). Small nutrient arterioles supply the fibroid and penetrate the fibroid pseudo-capsule. Despite this increased blood supply, fibroids themselves are hypo-vascular compared with the myometrium. This has been demonstrated by the injection of the radioisotope ^{133}Xe (Forssman, 1976b, Forssman, 1976a) during MRI imaging. When examined with colour flow Doppler ultrasound, uterine fibroid tumours will typically have an increased peripheral blood flow (peri-fibroid plexus) and a decreased central flow (Tranquart et al., 2002). The resistance index (a measure of diastolic blood flow) is usually decreased in the peri-fibroid plexus, compared with that in the surrounding normal myometrium (Tranquart et al., 2002).

Immunohistochemistry has been used to study fibroid vasculature. Antibodies against the endothelial cell markers CD31, CD 34, factor-VIII-related antigen (FVIII) and Ulex europaeus agglutinin (UEA) lectin have been used to identify blood vessels and calculate the vascular area (by measuring the proportion of tissue staining as vessels), micro-vessel density (MVD vessels per mm^2) and the vascular luminal diameter (µm). Using UAE and FVIII it was confirmed that all fibroids had a smaller vascular area and lower MVD compared with the surrounding myometrium (Fleischer et al., 2008). Blood vessels in smaller diameter fibroids had smaller luminal diameters than vessels in larger fibroids, or myometrium (Casey et al., 2000). After the menopause the myometrial MVD increases, whereas the fibroid MVD remains unchanged (Weston et al., 2005). It is possible that the different properties of the extracellular matrix within fibroid tissue compared with the surrounding myometrium accounts for this increase in relative vascular density within the myometrium compared with the uterine fibroids (Fleischer et al., 2008). Detailed study of the structure of vessels with in the myometrium and fibroid tissue has shown that normal human myometrial tissue has a vascular spatial gradient which is absent in small fibroids (Aitken et al., 2006). It has been suggested that there may be an increased expression of anti-angiogenic factors or a deficiency of pro-angiogenic factors that result in these abnormal vessels (Aitken et al., 2006).

Understanding the blood supply of fibroids has allowed the development of interventional radiological techniques to treat uterine fibroids non-surgically (by uterine artery embolism), and increased understanding of the angiogenesis involved in fibroid development may lead to further improvement of fibroid treatment modalities in the future.

Fibroid Histology

Uterine fibroids are formed from mesenchymal connective tissue, a type of undifferentiated loose connective tissue that is derived mostly from mesoderm. Despite the term fibroid, these tumours are composed of elastin, collagen and extracellular matrix proteins in addition to smooth muscle (Shaw et al., 2003). Fibroids contain bundles of elongated smooth muscle cells, with a whorl-like appearance, mixed with

connective tissue. Uterine fibroids are often well circumscribed, and although there is no true capsule, a pseudo-capsule is present (Novak et al., 2002). Degeneration of the fibroid tissue may occur, often as a consequence of the relatively poor blood supply. In particular, hyaline degeneration can occur resulting in a smoother and more homogenous appearance. Occasionally liquefaction can occur resulting in cystic changes within the uterine fibroids. Red degeneration is associated with pregnancy and occurs secondary to venous thrombosis within the fibroids, or leakage from the vessels with the fibroids (Murase et al., 1999). Myxoid degeneration appears as soft mucoid areas within the uterine fibroid tissue, sometimes with cystic change. It involves the presence of gelatinous foci at macroscopic examination, which contain hyaluronic acid-rich mucopolysaccharides (Prayson and Hart, 1995).

There are histological variants of fibroids depending on their cellular appearance. Cellular leiomyoma refers to a uterine fibroid with a significantly denser arrangement of smooth muscle cells, and little or no intervening collagen. The appearance of lined-up cell nuclei within the fibroid is referred to as a neurilemmoma-like leiomyoma. Uterine fibroids are termed symplastic, bizarre or atypical when uterine fibroids are composed of polygonal cells rather than spinal cells, with associated giant cell formation (Shaw et al., 2003). Uterine fibroids may have a similar macroscopic appearance to adenomyosis, however in the case of adenomyosis there is no clear demarcation from the myometrium.

Leiomyosarcoma is a rare malignant smooth muscle tumour of the uterus, representing only 1% of all uterine malignancies (Ip and Cheung, 2011).

The use of a gonadotropin releasing hormone analogue (GnRHa) can lead to the appearance of a densely cellular tumour and may mimic the appearance of Leiomyosarcoma. However numbers of mitoses should remain normal on further histological examination of these cases, allows for this important distinction (Crow et al., 1995). Unlike many other malignant tumours there are no specific measurable biochemical markers for uterine Leiomyosarcoma. Since the MRI appearances may be similar to degenerating uterine fibroids timely diagnosis can be problematic (Mazziotti et al., 2012).

Fibroid diagnosis

Most initial diagnoses of uterine fibroids are made in the community by general practitioners (GPs) based on the clinical history and abdominal palpation. Usually a mobile, firm, non-tender mass is palpable in the supra-pubic area. Confirmation of this diagnosis is usually made on 2-dimensional (2D) ultrasound (US). US has limited ability to diagnose coexisting pathology (such as pelvic masses), and may be limited by increased body mass index (BMI). In addition, 2D US has a relatively poor sensitivity for assessing fibroid size,

degeneration of fibroids, adenomyosis, and the position of the uterus and uterine fibroids in relation to other organs (Kirby et al., 2011).

Magnetic resonance imaging (MRI) is a safe, accurate, but relatively expensive method to assess fibroid size, location and appearance in terms of homogeneity and signal intensity (Murase et al., 1999). MRI utilises the principles of nuclear magnetic resonance (NMR), a physical phenomenon in which magnetic nuclei in a magnetic field absorb and re-emit electromagnetic radiation. The body is composed of 75-80% water molecules, and each water molecule has two hydrogen nuclei or protons. Hydrogen protons are charged particles that spin, and therefore have their own magnetic properties. When inside the powerful magnetic field, these protons align themselves to that field, oscillating about the field axis with a motion known as precession. Magnetic resonance (MR) images are produced using a pulse sequence, containing radiofrequency pulses and gradient pulses which have controlled durations and timings (McRobbie, 2007). These gradient pulses make the characteristic knocking noise during the MRI scan. The radio frequency pulse produces a varying electromagnetic field, applied at 90 degrees to the MR Field, that at the correct frequency causes the axes of all the protons momentarily align with or against that field. This addition field results in the protons acquiring energy and movement in the form of spin, as the protons are in phase. Once the radiofrequency pulse stops the protons shed their new energy to the surrounding chemical lattice (McRobbie, 2007, Brown and Semelka, 1999). This is known as the spin-lattice time or T1 relaxation. In addition, the protons also stop spinning synchronously. This is known as the spin-spin time or T2 relaxation. It is this relaxation that generates a radio frequency signal, which can then be measured with receiver coils. T1 weighted MRI is the basic standard scan, particularly useful in differentiating fat from water, with water darker and fat brighter. T2-weighted MRI is the other basic MRI imaging, and as with the T_1-weighted scan, fat is differentiated from water, but in this case fat shows darker, and water lighter.

MRI enables very accurate demonstration of the uterine size, position, and the classification of individual fibroid masses as SM, IM or SS (Dudiak et al., 1988). The usual MRI appearances of a non-degenerated uterine fibroid (see figure 6) are of a well-circumscribed homogeneous mass within the uterine corpus with decreased signal intensity on T2-weighted MRI compared with the surrounding myometrium (Murase et al., 1999).

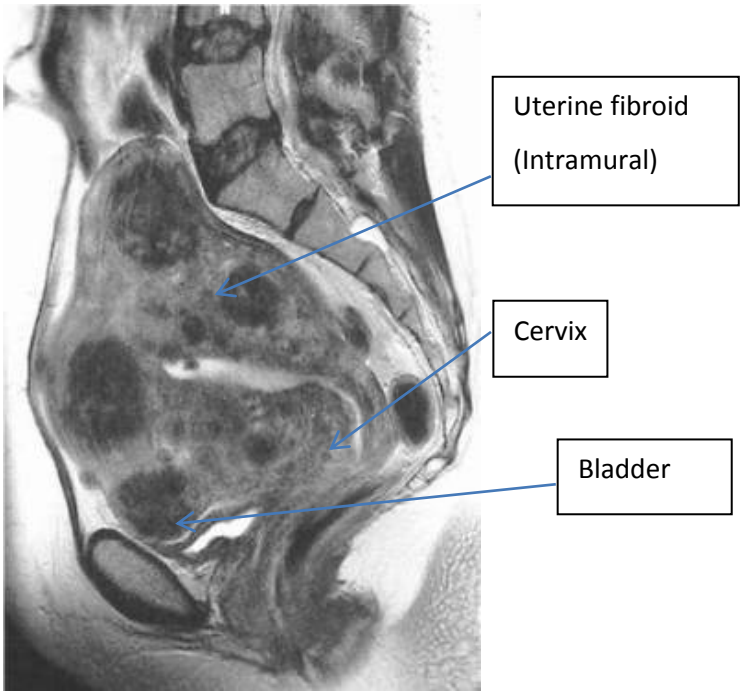


Figure 6: Sagittal T2 weighted image of a pelvis containing a multi-fibroid uterus

Cellular fibroids have a structure of more compact smooth muscle cells and reduced collagen, and as a result have a higher signal intensity on T2 weighted images and also show enhancement on post-contrast images (Takeuchi et al., 2009) (Figure 7). Degenerated fibroids have a heterogeneous appearance on T2-weighted images. Calcific or hyaline degeneration has low signal intensity on T2-weighted images, similar to that of normal fibroids. Areas of cystic degeneration show high signal intensity on T-2 weighted images that do not enhance post-contrast. Myxoid degeneration appears as very high intensity on T2-weighted images, and do show some enhancement post-contrast. Red degeneration of uterine fibroids appears as peripheral or diffuse high signal intensity on T1-weighted image, and variable signal intensity on T2-weighted images, occasionally with a rim of low intensity surrounding the mass. It is thought that high intensity appearances on T1 are due the proteinaceous content of blood (Murase et al., 1999). Some uterine fibroids have been found to demonstrate high signal intensity at the periphery of the lesion on T2 weighted images. This is thought to be caused by a pseudo-capsule of dilated blood vessels, lymphatic vessels or oedema around the fibroid.

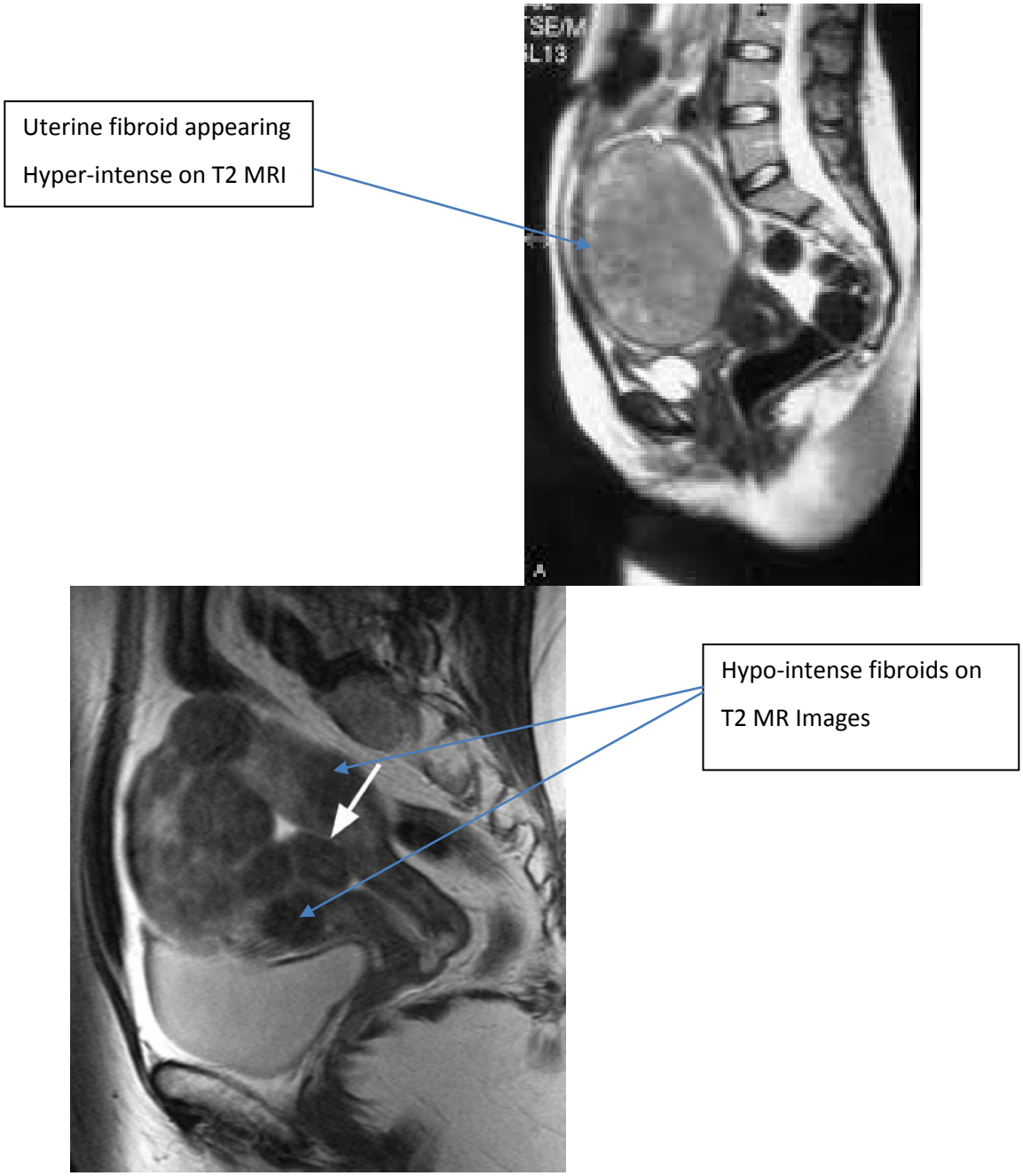


Figure 7: Hypo- and Hyper- Intense fibroids

Adenomyosis may appear similar to uterine fibroids on MRI imaging; however there are some distinguishing features. There is often a thickened junctional zone (comprising of the inner myometrium) on T2-weighted images. A junctional zone thickness of 12mm or more is highly predictive of Adenomyosis (Reinhold et al., 1996) (Figure 8).

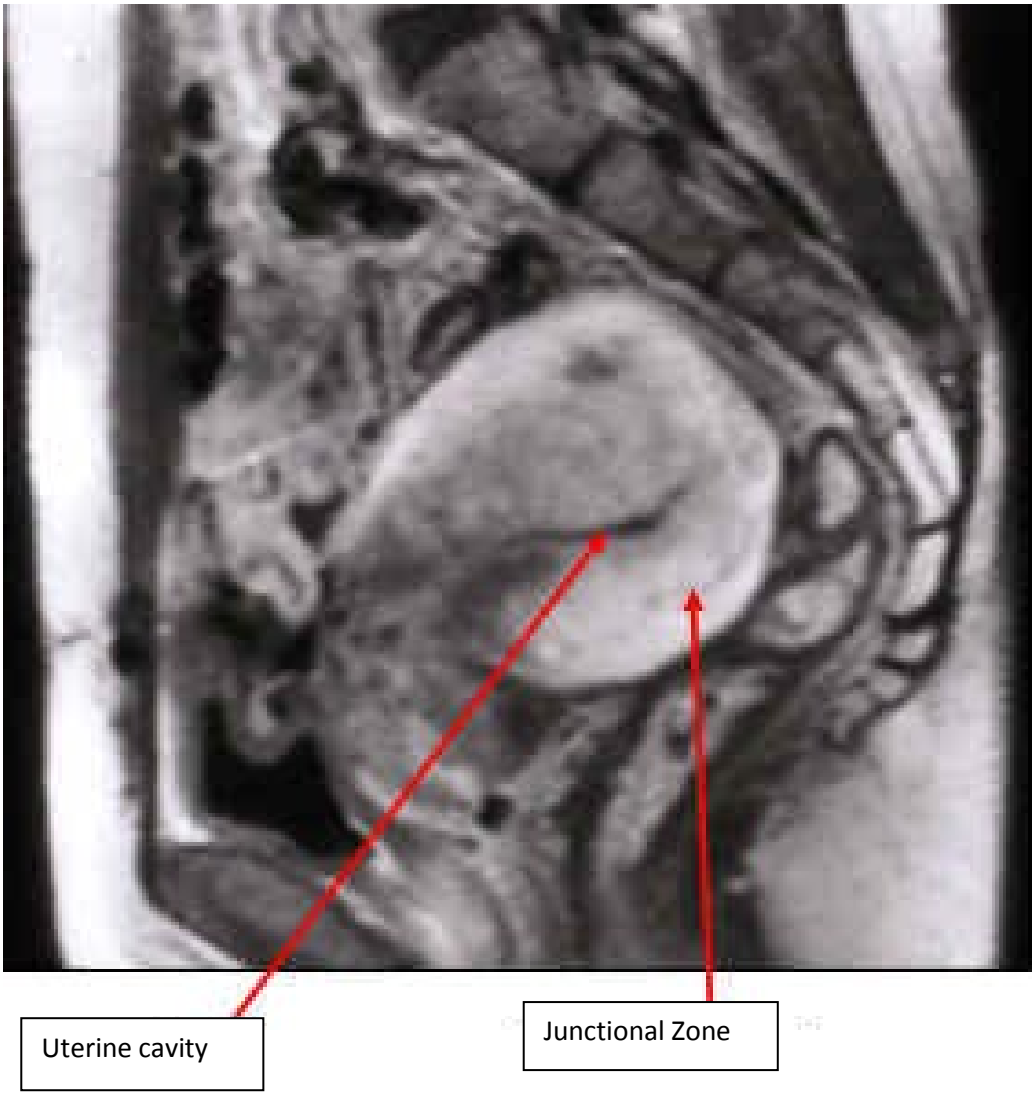


Figure 8: Thickened junctional zone on T1-weighted imaging

Adenomyosis usually appears as hypo-intense relative to skeletal muscle on T2-weighted imaging and this is thought to be related to the reactive, dense smooth muscle hypertrophy that surrounds the embedded endometrial glands found in Adenomyosis. Endometrial glands embedded within the myometrium appear as hyper-intense foci on T2-weighted images (Murase et al., 1999) (see Figure 9).

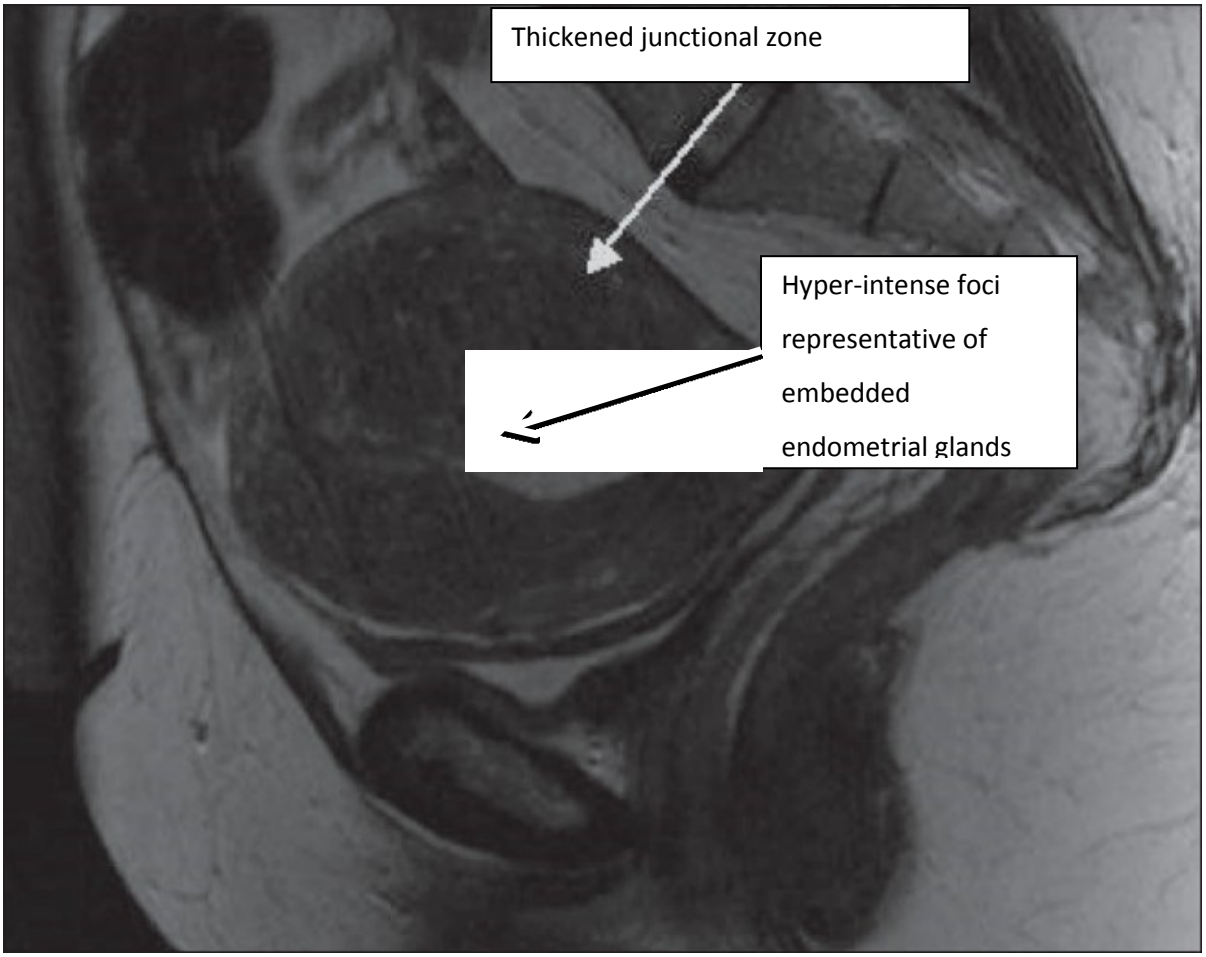


Figure 9: T2-weighted image of Adenomyosis

Leiomyosarcomatous lesions are difficult to distinguish from uterine fibroids on MR imaging. Histologically there are several MRI features associated with possible malignancy. These include greater than 50% high T2 signal intensity, small high T1 signal intensity foci, well-demarcated non-enhancing regions within the mass, indistinct tumour borders and invasion into adjacent structures (Griffin et al., 2010). However, the expectation that these malignant masses will appear as hyper-intense, poorly defined lesions on MRI does not correspond with clinical experience (Yamashita et al., 1993).

Attempts have been made to use diffusion-weighted MRI to differentiate between hyper-intense fibroids and malignant lesions, but without success (Takeuchi et al., 2009). The term smooth muscle tumour of uncertain malignant potential (SMTUMP) refers to those lesions where the degree of malignancy is pathologically characterised as being between a fibroid and a Leiomyosarcoma (Tanaka et al., 2004). These lesions display high signal intensity on T2-weighted images, and contain small areas of high intensity on T1-weighted images.

History of uterine fibroids and their management

References to the "uterine stone" were to be found as early as 5th century BC in Greek texts. The prominent Roman physician Galen of Pergamon referred to "scleromas" during the second century AD (Bozini and Baracat, 2007). The term Fibroid was first used by Rokitansky in 1860 and by Klob in 1863 (Bozini and Baracat, 2007). The term myoma was coined by Virchow, who demonstrated that these tumours originate from uterine smooth muscle (Shlansky-Goldberg, 2009).

The first documented account of an abdominal myomectomy was published in 1845 (Chamberlain, 2003). Dr Washington Atlee of Pennsylvania, US, a professor of medical chemistry, performed surgery on a woman initially believed to have an ovarian cyst. He made a sub-umbilical, midline incision without anaesthetic, whereupon he discovered an 18 inch pedunculated fibroid. Following this operation the women survived. With the development of abdominal surgery in the mid-19th century, the preferred method of treating uterine fibroids was hysterectomy. In 1898, a series of 11 cases of abdominal myomectomy, rather than hysterectomy was presented to a hostile reception at the British Gynaecological Society. This hostility was due to concerns over the risk of heavy bleeding with this surgery. It was only when the renowned surgeon Victor Bonney pioneered the idea of uterine conservation in the early part of the 20th century, that myomectomy became a more established treatment option for the treatment of uterine fibroids, as he proposed:

> *"…since cure without deformity or loss of function must be surgery's highest ideal, the general proposition that myomectomy is a greater surgical achievement than hysterectomy is incontestable"* (Bonney, 1928).

In 1980 myomectomy by laparoscopic surgery was described (Semm and Mettler, 1980) and since then has come to be regarded as a safe, effective method of removing fibroids, with a relatively shortened hospital stay and recovery period.

In additional to the surgical techniques described, numerous additional treatment options for treating uterine fibroids have been developed. The embolization of the uterine arteries following major post-partum haemorrhage has been in use since the 1970s (Worthington-Kirsch, 2004). In 1979 the first report of the use of trans-catheter embolization for life-threatening post-partum haemorrhage, after hysterectomy and hypogastric ligation had failed to control blood loss (Heaston et al., 1979). In this case haemostasis was achieved using a gelatin sponge to embolize a vaginal branch of the left internal pudendal artery.

In 1995, a group in Paris had initially requested the embolization of uterine arteries pre-operatively to reduce peri-operative complications, however they discovered that there was a significant decrease in fibroid volume and symptom severity following embolization (Ravina et al., 1995). The data that was published followed a group a patients specifically treated by embolization. Since then uterine artery embolization has become an established treatment option for women with symptomatic uterine fibroids.

Numerous thermo-ablative techniques have been developed to treat uterine fibroids. Laparoscopic myolysis using a Nd-YAG (neodymium-doped yttrium aluminium garnet) laser was developed in the 1980s (Nisolle et al., 1993a). Infrared laser energy is converted into heat within the fibroid. As the temperature rises above 55°C protein denaturation occurs, resulting in coagulative necrosis of the fibroid tissue. Complications such as adhesion formation led this technique to fall out of favour. Trans-cervical ablation of sub-mucous fibroids using bipolar diathermy has been used successfully, with fewer complications than traditional trans-cervical resection techniques (Clark et al., 2002). In addition to the application of heat, laparoscopic cryomyolysis has been used to ablate fibroid tissue (Ciavattini et al., 2004). The use of magnetic resonance imaging (MRI) to visualise the region of treatment and produce real-time thermal imaging of the tissue, together with the development of MRI-compatible needles and laser guide wires, led to the development of MR- guided Percutaneous Laser Ablation of Uterine fibroids (Law et al., 1999). This was in turn superseded by focused ultrasound technology, with the advantage of being completely non-invasive to the patient. The potential use of focused ultrasound has been studied since the 1940s (Lynn et al., 1942). By the turn of the millennium focused ultrasound was in use for the treatment of prostate, breast and hepatic tumours (Hill and ter Haar, 1995, Rowland et al., 1997, Gelet et al., 1999, Huber et al., 2001). These treatments used either ultrasound or MRI guidance to target the tissue. High intensity focused ultrasound to treat uterine fibroids was first described in 2003 (Stewart et al., 2003).

Fibroid management

Approximately half of all women with uterine fibroids will require some form of medical intervention. These can range from medical treatments to control the symptoms experienced, through to surgical removal of the uterus (hysterectomy). As fibroids are a benign condition, the primary aim of fibroid management is to improve quality of life while meeting the individual woman's expectations. For some women the idea of a hysterectomy may be abhorrent, whereas for others, especially those who have completed their families, this may feel like the most sensible way to deal with their fibroids. Future fertility expectations will dictate treatment modality and must be considered by the treating physician.

The current treatments for uterine fibroid are summarised in Table 1 and will be discussed in greater detail later in this chapter.

Treatment option	Anaesthetic/ Analgesia	Recovery	Benefits	Side-effects/ disadvantages
Hysterectomy	General anaesthetic	6-8 weeks*	Complete removal of uterus, fibroids and an end to symptoms	Risk of surgery/anaesthetic End of fertility Social stigma/concerns Hospital stay
Myomectomy	General anaesthetic	6-8 weeks*	Removal of the fibroids while preserving the uterus May be possible to perform at laparoscopy	Risk of surgery/anaesthetic Risk of recurrence of uterine fibroids Potential risk of Hysterectomy Potential risk to fertility Hospital stay
TCRF	General anaesthetic	2-4 weeks*	Removal of fibroid tissue within the endometrial cavity Improvements to fertility Day-case procedure	Risk of surgery/anaesthetic Risk of recurrence of uterine fibroids Potential risk of Hysterectomy (1%) Potential risk to fertility Risk of fluid overload Suitable for SM uterine fibroids only
UAE	Sedation Opioid PCA	<10 days*	Reduction of fibroid size -approx. 50% (Mara et al., 2012b) Avoidance of abdominal surgery Improvement in symptoms	Post-Embolization Syndrome Risks of premature menopause Risks to fertility Risk of recurrence of uterine fibroids/regrowth Risk of hysterectomy (1%) Hospital stay
MRgFUS	Opioids during treatment only	2-3 days*	Reduction of Fibroid symptoms Reduction in uterine fibroids size(up to 20% (Stewart et al., 2007) Avoidance of abdominal surgery No risk of hysterectomy No hospital stay	Risk of recurrence Painful during treatment

Table 1: Treatment options for uterine fibroids

*(Levy, 2008)

Surgical Treatments

Hysterectomy

Total abdominal hysterectomy (TAH) or depending on the size of the uterus, vaginal hysterectomies are the only definitive treatment option for uterine fibroids. Until recently this was the main treatment option for women with large symptomatic uterine fibroids. Aside from the risks associated with surgery and anaesthesia, these are well tolerated procedures. Surgical risks include the risk of infection, haemorrhage, injury to one of the surrounding organs, such as the gastro-intestinal tract (0.1-1%), genitourinary tract (1-2%), pain and neuropathy (0.2-2%), and vaginal cuff dehiscence (0.39%) - which is more common following laparoscopic hysterectomy (1.35%) compared with laparoscopic-assisted hysterectomy (0.28%) and total abdominal hysterectomy (0.15%), and total vaginal hysterectomy (0.08%) (Clarke-Pearson and Geller, 2013). A recent systematic review found no randomised control trials comparing hysterectomy with myomectomy for the treatment of uterine fibroids (Pundir et al., 2013b). From the observational studies that were identified by this study, the authors found no significant difference in the rates of major morbidity (RR 0.94; 95% CI 0.31-2.81) between abdominal hysterectomy and abdominal myomectomy, a larger overall blood loss following hysterectomy (869ml vs. 582ml), no significant difference in the operating time, requirement for blood transfusion or febrile illness (Pundir et al., 2013b). Vaginal hysterectomy is the safest route for hysterectomy, although with very large fibroid uteri may not be feasible (Ridgeway and Falcone, 2013). Minimal access surgery ("key-hole" surgery) is widely becoming the preferred approach to much abdominal and pelvic surgery, due to the reduction in trauma to the anterior abdominal wall and reduction in post-operative pain, smaller scars and shorter hospital stay. The first reported laparoscopic hysterectomy was described in 1988, and since then developments in energy devices, minimal access instruments, haemostatic agents and the development of structured laparoscopic training has improved the safety of this technique. A meta-analysis of laparoscopic assisted vaginal hysterectomy (LAVH) compared with vaginal hysterectomy found comparable clinical outcomes, however longer operating times (Guo et al., 2013). LAVH when compared with open hysterectomy was found to have longer operating times, but shorter hospital stay, fewer post-operative complications and a more rapid return to normal activities (Yi et al., 2011). Further developments to minimal access surgery and training are being developed with will likely further improve outcomes and reduce operating times. Single-port laparoscopic hysterectomy is currently being developed, and although initial studies are encouraging there is currently insufficient evidence for its routine use (Murji et al., 2013). Robotic-assisted hysterectomies have mainly been used for endometrial cancer cases or cases of severe endometriosis, and although studies suggest benefits of reduced hospital-stay and low

complication rate, the quality of current studies remains poor (O'Neill et al., 2013). As the cost of robotic-assisted laparoscopic surgery reduces and experience increases this may well become a more common approach to hysterectomy for uterine fibroids.

Over the last few decades the mean age of women becoming pregnant for the first time has increased significantly (Liu et al., 2011). For many women, hysterectomy will not be considered as a treatment option for their fibroids until they have completed their families. Even once fertility is no longer a concern, many women have very negative feelings toward hysterectomy due to the psychosocial effects of losing their uterus, especially for African women (Williams and Clark, 2000, Lewis et al., 2000), in particular concerns regarding the association of a perceived loss of femininity (Kunde and Khalaf, 2005). Other concerns regarding the perceived risk of sexual dysfunction following hysterectomy also affect a women's decision to undergo hysterectomy (García, 1993).

Myomectomy

Surgical removal of the uterine fibroids with preservation of the uterus can be performed abdominally by either the open (laparotomy) or laparoscopic routes. The decision regarding route of myomectomy depends on several factors, including the size, the number and the location of the fibroids in the uterus and the experience of the surgeon. Abdominal operations are also associated with the formation of post-operative adhesion formation which can result in pelvic pain, bowel obstruction, or occlusion of the fallopian tubes. There are no randomised control trial to support the theory that myomectomy improves fertility, however some longitudinal studies certainly suggest this (Rossetti et al., 2001, Hart et al., 2001).

At laparoscopic myomectomy, the myometrium overlying the fibroids is often injected with vasopressin in order to constrict the overlying blood vessels. This is usually associated with blanching of the uterus, indicating corresponding reduced local blood flow. A myometrial incision is made with a mono-polar electrode, scissors or harmonic scalpel in an effort to identify the tissue plane between the fibroid tissue and surrounding myometrium. The fibroid is grasped with a tenaculum or myomectomy screw and the surrounding myometrium is progressively dissected away, until it is possible to remove the entire fibroid. Once all of the uterine fibroids have been removed the myometrium is closed in layers using intra-corporeal knots. The fibroid is then broken down using a process of morcellation, as pieces of fibroid are suctioned from the abdomen (Luciano, 2009).

Myomectomy is associated with the risk of intraoperative blood loss, the potential need for an emergency hysterectomy in order to control excessive blood loss, distortion of the endometrial cavity and increased risk of uterine rupture in any subsequent pregnancies (Khaund and Lumsden, 2008).

A large cohort study of 628 women following abdominal myomectomy found a re-intervention rate at 5 years of 23% (Reed et al., 2006). The REST study randomised 157 women to either UAE or myomectomy and found a 4% re-intervention rate at 5 years (Moss et al., 2011). A multicentre retrospective study of 512 women undergoing laparoscopic myomectomy between 1995 and 2004 found an overall re-intervention rate of 6.7% at 5 years and 16% at 8 years (Yoo et al., 2007). A meta-analysis of laparoscopic vs. open myomectomy identified 6 randomised control trials found comparable rates of major complications, but significantly reduced peri-operative blood loss, reduced post-operative pain score, more rapid recovery overall, but a longer overall operation time (Jin et al., 2009). The authors concluded that where the required laparoscopic surgical skills are available the laparoscopic approach should be favoured.

Robot-assisted myomectomy has been introduced, and an initial meta-analysis of current observational studies suggest this technique has no benefits over laparoscopy and appear to increase the costs and requirement of blood transfusion in patients (Pundir et al., 2013a). There are, however no randomised control trials examining the use of robotic-assistance at myomectomy, and as surgical experience grows and costs reduce this may become a more acceptable method of myomectomy.

Vaginal myomectomy has been developed as an alternative to open or laparoscopic myomectomy, particularly in the presence of multiple, or large uterine fibroids (Davies et al., 1999). By surgically opening the anterior or posterior vaginal wall (colpotomy), sub-serosal and intramural fibroids have been accessed and successfully removed (mean diameter 5mm). Operating time was found to be shorter than laparoscopic myomectomy. Symptomatic relief was comparable to myomectomy by laparotomy, with the benefit of no abdominal wound. For vaginal myomectomy to be attempted adequate vaginal access, good uterine mobility, and moderate uterine size are essential prerequisites, in addition to the appropriate skills of the surgeon.

Trans-cervical resection of fibroid (TCRF)

The development of hysteroscopic visualisation of the endometrial cavity in the 1970s has allowed trans-cervical resection of sub-mucosal fibroids (Indman, 2006); prior to this the only surgical approach was abdominal myomectomy or hysterectomy. Clinical response following trans-cervical resection of fibroids (TCRF) depends on the number of uterine fibroids projecting into the endometrial cavity. Those women with

normal size uteri and no more than two fibroids seen on hysteroscopy had a five-year re-intervention rate of 9.7%; those with an enlarged uterus and three or more fibroids seen at hysteroscopy had a five-year re-intervention rate of more than 35% (Emanuel et al., 1999). Complications of TCRF include heavy bleeding, fluid-overload (secondary to the use of high pressure glycine to visualise the endometrial cavity) and intrauterine adhesions (Asherman's syndrome). Where fertility is not desired endometrial ablation can be used following TRCF to improve the reduction in menstrual blood loss.

There have been reports in the literature about the embolization of larger sub-mucosal fibroids prior to TCRF, however evidence is currently limited (Serradilla et al., 2011). Hysteroscopic resection of sub-mucosal fibroids can be limited by bleeding with subsequent loss of adequate views during the procedure, and resection may involve multiple procedures to excise large sub-mucosal fibroids.

The technique of "cold-loop" hysteroscopic myomectomy has been described using a diathermy cutting loop to first excise the portion of the fibroid within the endometrial cavity and identifying the cleavage plane between the fibroid and normal myometrium (Di Spiezio Sardo et al., 2008). Once this plane has been identified a non-diathermy rectangular loop is used to mechanically dissect the fibroid away from the surrounding normal tissue. This technique allows a more complete resection of the fibroid, however requires a higher level of training and surgical skill.

One of the problems encountered with TCRF is that resected pieces of the fibroid may obscure the hysteroscopic view, increasing the risk of uterine perforation. Resected fibroid pieces can be removed using forceps however it may be difficult to remove all tissue and this carries the risk of uterine perforation; or alternatively the remaining tissue may be removed under direct vision using the electrode-loop of the resectoscope, however this may significantly prolong the operation time. The hysteroscopic morcellator was developed to potentially solve this clinical problem, in addition to reducing the operation time and the risk of fluid overload. The TRUCLEAR™ (Smith & Nephew Endoscopy, Andover, MA) hysteroscopic morcellator (THM) device uses two tubes, an external stationary tube, and an inner rotating cylindrical blade that is inserted through the operating hysteroscope (Pakrashi et al., 2013). While the inner blade cuts the fibroid tissue the pieces are aspirated through the tube via a connection to a vacuum device. As the device uses no diathermy there is no risk of lateral thermal spread; haemostasis is achieved by spontaneous myometrial contraction.

Electrosurgical vaporization has been used to resect fibroid tissue, having been first used by urologists in the treatment of prostate disease (Glasser, 1997). This technique uses hysteroscopically inserted electrodes that

are slowly moved over the visible portion of the fibroid. Electrosurgical vaporisation is less time consuming than standard resection and due to the vaporisation of fibroid tissue there is less obstruction of the view during surgery. This vaporization also means that no specimens are obtained for histology and carries a significant risk of uterine perforation.

Uterine artery embolism (UAE) or uterine fibroid embolization (UFE)

Uterine artery embolism (UAE) or uterine fibroid embolization (UFE) has become a well-established alternative treatment for those women wishing to avoid the risks of surgery while preserving their uterus. Women undergoing UAE are usually administered conscious sedation. In most cases a single groin puncture is needed. UAE is carried out by an interventional radiologist in an interventional radiology suit with an overnight stay post-procedure. UAE involves occlusion of either one or both uterine arteries with particulate emboli resulting in ischaemic necrosis of the fibroids. A catheter is used to enter the internal iliac artery. Antero-posterior and bilateral oblique arteriograms are used to visualise the origin of the uterine artery, and micron polyvinyl alcohol (PVA) is used to embolize the vessels. Other embolic agents used include trisacryl microspheres and gelatin sponge particles. Occlusion of the uterine blood vessels is confirmed by angiography. The woman is exposed to approximately 20 rad (20cGy) of ionising radiation to the ovaries (Hirst et al., 2008). Following embolization there is rapid decrease in the perfusion of the whole uterus including the fibroids, however the perfusion of the normal myometrium rapidly return to normal, leaving the fibroids non-perfused (deSouza and Williams, 2002a).

Following UAE procedures subjects usually remain in hospital for 24-48 hours and use a patient-controlled analgesic (PCA) opioid-containing device for the first 12-24 hours. Pain and discomfort following UAE is the most commonly reported side-effect following this treatment modality. The pain experienced is due to the ischaemic necrosis of the fibroid tissue and surrounding myometrium. In addition to pain, a frequent morbidity of UAE is post-embolization syndrome, which includes high fever, malaise, nausea and vomiting. Post-embolization syndrome (PES) can last from a few hours to a few days and is thought to be an immune-mediated response. It has been previously been reported to occur in approximately half of women undergoing UAE and is controlled with analgesics, antipyretics, and anti-inflammatories (Carrillo, 2008). A recent meta-analysis of UAE studies found the combined rate of PES to be much lower at 2.857 % overall (Martin et al., 2013). The combined rates of complications are summarized in table 2. The most serious complication encountered following UAE is uterine infection (1.143%). This may present with severe acute pain, vaginal discharge and bleeding. Infection may lead to a systemic sepsis and may occasionally require emergency hysterectomy. Infection is very rarely associated with death due to overwhelming sepsis (less

than 1%) (Tropeano et al., 2008, Vashisht et al., 1999). A larger meta-analysis and systematic review found the risk of hysterectomy to be 0.7%, and a risk of venous thromboembolism to be 0.2% (Toor et al., 2012).

Complications	No. of cases	Rate (%)
Bilateral-failure UAE	14	4.000
Discharge, fever	14	4.000
Post-embolization syndrome	10	2.857
Pain	10	2.857
Groin complications	10	2.857
Repeat UAE	8	2.286
Fibroid expulsion	6	1.714
Uterine infection	4	1.143
Uterine artery dissection	2	0.571
Amenorrhea	2	0.571
Vesicovaginal fistula	1	0.286
Stress incontinence	1	0.286
Dyspareunia	1	0.286
Deep vein thrombosis	1	0.286
Severe vasovagal event	1	0.286
Haematometria	1	0.286
Pelvic abscess	1	0.286
Death	0	0.000
Unilateral-failure UAE	0	0.000
Total	87	24.86

Table 2: Complication rates of UAE in eight clinical trials (n = 350) (From (Martin et al., 2013)

The EMMY (embolization verse hysterectomy) trial was a multicentre randomised control trial of 177 women randomised to either UAE or hysterectomy (van der Kooij et al., 2010). Of the 89 women randomised to the hysterectomy group 63 women had their hysterectomies by the abdominal route, nine underwent vaginal hysterectomies and three had laparoscopic hysterectomies. Reported patient satisfaction was not significantly different between the UAE and hysterectomy groups; however the reported satisfaction may have been affected by the mode of hysterectomy performed. They found that by five years following embolization 28.4% of women required an additional surgical procedure to treat their fibroid symptoms. Other authors have highlighted that this study was performed by radiologists who were relatively inexperienced with UAE as a technique and that this may have contributed to the high re-intervention rate (Spies, 2006).

In 2011 the results of the REST trial (Randomised comparison of uterine artery embolization with surgical treatment in patients with symptomatic uterine fibroids) were published (Moss et al., 2011). They found similar improvements in symptom severity scores and similar safety profiles, but found a five year re-intervention rate of 32% compared with 4% in the surgery group. Further analysis of the five year data from this study found re-intervention was related to the degree of fibroid infarction produced as a result of

embolization, or non-perfused volume (NPV) observed following contrast enhanced MRI (Ananthakrishnan et al., 2012). Those women with less than 90% NPV at follow-up MRI had a 33% re-intervention rate at five years compared with a 19% and 10% re-intervention rate for the 100% NPV and 90-99% NPV cases. Assessment of NPV was made by a subjective assessment ("eye-balling") by two radiologists which would lead to significant biases in the assessment of NPV.

This study involved 106 women in the UAE group and 51 women defined as undergoing surgery for their fibroids, which included both myomectomy and hysterectomy. Since those women undergoing hysterectomy would not require further treatment for their fibroids, this would have introduced bias into this study's findings in terms of re-intervention rates, which could have been avoided had myomectomy alone been compared with UAE.

The HOPEFUL study was a multicentre retrospective cohort study looking at outcomes from 649 UAE cases and 459 hysterectomies performed for women with symptomatic uterine fibroids. They found fewer complications in the UAE cohort compared to the hysterectomy cohort. They defined severe complications as death, pulmonary embolus, myocardial infarction, stroke, and organ failure. The UAE group had a 0.2% rate of severe complications compared with 1.1% in the hysterectomy group. Major complications were defined as permanent amenorrhoea (over 40 years), blood transfusion, and damage to other abdominal organs, septicaemia and thrombosis. The rates of major complications following UAE were 4.3%, and 13.7% following hysterectomy. Among the 13.7% in the hysterectomy group, 7.4% had a major side effected listed as blood transfusion, and a further 2% simply classed as other, and not defined elsewhere in the document. Hysterectomy when performed for uterine fibroids is known to be a procedure involving significant blood loss and as such the requirement for blood post-operatively may not truly represent a major side-effect. The overall re-intervention rate by five years was 19.8% for UAE (Hirst et al., 2008). This data was retrieved retrospectively from nine radiologists from different units around the United Kingdom; at least one of these radiologists had subsequently stopped performing UAE after 2000. The data was from UAE procedures performed from the late 1990's until as late as 2002.

A recent prospective cohort study of women requiring treatment for their uterine fibroids reported that the re-intervention rate related to UAE was dependent on the percentage of fibroid devascularisation seen post-procedure at follow-up contrast enhanced MRI (non-perfused volume (NPV)) (Scheurig-Muenkler et al., 2012). Those with a complete or almost complete infarction of their fibroids had a 4 year re-intervention rate of 8-13%, compared with 43% re-intervention rate for those women with less than 90% NPV. Assessment of the degree of infarction was made by two independent radiologists blinded to the others findings. They were asked to quantify the fibroid NPV as a percentage between 0 and 100, based on their subjective estimation, rather than a more accurate, objective measurement of volume.

Evidence for the effects of UAE on fertility has been difficult to establish due to differences in procedure (varying size and material of particles) and problems with study design, as no randomised control trials with fertility as an endpoint have been performed. A recent review of cumulative data for 215 pregnancies following UAE in the literature (Homer H, 2009) found a cumulative live birth rate of 64.%, a 16.1 % preterm birth rate, 67.2% caesarean section rate and a 7.3% rate of fetal growth restriction. The miscarriage rate following UAE is 35.2% compared with women with fibroid-containing pregnancies matched for age and fibroid location 16.5% (Homer and Saridogan, 2010).

Premature ovarian failure is a recognised complication of UAE, and reports of amenorrhoea following treatment vary from 1-2% to 14% (Goodwin et al., 2006, Pron et al., 2003, Chrisman et al., 2000). Most recently a subgroup of the women in the REST study, examined 73 women undergoing UAE and 23 women undergoing surgery with ovarian conservation. Serum follicle-stimulating hormone (FSH) measurements were taken on day three of the menstrual cycle prior to treatment and at six and 12 months post-treatment. For women under 45 years, they found no significant difference in the ovarian failure rate at 12 months between UAE (11%) and surgical patients (18%) (Rashid et al., 2010).

Hysteroscopic visualisation of the endometrial cavity following UAE was described in 127 women (Mara et al., 2012a). Uterine fibroids were seen to protrude into the endometrial cavity in 35.4%, with 35.4% also demonstrating appearances of necrosis on histological examination. The authors suggested that hysteroscopic examination following UAE should be recommended in those women with persistent menstrual symptoms following UAE, or those women with fertility concerns.

Medical treatment

These treatments are generally based on improving the symptoms experienced, in particular heavy menstrual blood loss and pain and thereby improving overall quality of life. Medical treatment are currently recommend for use prior to using more invasive treatments, especially when treating heavy menstrual loss (guideline, 2007).

Non-steroidal anti-inflammatory drugs (NSAIDS)

Non-steroidal anti-inflammatory drugs (NSAIDS), in particular Mefanamic acid are very effective at reducing pain during periods and may reduce menstrual blood loss by 28.1% (Fraser et al., 1981). Irrespective of the presence of uterine fibroids, women with menorrhagia have higher levels of prostaglandin E2 and prostaglandin F2a compared with women with reportedly normal menstruation (Lethaby et al., 2007).

NSAIDS inhibit the enzyme cyclooxygenase, reducing prostaglandin levels, thereby reducing overall menstrual blood loss. The side effects of NSAIDs include gastrointestinal effects such as diarrhoea, constipation, heartburn and dyspepsia.

Anti-fibrinolytic agents

Tranexamic acid is an anti-fibrinolytic agent that has been proven to be effective at reducing menstrual blood loss by up 54% (Bonnar and Sheppard, 1996). Tranexamic acid is a synthetic lysine that results in the reversible blockade of lysine-binding sites on plasminogen molecules. It inhibits endometrial plasminogen activator and therefore prevents fibrinolysis and clot breakdown. The use of Tranexamic acid is associated with an increased incidence of infarct-type necrosis and thrombosis of leiomyoma (Ip et al., 2007).

Aromatase Inhibitors

Aromatase is the enzyme that catalyses the conversion of androgens to oestrogen via hydroxylation. Aromatase inhibitors therefore block the synthesis of oestrogen with a resultant decrease in fibroid size and symptoms (Gurates et al., 2008). A recent Cochrane review identified one un-blinded randomised control trial using the aromatase inhibitor Letrozole and although there appeared to be a reduction in fibroid size, and side-effects experienced compared favourably with Gonadotropin releasing hormone agonists (GnRHa), these differences were not significant (Parsanezhad et al., 2010). The authors of this review concluded that at present there is insufficient evidence for the routine use of aromatase inhibitors for uterine fibroids (Song et al., 2013).

Progestogens

The synthetic Progestogens norethisterone, levonorgestrel and medroxyprogesterone acetate have all been demonstrated to be effective at reducing total menstrual blood loss and can be used to stop bleeding altogether in some cases. The efficacy of oral progestogens is dependent on the timing of use. A Cochrane review found that progestogens given between day 15 or 19 until day 26 of the cycle offered no advantage over other medical treatments (e.g. Tranexamic acid), but progestogen therapy for 21 days of the cycle does

result in significant reduction in menstrual loss (Lethaby et al., 2008). The levonorgestrel-containing intrauterine system (IUS or Mirena) is a T-shaped device that delivers 20 micrograms of levonorgestrel per day and maintains its contraceptive effectiveness for at least five years (Varma et al., 2006). The contraceptive effects of the IUS are comparable to female sterilisation and are completely reversible (French et al., 2004). Assessment of effectiveness of the IUS in controlling blood loss secondary to uterine fibroids has demonstrated significant decreases in overall menstrual loss and increases in haemoglobin levels in those women using the IUS at one year (Varma et al., 2006). There is currently no good evidence that IUS effects fibroid size (Varma et al., 2006). One large multicentre RCT has suggested that there may be a decreased incidence of fibroids with the IUS compared with the non-hormonal copper intrauterine device, however this finding has not been found since (Sivin and Stern, 1994). Some papers have suggested that the IUS should be contraindicated in those women with significant intra-cavity sub-mucosal or intramural uterine fibroids, due to the potential risks of vaginal spotting or painful expulsion of the IUS (Hoellen et al., 2013), however there is currently no good evidence to support this. Women with intramural fibroids may also expel the IUS and at present no work has been done to assess whether the size or location of the uterine fibroid contributed the likelihood of IUS expulsion (Talaulikar and Manyonda, 2012). In a trial of 56 women on waiting lists for hysterectomy, there was open randomisation to either continue medical treatment or use the IUS (Lähteenmäki et al., 1998). This study found that 64% of women using the IUS cancelled their surgery compared with 14% not using the IUS. This study was limited by its relatively small size and recruitment of women with small fibroids (less than 3cm) and fewer than four fibroids in total.

Gonadotropin releasing hormone agonists (GnRHa)

Gonadotropin releasing hormone agonists (GnRHa) were initially developed in the 1970s (Hayden, 2008) and have been used to treat prostate cancer, endometriosis, hirsutism, dysfunctional uterine bleeding, premenstrual syndrome and have been used in assisted reproduction. GnRHa reduce menstrual blood loss, reduce some fibroid related symptoms and reduce fibroid and uterine size (Lethaby and Vollenhoven, 2005). Due to their oestrogen suppressive effects they do lead to menopausal symptoms and reduced bone mineral density, which make them unacceptable for long term use. These effects are, however reversible with cessation of use. GnRHa have been used for 2-4 months before myomectomy or hysterectomy, in order to reduce perioperative blood loss and surgical trauma by significantly reducing fibroid volume and vascularity of the fibroid uterus (Crosignani et al., 1996). A systematic review assessed GnRHa use 3 months prior to myomectomy or hysterectomy compared with placebo or no treatment (Lethaby et al., 2002). GnRHa compared with placebo or no treatment improves preoperative haemoglobin concentration (weighted mean

difference (WMD) 0.98g/dl), reduces intraoperative blood loss (WMD 67ml) and reduces the duration of the surgery (WMD 5.2 minutes) (Lethaby et al., 2000). A subsequent RCT of 162 women undergoing laparotomy and myomectomy found a reduced mean blood loss of 31ml between those receiving two doses of Triptorelin (a GnRHa) pre-operatively and those who did not. This was not a statistically significant reduction in intraoperative blood loss (Vercellini et al., 2003). Some papers have raised concerns regarding loss of the cleavage plane following GnRHa use and histologically there is evidence of a blurred interface between the myometrium and fibroids, with possible loss of the pseudo-capsule (Deligdisch et al., 1997). One small prospective observational study suggested a reduction in postoperative adhesion formation following GnRHa use before laparoscopic myomectomy (Imai et al., 2003). They demonstrated a significant decrease in plasminogen activator inhibitor (PAI) following GnRHa use and suggested that increased fibrinolytic capacity in peritoneal fluid reduces adhesion formation. A survey of 852 UK consultants in 2005 found that 87% prescribed GnRHa prior to open myomectomy and 58.6% used GnRHa prior to laparoscopic myomectomy (Taylor et al., 2005).

Selective progesterone receptor modulators (SPRMs)

Selective progesterone receptor modulators (SPRMs) have a structure that differs from endogenous progesterone, but are able to bind to progesterone receptors. Selective progesterone receptor modulators (SPRMs) include Ulipristal acetate (CDB-2914), which has minimal in vivo anti-glucocorticoid activity (Attardi et al., 2004). A recent RCT of once daily Ulipristal acetate verses placebo for 12 weeks found a reduction in fibroid volume of between 17-24% depending on the dosage (Nieman et al., 2011). Amenorrhoea occurred in 20 of the 26 women taking Ulipristal acetate by 12 weeks. This study investigated the endometrial biopsies of 21 women taking Ulipristal acetate and found that five of these 21 had some form of endometrial irregularity. One woman demonstrated cystic glandular hyperplasia, two women had progesterone receptor modulator-associated endometrial changes without atypia and another had cystic glandular changes at 3-6 months. A larger study examined endometrial biopsies of 156 women taking either 5mg or 10mg of Ulipristal acetate. Histological examination of the endometrial biopsies obtained at 13 weeks including altered structural glandular features and abnormal stromal vessels in many of these women, however by six months the endometrium had returned to a normal appearance (Williams et al., 2012). The long term endometrial effects of these medications need to be further investigated. Abnormal liver function tests and prolactin levels have also been demonstrated following short-term use, however initial reports suggest that these normalise rapidly after treatment cessation (Nieman et al., 2011). Although the initial licence for use of SPRMs is as a pre-operative adjunct in order to reduce fibroid size and symptoms prior to surgery, it is

possible that as experience with SPRMs growth, these may become part of the longer-term strategy for treating uterine fibroids.

Lifestyle

Many of the symptoms experienced by women with uterine fibroids may be described as subjective. The extent to which women find their menstrual bleeding problematic may vary considerably, and it is vital that the physician assesses how these symptoms are interrupting the women's normal quality of life. By doing so, an individualised plan of treatment may be made.

General lifestyle measures such as weight-loss and smoking cessation in the first instance may significantly help improve quality of life issues. In addition, by reducing BMI and improving the general health of the women, many of the risks associated with medical and surgical interventions can be reduced.

Alternative treatments

Chinese and Korean herbal treatments using Kuei-chih-fu-ling have been used for many years to treat the symptoms of uterine fibroids (Liu et al., 2013). These preparations contain cinnamon bark, cassia root of Peonia lactiflora, seeds of prunus persica, carpophores(stalks) of Poria Cocos and root bark of Paeon suffruticos and have been used to decrease symptoms of menorrhagia and reduce fibroid size (Brosens and Pinn). One study suggested that the effects of these treatments may be based on Luteinising hormone releasing hormone (LHRH) antagonist properties and a weak anti-oestrogen effect detected by animal models (Sakamoto et al., 1992). No robust evidence has as yet been provided for these treatments. A recent Cochrane review of 21 randomised control trials using these treatments for three to six months found insufficient evidence to support the routine use of these preparations, but suggested more robust study of their effects was warranted (Liu et al., 2013).

Thermal ablation techniques

The destruction of tissue by thermal injury has been achieved by using radiofrequency energy, focused ultrasound and microwave energy. Whereas uterine artery embolization results in tissue infarction with associated disruption of cell membranes and the leakage of intracellular contents, thermal ablation results in thermal fixation, resulting in coagulative necrosis and preservation of cellular architecture (Jones et al., 2012). The first use of high temperatures to ablate fibroid tissue was myoma coagulation or myolysis

involved the use of a neodymium-doped yttrium aluminium garnet (Nd:YAG) laser, used laparoscopically or hysteroscopically (Donnez et al., 1990, Goldfarb, 1995). This technique results in destruction of the fibroids local vascular supply and produces a coagulative necrosis. Usually women would be given three months of GnRHa prior to treatment, and would require an average of 30-50 needle insertions per fibroid, over a period of 20 to 30 minutes (Goldfarb, 1995). This method of treating uterine fibroids was however associated with a high incidence of post-operative adhesion formation, presumed to be related to the multiple incisions through the serosa required to treat the uterine fibroids (Nisolle et al., 1993b). The risk of adhesion formation in addition to the reported cases of uterine rupture in pregnancy following this treatment have, limited the appeal of this modality (Arcangeli and Pasquarette, 1997, Vilos et al., 1998). Other concerns regarding this method of treatment include the inability to accurately predict the size of ablated tissue, or to visualise the effect of heating on tissues during the treatment process, leading to concerns regarding the reproducibility of treatments and safety.

MRI-guided percutaneous insertion of diode lasers were developed in order to better visualise the area under treatment, and perform a more controlled procedure (Law et al., 1999) . These use light in the near infrared spectrum at a wavelength of 810 nm, and 18-gauge Turner needles introduced into the centre of the targeted fibroid under MRI guidance in a square configuration 1 cm apart. Mean treatment times are around 15 minutes and initial results were very encouraging with a mean reduction of uterine fibroids volume of 31% (Hindley et al., 2002). By using MR imaging to allow real-time thermal mapping of the uterus throughout the treatment, operators were able to achieve increased ablation volumes within the target tissue with minimal risk of damage to the serosa or any adjacent structures, which can be clearly visualized in relation to the area of treatment.

A trans-cervical radiofrequency ablation device has been developed, known as the VizAblate System™ (Gynesonics; Redwood City, CA). This system uses an intra-uterine ultrasound probe and an intra-uterine radiofrequency generator, which allows real-time ultrasound imaging of the uterus and fibroid tissue while the VizAblate needle electrodes pass under ultrasound guidance into the target fibroids. When a safe position has been confirmed the radiofrequency energy is delivered directly into the fibroid for a set period of time, depending on the fibroid size (Garza-Leal, 2011). This system produces ellipsoidal ablations of up to 5x4cm in size and heats the tissue to a temperature of 105°C, and the size of these ablations can be adjusted depending on the depth of the needle. This system calculates a thermal safety zone around the fibroid which prevents ablation of myometrium or uterine serosa and therefore minimises the risks of adhesion formation or uterine damage. Fibroids of up to 5cm in size can be treated and the procedure takes around 30 minutes to complete (Garza-Leal, 2011). A clinical trial of this device is on-going (Gynesonics, 2013).

High intensity ultrasound can be focused into a small point to produce a rise in tissue temperature sufficient to cause irreparable cell damage in the target at depth within the body (Lynn et al., 1942). Magnetic resonance guided focused ultrasound (MRgFUS) is a non-invasive thermo-ablative technology which has been used in the treatment of various benign and malignant soft tissue tumours (Jolesz and Hynynen, 2007). The first report of the use of MR imaging to direct focused ultrasound in the treatment of tumours was described in 1995 (Cline et al., 1995). MRgFUS has been proven to be a safe, cost-effective treatment option for uterine fibroids (Zowall et al., 2008). Initial research suggests that MRgFUS is a fertility-sparing treatment, with encouraging results for those women conceiving after treatment (Rabinovici et al., 2010).
MRgFUS uses ultrasonic waves created in tissue as a source of thermal injury. Ultrasound is cyclical sound pressure at a frequency greater than the upper limit of human hearing (approximately 20,000 hertz). Ultrasound waves are generated by a transducer, a device that converts electrical transmission pulses into ultrasonic pulses. The transducer contains a piezoelectric plate that generates the ultrasound pulse. Piezoelectric materials expand or contract proportionally when a positive or negative charge is applied (Hoskins et al., 2010). It is this expansion and contraction that results in the production of ultrasound waves. When an ultrasound wave passes through a medium, the particles of that medium oscillate back and forth along the direction of propagation of the wave. As particles cannot move instantaneously, they are unable to move in complete synchronicity with the passing wave. Because the particles of the medium are unable to return all of the energy associated with their movement back to the wave, a small amount of the total energy of the wave is retained by the medium, which is then manifest as heat. Diagnostic medical ultrasound commonly uses ultrasound frequencies in the range 2-15 MHz. In MRgFUS frequencies of between 0.9 and 1.15MHz MHz are used. MRgFUS uses 5-10,000 times the power of diagnostic ultrasound. The intensity of the ultrasound beam is a measure of power flowing through an area of the beam, which is measured in Wcm^2. The intensities of typical diagnostic ultrasound scanning (B-mode, pulsed or continuous Doppler) can be up to 720 $mWcm^{2.}$ In contrast, the intensity of focused ultrasound in the focal region is about 100-10 000 Wcm^2 (Zhou, 2011), over 10,000 times the power of conventional diagnostic ultrasound.

Ultrasound waves can be focused either via a lens, a curved transducer, or a phased array into a small focal zone, in a similar way to light being focused through a magnifying glass to a single point. A phased array transducer produces either a fan-like spread outwards from the transducer face, or in the case of MRgFUS a convergence of a beam to a focal point (Figure 10).

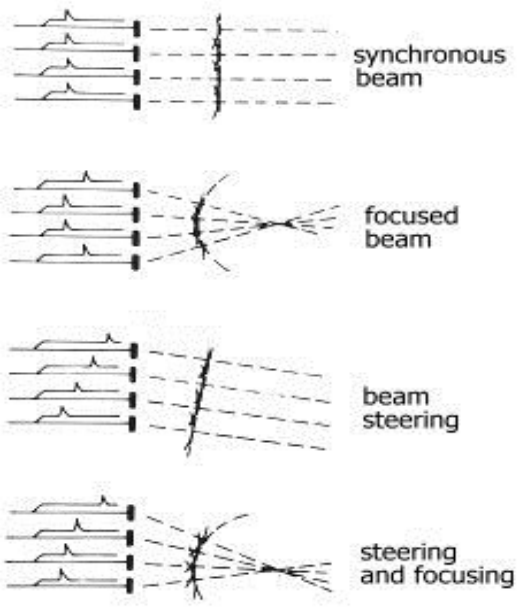


Figure 10: Beams with differing phases results in beam shaping

The phased array transducer is constructed using multiple parallel transducer units (Hoskins et al., 2010). The ExAblate system used at St Mary's uses a 211 element phased array transducer (InSightec, 2010). Each beam is at a specifically different phase to the preceding beam. As the different signals pass through a transducer array at their different phases, they are each refracted by a different degree; therefore it is possible to have these signals converge at a single point. By using ultrasound with energies of up to 7000 Joules focused into a cigar-shaped focal spot of between 2x2x4mm^3 and 10x10x70mm^{3,} the tissues within the focal point are heated to greater than 58°C for a period of one second. These focal spots are often referred to as sonications and will be in the remainder of this thesis. The thermal threshold of 58^{o}C was established as the minimum temperature at the edges of a sonication (Hill et al., 1994). Temperatures at the centre of the sonication may be greater than 90^{o}C (ter Haar, 1995). This results in coagulative necrosis and apoptotic cell death of the targeted tissue. The local tissue structure and vascular content, through which the beam passes, have a large effect on the tissue temperatures reached. In soft tissue the rate at which energy is absorbed per unit volume (q_v), depends on the amplitude absorption coefficient of the tissue (α_0) the acoustic frequency of the pulse(f) , and the intensity (I) (Hoskins et al., 2010).

$$q_v=2\alpha_0 fI$$

The absorption coefficient of tissues vary significantly with bone being the tissue that absorbs most ultrasound energy, fluids such as blood absorbing very little ultrasound energy and soft tissue like myometrium falling someway in between (Hoskins et al., 2010). When the ultrasound is applied, the energy is absorbed at a rate that is directly proportional to the local intensity, meaning that the highest rise in temperature will be at the focus of the beam. The temperature continues to rise as more energy is absorbed; although some heat is lost by the process of conduction to surrounding cooler areas. The rate of heat loss by conduction will nearly cancel out energy absorption after about 30 seconds. Blood vessels are effective conductors of heat, moving heat away from the point of delivery, thereby acting as an effective cooling system. It is known that the signal intensity of fibroids can correspond to the density of vasculature within the fibroids (Yamashita et al., 1993). The signal intensity, and hence the possible vascularity of uterine fibroids is a known predictor of the success of MRgFUS (Lénárd et al., 2008).

In addition to the thermal effects, focused ultrasound may also have mechanical effects in tissue. At very high temperatures high intensity ultrasound can lead to the creation of a gas cavity within the tissue known as a cavitation. Gas cavities are the result of the alternating compression and expansion of tissue as an ultrasound burst propagates through it. There are two forms of cavitation: stable and inertial cavitation (Zhou, 2011). Stable cavitation refers to stable oscillation of the size of the gas bubble when exposed to a low-pressure acoustic field. The oscillating motion of stable cavitation causes the rapid movement of fluid near the bubble, known as the micro-streaming effect. The micro-streaming of fluid results in high shear forces that can result in transient damage to cell membranes. Inertial cavitation involves violent oscillations of the gas bubble and rapid growth of the bubble eventually leading to their violent collapse and destruction. The violent collapse results in shock waves of very high pressure and temperature that are transmitted to the surrounding tissues. In addition to these two forms of cavitation, radiation force is developed when an acoustic wave is either absorbed or reflected (Zhou, 2011).

High intensity focused ultrasound has been used with both ultrasound guidance and MRI guidance.
MRI enables assessment of the pelvic anatomy in three dimensions, allowing accurate targeting of the uterine fibroids. By using MRI the ultrasound beam path can be visualised, therefore enabling the avoidance of such structures as the bowel that may be damaged (Figure 11).

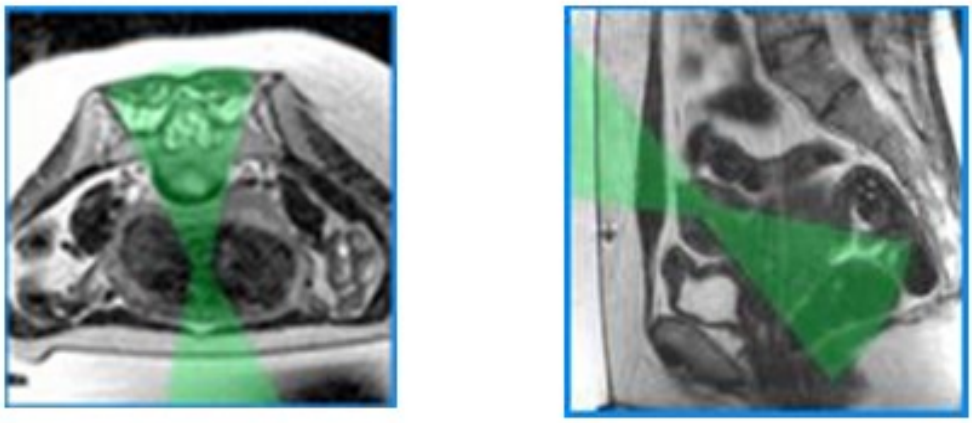


Figure 11: Visualising the beam path

MRI allows real time thermometry of the tissue to achieve the planned outcome (Figure 12). MR thermometry is based on the temperature dependence of the proton-resonance phase shift (McDannold, 2005, Ishihara et al., 1995). As the temperature of tissue rises, the phase changes accordingly.

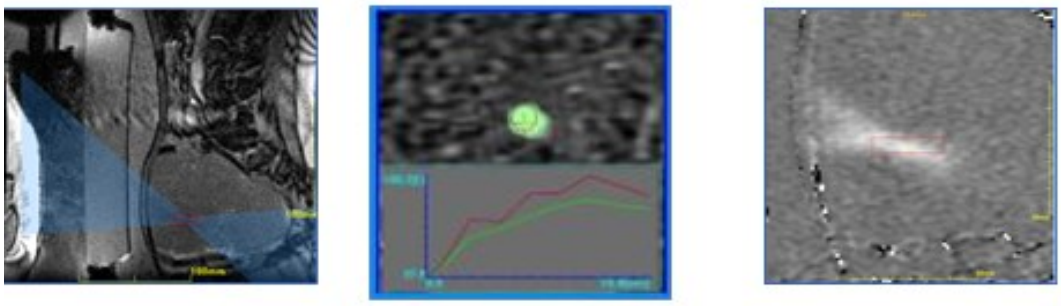


Figure 12: Real time thermometry employed during MRgFUS

MRI allows post-treatment assessment of treatment outcome. Following the completed sonications an intravenous contrast agent is given and T1-weighted images of the pelvis are obtained. From these the degree of non-perfused volume of the fibroid can be assessed (Figure 13).

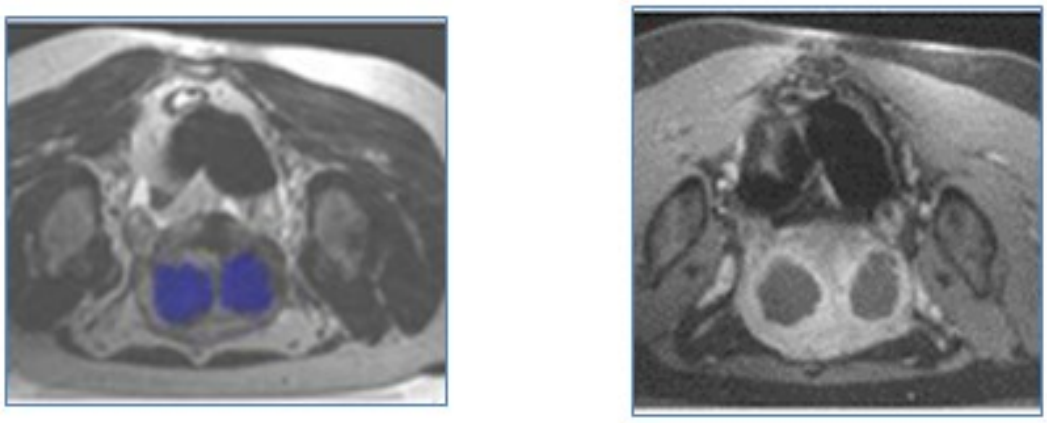


Figure 13: Post treatment Non-perfused Volume (NPV)

The aim of MRgFUS is to treat the largest possible volume of fibroid tissue with the greatest safely. Following treatment, contrast agents are given to measure the non-perfused volume (NPV) of the fibroid tissue; which is the area of fibroid tissue with no functioning blood supply. At present, NPV of approximately 40-80% of the fibroid volume is usually achieved. The remaining fibroid volume that is not treated will continue to grow. In addition to this area of fibroid tissue, any remaining smaller fibroids may also continue to grow post treatment.

At present evidence of efficacy of this treatment comes from cohort studies only, and long-term follow-up data is restricted to two years data only (Funaki et al., 2009a).

The first MRgFUS treatment of uterine fibroids using the ExAblate 2000 system was performed in 2001. Since then, more than 5000 treatments have been performed in more than 60 different units around the world. The experience accumulated in MRgFUS was collected by the company InSightec and implemented into software and hardware updates and guidelines, all aimed to improve the clinical results, while maintaining a high level of safety.

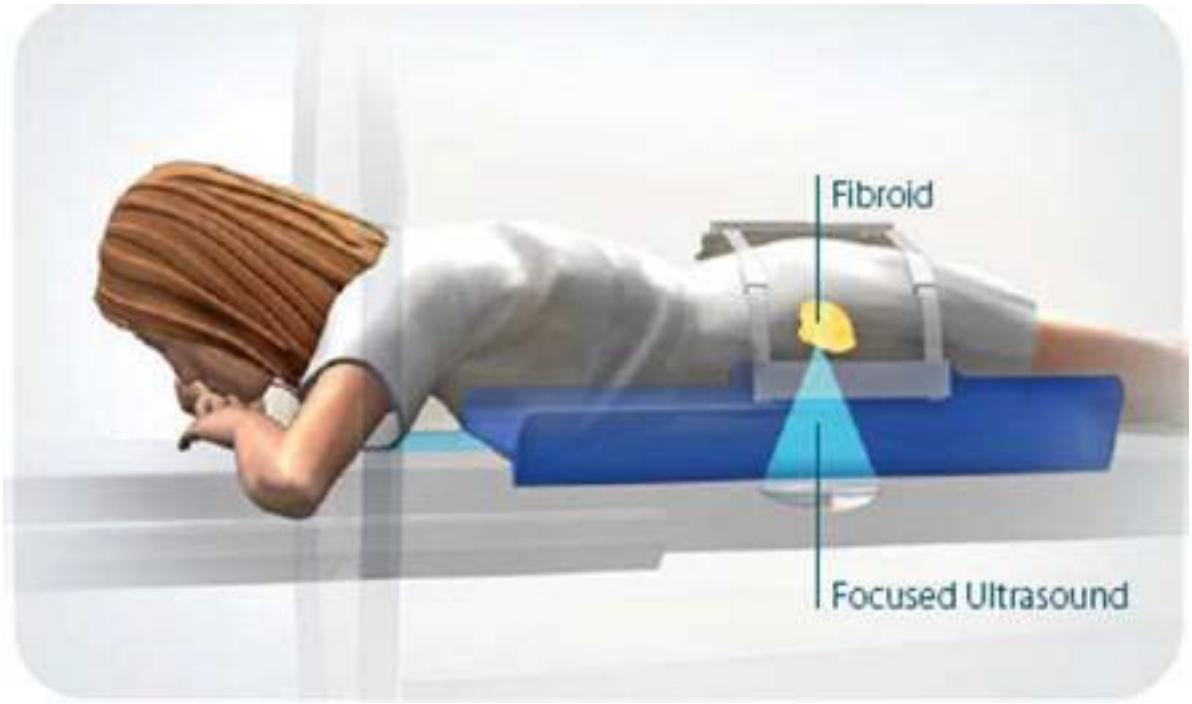


Figure 14: MRgFUS (image from InSightec)

Based on clinical experience, research and development, changes have been made to the current ExAblate system. This modified ExAblate system version is designated as the ExAblate 2100 UF V2 system. In January 2011 the new ExAblate 2100 UF V2 system was introduced at St Mary's Hospital, London.

Aims and Objectives

In this study I will:

1. Investigate the most accurate method of measuring fibroid volumes. I will also propose a new classification system for describing the appearance of fibroid uteri.

2. Retrospectively review the demographics and fibroid characteristics of a cohort of uterine fibroid subjects seen at a London tertiary referral centre. Fibroid uteri will be classified by volume, number of fibroids, signal intensity and pattern of fibroid distribution. Treatment used will be assessed in relation to these features.

3. Perform a longitudinal analysis of MRgFUS results, in terms of re-intervention rate, symptom severity and patient satisfaction. In addition to the outcomes a review of the safety and adverse outcomes since the introduction of this treatment at St Mary's Hospital. Treatments with the new ExAblate 2100 UF V2 system will be compared to results from the UF 1 system.

4. Assess the relationship between fibroid volume treated, patient pain scores and circulating cytokine levels following MRgFUS and UAE.

5. Assess the circulating vascular endothelial growth factor levels (VEGF), following UAE and MRgFUS and examine whether this relates to the fibroid volume treated by these methods and tumour regrowth following treatment.

2. Classification of uterine fibroids and measurement of fibroid volumes and non-perfused volume following MRgFUS

Introduction

Although the most common tumour affecting women, the clinical types of fibroid uterus encountered varies significantly, and these different types of fibroid uteri may present with different symptoms requiring potentially different treatments. Women presenting with a solitary sub-mucosal (SM) fibroid will require different management from an individual with over 30 fibroids scattered throughout the uterus. Information from MRI and ultrasound regarding the fibroid volume and positions will inform the management of these women. Beyond the simple description of a fibroid in relation to the uterine wall, number and sub-classification of SM fibroids there is little consensus on how best to describe fibroid subgroups (Lasmar et al., 2011). The size and shape of uterine fibroids vary considerably, and this makes measuring the volumes of these tumours particularly problematic. Following treatment by MRgFUS an intra-venous contrast agent (e.g. Gadolinium) is given to assess the area of non-perfused tissue following ablation, the non-perfused volume (NPV) of the fibroid. Throughout this thesis I was required to measure the volumes of individual fibroids, fibroid uteri and the NPV of fibroids post-treatment. I also sought to establish whether a classification system of fibroid uteri based on the numbers and sizes of fibroid would be a reproducible method of describing fibroid uteri. To this end I set up two experiments, firstly to establish which method of volume estimation of irregular objects was the most accurate, and secondly whether the intra-observer agreement between volume calculations for fibroids and fibroid uteri classification was acceptable.

Volume calculation methods

Traditionally the size of fibroids and uteri have been described by either their maximum diameter, or volume as calculated by ellipsoid formula or parallel planimetric area method (Cavalieri method) (Geirsson et al., 1982).

The ellipsoid formula estimates the volume of an object by assuming an ellipsoid shape (Figure 15). The volume of an ellipsoid (v) is calculated as: $v = 4/3\pi abc$ where a, b and c are the maximum diameters in three planes (Figure 15). To calculate the volume using the ellipsoid method, the maximum diameter of each object in three separate planes was recorded for each object and the volume calculated using the formula above.

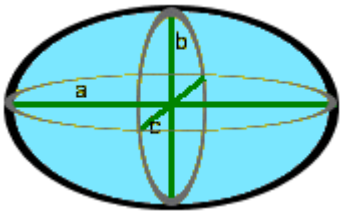


Figure 15: An ellipsoid

The parallel planimetric area method (Figure 16) calculates a volume based on the sum of the multiple areas recorded on either MRI or computerised tomography (CT) images, multiplied by the thickness of each individual slice. An interpolation formula is then applied by the software to smooth the boundaries of the object between slices, a feature which more accurately represents the true shape of the object.

The total volume (V_{total}) is equal to the sum of the volumes of each individual slice.

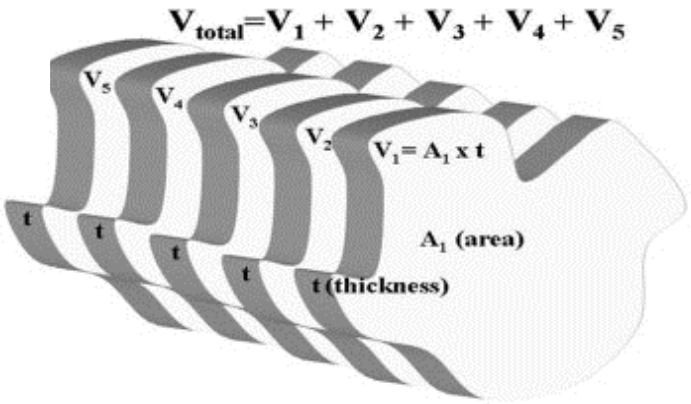


Figure 16: Parallel Planimetric Method of calculating total volume of an irregular shape

This first study was conducted in three parts:

1. I compared the parallel planimetric area and ellipsoid formula to a gold standard (volume of water displacement) to assess the relative accuracy of each method. I also assess the inter-observer variability of these measurements as part of this evaluation.

2. I compared the inter-observer correlation of the parallel planimetric area method in estimating the volumes of a variety of randomly chosen fibroid uteri and non-perfused volumes post MR guided focussed ultrasound treatment.

3. I produce a new classification system for fibroid uteri based on the numbers of uterine fibroids within the uterus and the pattern of fibroid presentation. I then investigated the intra-observer agreement in describing these fibroid uteri.

Materials and Methods

The volumes of 50 different irregularly shaped objects were calculated using the water-displacement method. To reproduce the organically irregular shape of fibroid tumours, a variety of differently shaped fruit and vegetables were measured (potatoes, courgettes, plums, apples, avocados and sweet potato –see Figure 17). The volume of water displaced by these objects was recorded by two independent observers (SQ and JV), blinded to the others results (see Figure 18). The volume of normal saline displaced by the objects was calculated using a 1000ml plastic measuring cylinder with 5ml gradations. Following this, all objects were scanned by tri-planar T2 weighted MRI (GE Healthcare, Milwaukee, USA). MRI was performed within four hours of the volume displacement measurements.

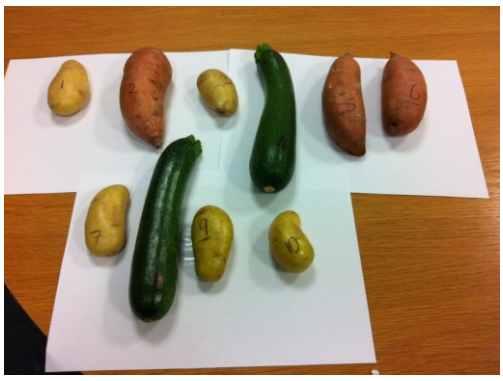


Figure 17: Examples of objects used in initial assessment of methods

Figure 18: Volume estimation using displacement method

The MRI images were then uploaded into a software package for planimetric volume assessment, (GE Reportcard©). Using this software the area of each slice was calculated by assigning multiple points on each

sagittal slice around the perimeter of the object and a total object volume calculated automatically by the program using the software's interpolation formulae (Figure 19).

The inter- and intra-observer agreement was described using the coefficient of variation. Statistical graphs and calculation were produced using MedCalc Software Version 12.1.0 - © 1993-2011.

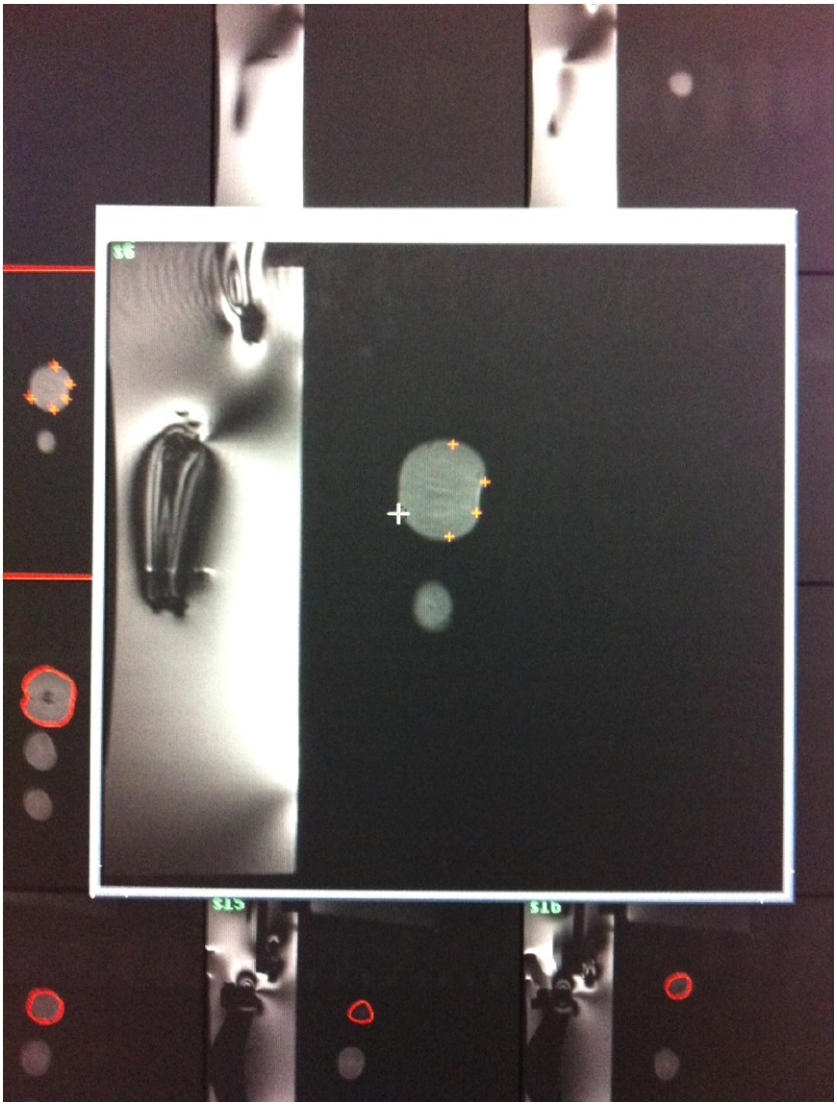


Figure 19: Reportcard© software and outline of marking the periphery of each object (red line surrounding object in background) for planimetric volume assessment.

Following this initial assessment of methods, the MR images of 50 women with uterine fibroids undergoing treatment for MRgFUS were then examined by two independent investigators at different times (SQ and JV), blinded to the other`s findings. Each observer used the parallel planimetric method to calculate the total uterine volume (TUV), volume of the largest fibroid (VolFib) (see figure 20) and final non-perfused volume apparent following administration of the gadolinium contrast agent post procedure (see figure 21). The area

of uterus, fibroid or NPV was marked on each sequential sagittal slice. The volume was then calculated by multiplying the area by the slice profile (3mm slice thickness plus 3mm intersection gap). A total volume was automatically calculated by the Reportcard© software by summation of the adjacent volumes and application of interpolation formulae.

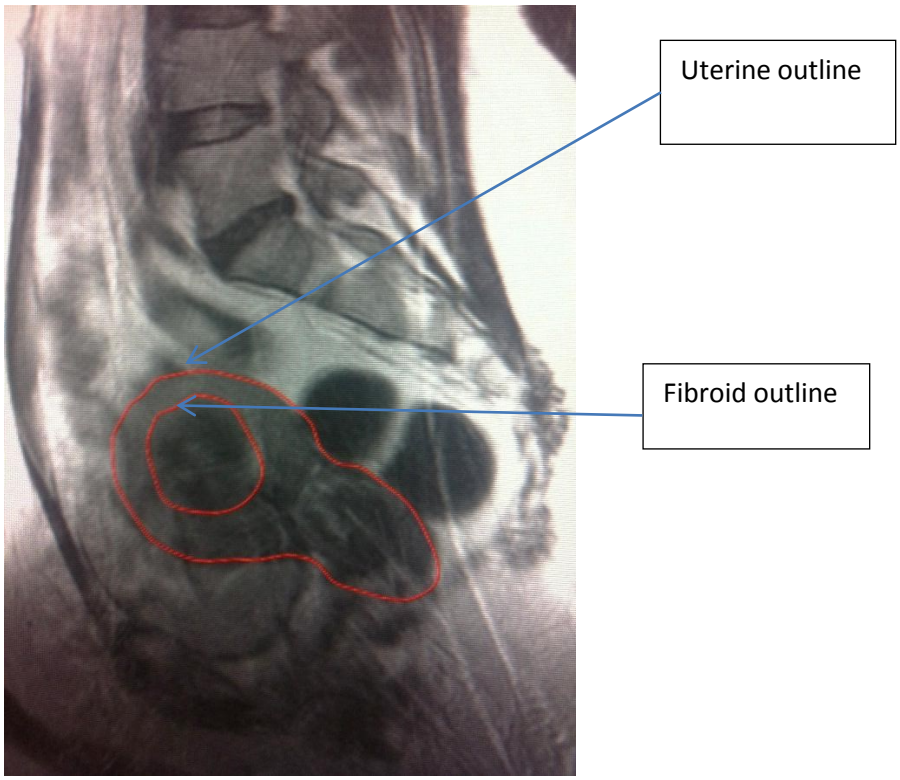


Figure 20: Uterine and fibroid area on Reportcard© software

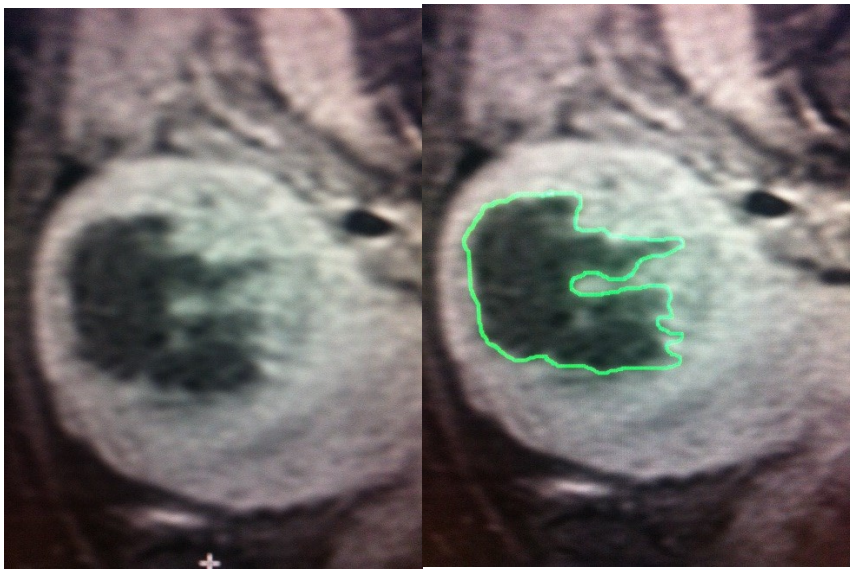


Figure 21: NPV outline marked post-contrast

In addition, both investigators were required to assign each uterus a category based on the number of fibroids and the presence of a single dominant fibroid, or multiple fibroids of similar sizes (see Table 3).

Uterine category	Description
1	Single fibroid within the uterus
2a	2-5 fibroids with a single dominant fibroid, and additional fibroids of less than 2cm diameter
2b	2-5 fibroids with multiple fibroids of greater than 2cm diameter
3a	6-10 fibroids with a single dominant fibroid and additional fibroids less than 2cm in diameter
3b	6-10 fibroids with multiple fibroids greater than 2cm diameter
4	11-20 fibroids
5	Over 20 fibroids

Table 3: Uterine category classification

Examples of MRI images for the different uterine categories are given below, on the next pages.

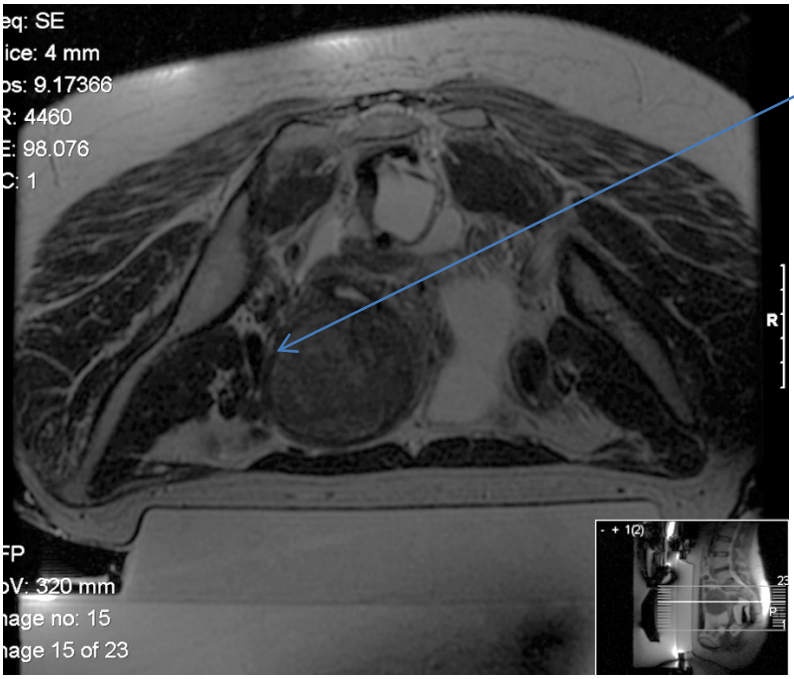


Figure 22: Category 1 Fibroid Uterus on axial MR image

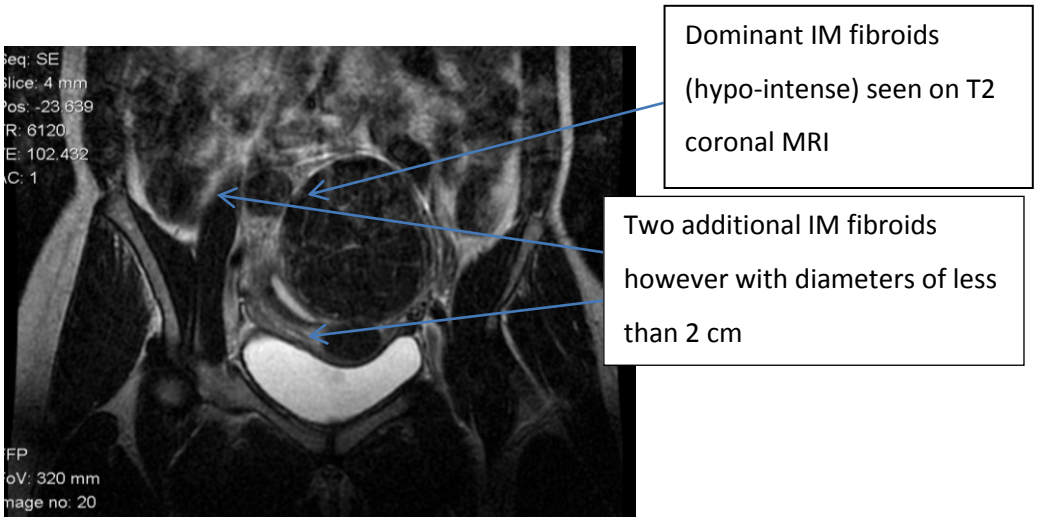


Figure 23: Category 2a Fibroid Uterus on coronal MR image

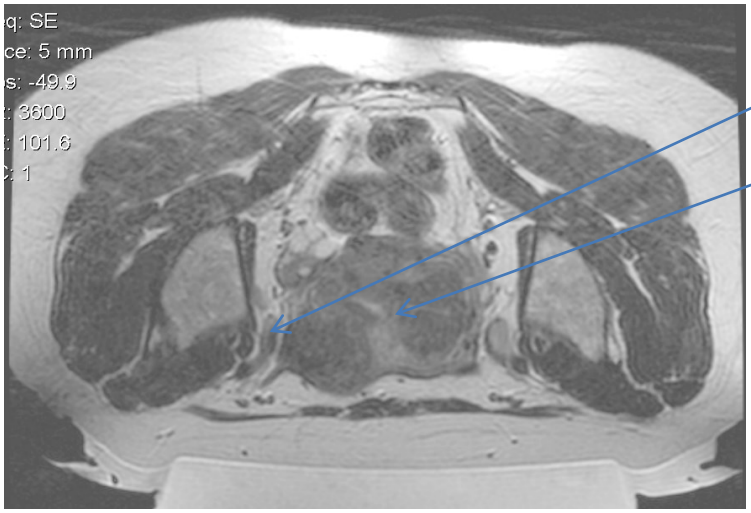


Figure 24: Category 2b Fibroid Uterus on T2 Axial MRI

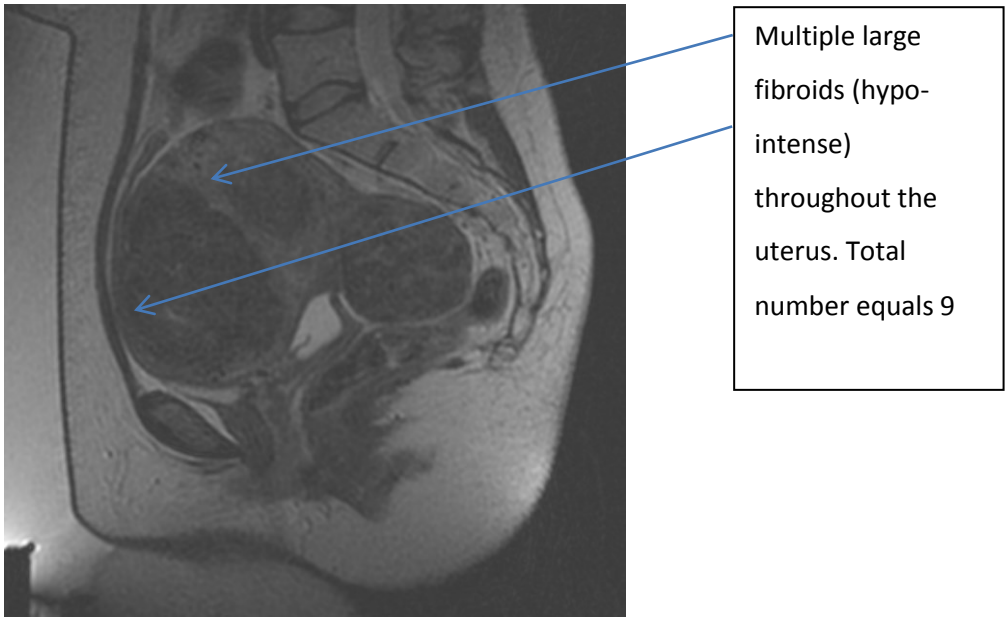
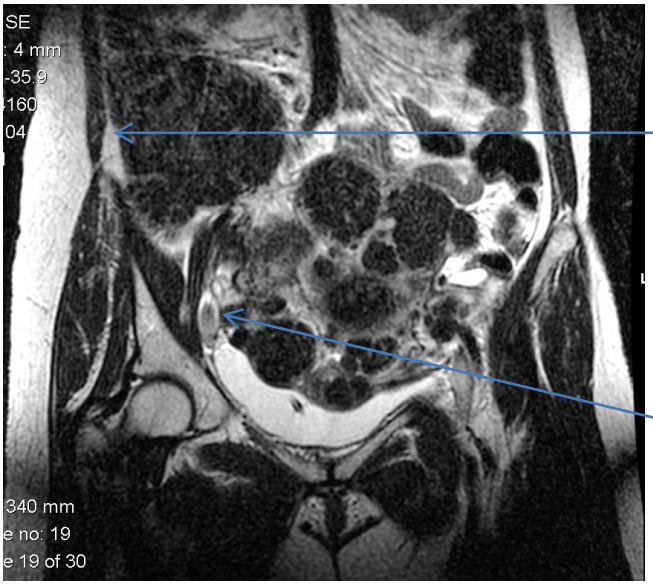


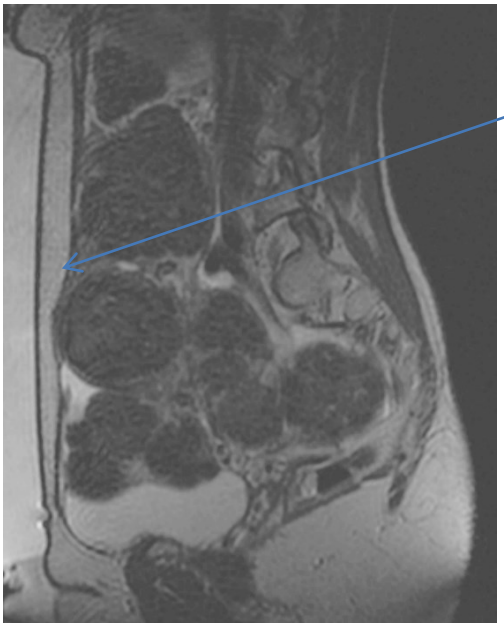
Figure 25: Category 3b Fibroid Uterus on sagittal T2 MRI



Large (7cm) SS fibroid on right, demonstrated by T2 coronal MR Image

Multiple smaller intramural and sub-mucosal fibroid throughout the uterus (hypo-intense).

Figure 26: Category 5 Fibroid Uterus on coronal T2 MRI



Same fibroid uterus as figure 26, however on T2 sagittal imaging.

Figure 27: Category 5 Fibroid Uterus, sagittal T2 MRI

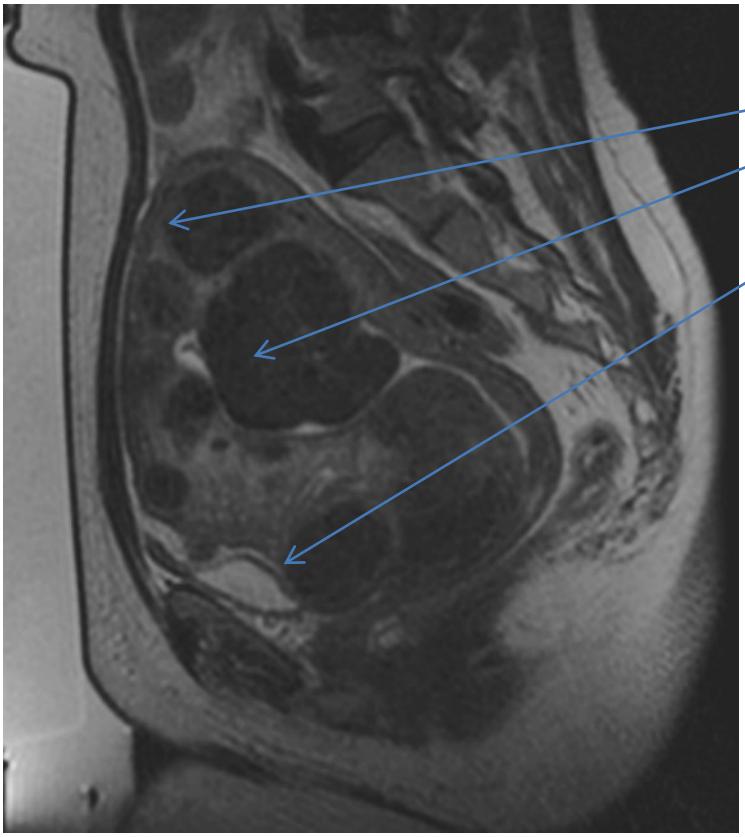


Figure 28: Category 4 Fibroid Uterus

Statistical analysis

The degree of agreement between different methods of volume calculation was assessed according to the method of Bland and Altman (Bland and Altman, 1986), plotting the difference between each result versus the mean of the two results. Pearson correlation coefficients were also used to compare measurements made by the two readers, and linear regression was used to obtain the individual slope and 95% confidence intervals (CI). A p-value of less than 0.05 was considered statistically significant. Statistical agreement between the nominal values of uterine category between observers was calculated using Cohen's Kappa coefficient. Data analysis was performed using commercially available software (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp and GraphPad Prism 5.0, CA, USA).

Results

The range of volumes measured in the 50 objects (fruit and vegetables) group was 35ml to 270ml as measured by volume displacement method.

A summary of the measured volumes is given in table 4. Measurement by the Ellipsoid calculation gave lower mean volume overall.

	Volume Plannimetric SQ	Volume Plannimetric JV	Volume Displacement SQ	Volume Displacement JV	Volume Ellipsoid SQ	Volume Ellipsoid JV
Mean (mL)	99	99	102	101	86	85
Standard Deviation	55	54	55	55	38	42
Range (mL)	31-269	35-265	36-267	35-270	20-165	28-229

Table 4: Measurements by SQ and JV by alternative methods

The difference between the volume calculated by observer, a radiologist (JV), and a gynaecologist (SQ) was plotted against the mean of their two results (Figure 29. NB Bland Altman plots for other investigations are included in Appendix C). Volume calculated by the displacement method by JV (VolDisJV) and the volume calculated by the displacement method by SQ (VolDisSQ) is represented by Figure 29. In all cases, the difference between observed volumes fell between 1.96 standard deviations of the mean (0.01ml). From this we concluded that there was no significant disagreement between investigators by the volume displacement method.

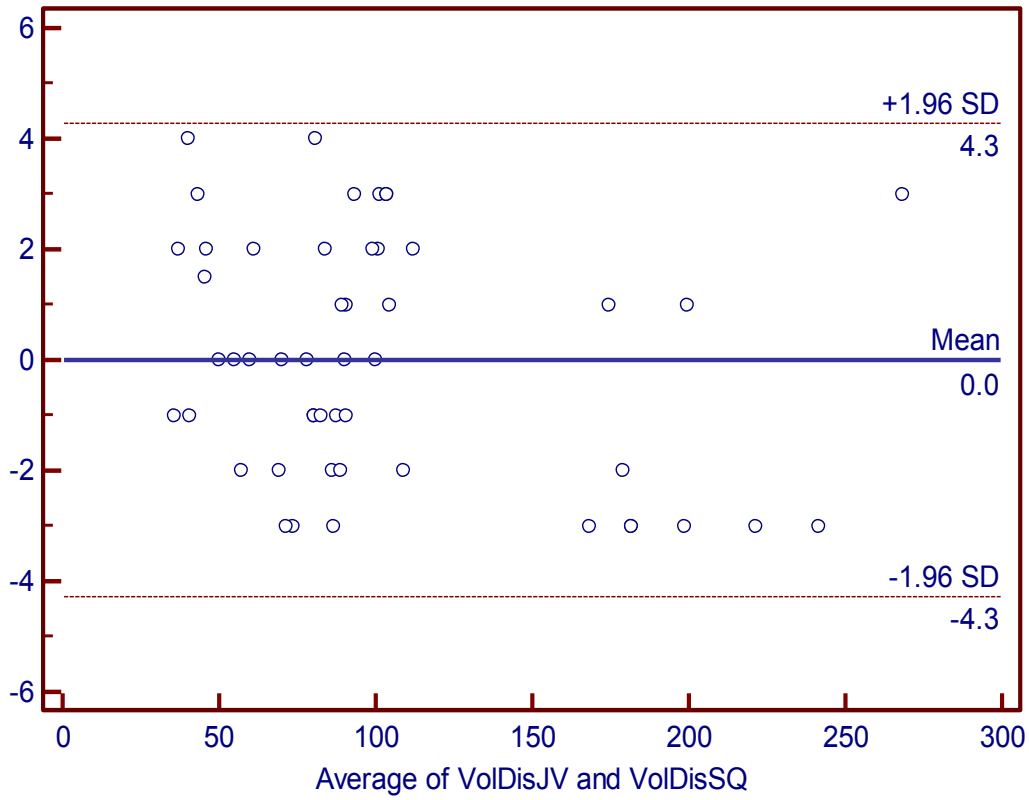


Figure 29: Bland Altman for volume of object calculated by saline displacement method

The mean difference was 0.01ml, with 95% confidence intervals (CI) of -0.6101 to 0.6301 and a standard deviation (SD) of 2.1818. The mean volume calculated by SQ and JV for each object was used as the standard by which we assessed the other methods of volume calculation.

When we compared the mean volumes between observers by the displacement methods and parallel planimetric methods we constructed a Bland-Altman plot (Figure 30) and found the mean difference was 2.63ml, with 95% confidence intervals (CI) of 1.92 to 3.34 and a standard deviation (SD) of 2.58.

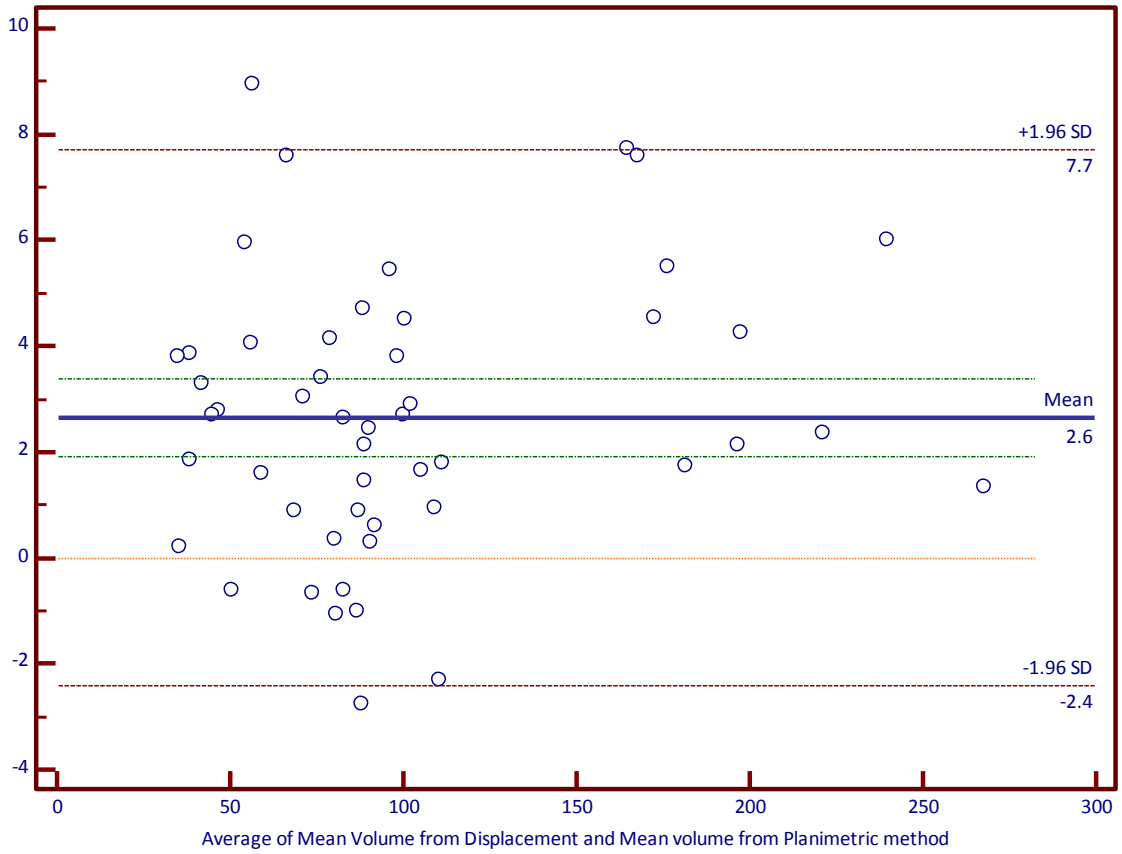


Figure 30: Bland-Altman plot of Mean Displacement volume verses Mean Planimetric volume

Comparing the mean volume by the displacement method with the mean volume by the ellipsoid method gave a mean difference was -16.08ml, with 95% confidence intervals (CI) of 9.97 to 22.19 and a standard deviation (SD) of 22.05. The Bland-Altman plot for this comparison is given in Figure 31.

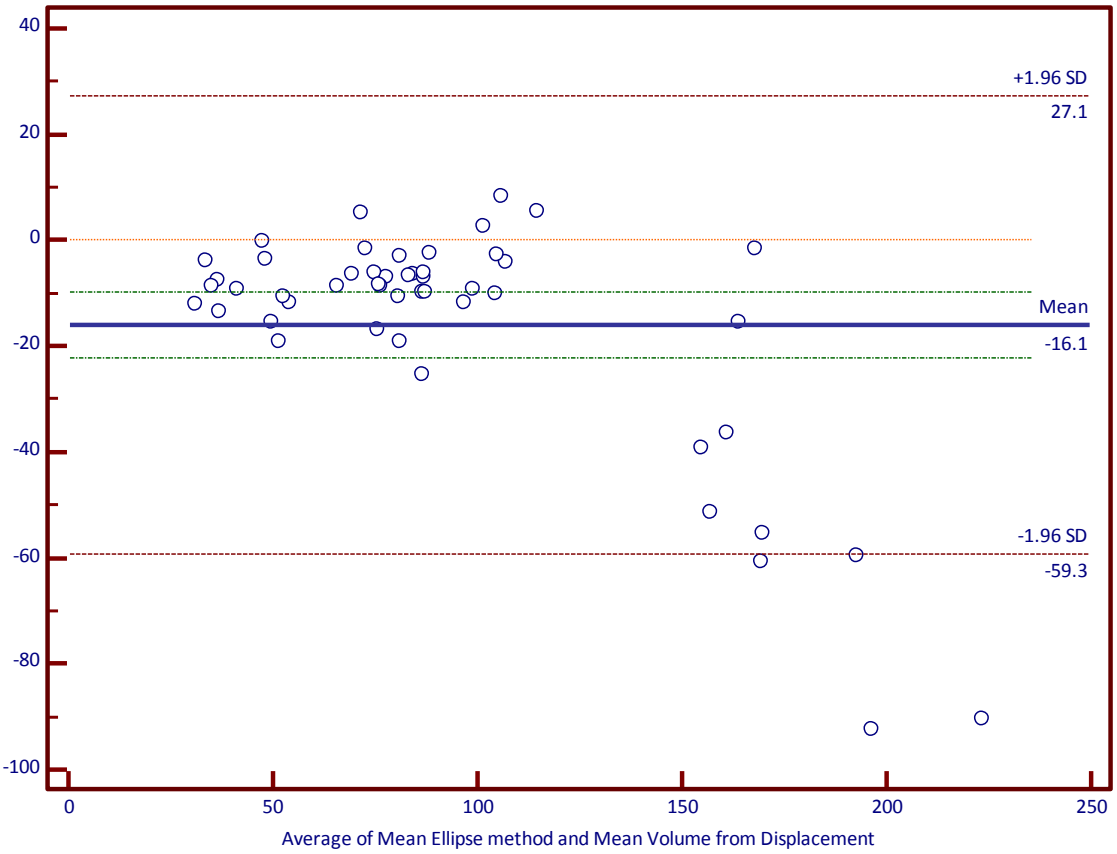


Figure 31: Bland-Altman plot for Mean volume by displacement method verses mean volume calculated by ellipsoid calculation

We performed seven pairwise comparisons of method, firstly comparing the results between investigators for each method, and then for each observer between their volume calculations by displacement method and parallel planimetric method and ellipsoid method respectively (Table 5).

Comparison	Mean Difference*	ICC[Ŧ]	95% Confidence intervals for ICC	Bland Altman Analysis		
				Bias	SD of Bias	95% limits of agreement
Volume displacement method JV vs. SQ	-0.05 ± 4.59	0.997	0.994-0.998	0.05	4.58	-8.94-9.04
Planimetric method JV vs. SQ	-0.28 ± 6.59	0.993	0.988-0.996	0.28	6.593	-13.20-12.64
Ellipsoid method JV vs. SQ	-0.4 ± 20.3	0.878	0.794-0.929	0.40	20.3	-40.19-39.39
Volume displacement vs. Planimetric Method						
SQ	2.52 ± 4.14	0.997	0.995-0.998	2.52	4.138	-5.59-10.63
JV	2.75 ± 3.14	0.998	0.997-0.999	2.75	3.145	-3.414-8.39
Volume displacement vs Ellipsoid method						
SQ	15.90 ± 28.75	0.822	0.706-0.895	15.91	28.73	-40.41-72.22
JV	16.25 ± 19.06	0.926	0.874-0.958	16.26	19.06	-21.10-53.61

*Data are means ± SD [Ŧ]Interclass Correlation Coefficient (ICC)

Table 5: Correlation between methods and observers for objects

Comparing the two different methods the planimetric method was, by a considerable margin, the method which showed not only closest approximation to the 'gold standard' of volume of water displaced, but also the least inter-observer variability. There was very little variability between the observers when measuring the volume of each object by the water displacement method. There was also very good correlation within each individual observer between the volume measured by water displacement and the volume as measured by the planimetric method. Very good inter-observer correlation was also seen when measuring objects by the planimetric method. Conversely, the ellipsoid measurements resulted in vastly different volume calculations between observers. Those volumes obtained were not at all well correlated with the 'true' volume as measured by water displacement. For this reason parallel planimetric method was used as the method to describe uterine, fibroid and non-perfused volume in fibroid patients.

Following this initial assessment of methods, the MR images of 50 women with uterine fibroids undergoing treatment by MRgFUS were examined by two investigators at different times, blinded to each other's findings. Each observer was asked to use the parallel planimetric method to calculate the overall uterine volume, the volume of the largest fibroid (agreement on which fibroid was checked by pictorial location of the largest fibroid) and the total non-perfused volume achieved following administration of gadolinium dye post procedure (Table 6). In addition each observer was asked to classify each fibroid uterus by the classification system described in Table 3.

	Uterine Volume SQ	**Uterine Volume JV**	**Fibroid Volume SQ**	**Fibroid Volume JV**	**NPV SQ**	**NPV JV**
Mean (mL)	727	726	315	320	139	141
Standard Deviation	383	386	266	276	97	99
Range (mL)	2161905	220-1894	25-1169	26-1201	7-530	10-524

Table 6: Measurements from pelvic MRI by planimetric method by SQ and JV

The results of this study are given in Table 7. As expected the standard deviation of bias was greatest in the uterine volume calculations where the range of values was largest.

Comparison	Range (ml) n=50	Mean Difference (ml)*	ICC[Ŧ]	95% Confidence intervals for ICC	Bland Altman Analysis		
					Bias	SD of Bias	95% limits of agreement
Uterine volume calculation JV vs. SQ	220-1905	0.93 ± 19.85	0.999	0.998-0.999	-0.92	19.85	-39.82-37.97
Fibroid volume calculation JV vs. SQ	25-1201	5.24 ± 16.45	0.998	0.997-0.999	5.24	16.46	-27.01-37.50
NPV JV vs. SQ	7-534	2.11 ± 9.01	0.996	0.993-0.998	2.11	9.01	15.56-19.77

*Data are means ± SD [Ŧ]Interclass Correlation Coefficient (ICC)

Table 7: Correlation between observers for pelvic MRI findings

Both observers described the uterine category based on Table 3. We used Cohen's Kappa co-efficient as a measure of the agreement between our two observers in assigning these categorical values. Kappa is used rather than simple percentage agreement as Kappa takes into account the probability of agreement occurring by chance. The correlation between observers for uterine category had a weighted kappa of 0.851 with a standard error of 0.061 and 95% confidence intervals of 0.731 to 0.971. This degree of correlation falls within the range of almost perfect agreement (Landis and Koch, 1977).

Discussion

Accurate determination of the volume of a uterine fibroid tumour is of importance because its size may be a factor in determining appropriate management. Large fibroids may be considered a relative contra-indication for focussed ultrasound therapy, or laparoscopic myomectomy. Excessively rapid growth of a lesion may raise suspicion of more sinister non-leiomyomatous pathology, or indicate the need for earlier

follow up or intervention. Monitoring the efficacy of embolization, hormonal or focussed ultrasound therapy also relies on accurate determination of fibroid and uterine volumes.

Our results suggest that, although it is the quickest and most simple method available, there is wide inter-observer variability when using the ellipsoid method of volume calculation. Further, that this method frequently fails to reflect the true volume of an irregular ovoid structure. This significant degree of variability could lead to inaccurate assessment of fibroid volume, growth or regression, before or after therapy. We therefore contend that the optimum method of pre and post treatment fibroid volume assessment is the planimetric method.

Describing the NPV of a fibroid following MRgFUS or UAE provides useful information as to the success of that treatment. This makes the accuracy of measurement of increased importance, in enabling the physician to assess how successful a treatment has been and whether a repeat procedure or alternative treatment is required.

The planimetric method has been validated in volume measurement of other structures and organs in the body, including measurement of the chambers of the heart and the prostate (Jia et al., 2005, Katz et al., 1988). These validation studies have been performed using both ultrasound and MRI modalities. A potential disadvantage of the planimetric technique is the amount of time required to calculate the volume of an object using this method. On each slice, multiple points outlining the perimeter of the object must be marked with callipers manually, until the entire perimeter has been circumnavigated and defined. This represented the most time consuming part of the process. In our experience the planimetric method may take many times longer, or depending on the size of the structure, possibly more, than the simple application of a the ellipsoid formula to the three diameters in the a, b and c axes (Figure 15: An ellipsoid). In the future, software advances which automatically define the perimeter of an object may be of great use in streamlining this process, and allow the radiologist or gynaecologist to perform the volumetric calculation in a fraction of the time currently required.

Our study was limited by several technical constraints which could have potentially introduced small, and we believe insignificant, sources of error. The interpolation algorithm of the software defines the outline of an object between two points which have been marked manually by the observer on the perimeter of the object. Depending on where these points are defined, and how many, we noticed that occasionally the automatically calculated trace of the outline of the object could be drawn deep to its surface, leading to an underestimation of its volume. Irregular edges or margins of certain objects would exacerbate this effect.

We endeavoured to re-draw any instances where this occurred. When measuring volume of water displacement, we were also unable to confidently perform measurements using the measuring flask to the nearest millilitre, because the gradations on the side of the flask were at 5mL intervals. Our measurements represented our best interpretation, taken at the horizontal limit of the meniscus of fluid, to the nearest millimetre. Such potential sources of error we believe are very minor when compared to the differences between two observers using the formula for an ellipsoid volume; firstly, defining exactly which measurements constitute largest dimensions; secondly, at which angles such lines along the object should be drawn and finally on which slices are all factors open to significant inter and intra-observer variation and likely accounted for the large degree of variation between the two observers.

Conclusion

A variety of methods have been used to calculate the volume of uterine fibroid tumours. Our study indicates that, when comparing the method for calculating the volume of an ellipsoid sphere to the planimetric method, it is the planimetric method which most accurately defines the volume of an irregular object and demonstrates the least inter-observer variability. Use of this method, whilst more time consuming than applying the formula for an ellipsoid sphere, results in more accurate reporting of fibroid volume for treatment planning and monitoring, and is recommended. Our method of categorising fibroid uteri by the numbers and sizes of uterine fibroids has good intra-observer agreement, and will be used throughout this thesis.

3. Demographics, fibroid classification and symptom measurement at a Tertiary Fibroid clinic and treatment

Introduction

As stated in chapter one, up to 74% of pre-menopausal women will have uterine fibroids, and nearly half of these women will require some form of treatment (Cramer and Patel, 1990). Women will present to their physicians with a variety of different symptoms, fertility needs and social circumstances, as well as a wide variety of the size and numbers of fibroids within their uteri. There are many treatment options available for treating fibroids (page47) and these will be tailored to the individual woman based on the assessment of the physician and the woman's personal wishes. In many cases numerous different treatments for uterine fibroids may be required. Re-intervention rates following myomectomy range between four and 23% by five years (Reed et al., 2006, Moss et al., 2011). One study found that 20% of women with a palpable uterine size corresponding to greater than 8 weeks gestation underwent a hysterectomy within 1 year of presentation (Carlson et al., 1994). Factors associated with early surgery include large uterine volume, menorrhagia, and prior pelvic surgery (Weber et al., 1997). Attempts have been made to correlate the size and numbers of fibroids, symptoms, and pain scores with the need for therapeutic intervention and the type of treatment required. The Fibroid Growth Study (Davis et al., 2009) found that bleeding and pain, rather than the size or number of fibroids determined the choice for any type of intervention. This study population was however limited to 151 pre-menopausal women.

The St Mary's Fibroid clinic receives over 300 new referrals a year, and is the only centre in the UK that offers all described modalities for treating uterine fibroids. These women undergo clinical assessment, complete a fibroid symptom severity score (SSS) (Appendix A: UFSQOL) and fibroid questionnaire (Appendix D: St Mary's Hospital Fibroid Clinic Patient questionnaire) and if appropriate MR imaging. These MR images are then discussed at a weekly multidisciplinary team (MDT) meeting consisting of a panel of radiologists, radiographers and gynaecologists and a management plan produced.

In this chapter I will:

1. Describe the overall demographics of the women attending the fibroid clinic at St Mary's Hospital. This will include details regarding age, BMI, ethnicity, fertility, previous treatment, SSS as well as fibroid volume and number.
2. Examine how these demographic features change by fibroid uterine category, ethnicity and treatment group
3. Attempt to further refine the categorisation of fibroid uteri in order to better reflect the treatment pathways of these groups of women

Methods

Prospective recording of patient demographics, symptoms and treatments was performed from the fibroid clinic at St Mary's Hospital, Imperial College NHS healthcare trust, from October 2009 to December 2011. All women were asked to complete standardised symptom severity scores (SSS) (Spies et al., 2002a) (see Appendix A) at first visit and then subsequently following treatments. In addition to the SSS the women were asked to fill in a questionnaire (see Appendix D). This questionnaire asked specifically about bladder symptoms, bowel symptoms, fertility problems and any prior treatment for uterine fibroids.

370 women being considered for either MRgFUS or UAE had an MRI performed. These MRIs were reviewed by two investigators (John Vedalago, Radiologist and Stephen Quinn, Gynaecologist). The degree of agreement between these two investigators is described in chapter 2 and on Table 7. Each investigator was asked to describe each MRI in terms of the differing characteristics (Table 8). Those women who were either found to be not suitable for MRgFUS or UAE at the time of their initial consultation did not undergo MR imaging and were therefore excluded from this study. This group included those women with known sub-mucosal fibroids referred specifically for hysteroscopic trans-cervical resection of fibroids (TCRF), or those women specifically referred for surgical debulking by either myomectomy or hysterectomy.

Number of Uterine Fibroids within the uterus			
Total Uterine volume (ml)			
Characteristics of the 3 largest fibroids	Signal intensity		
	i	Hypo-intense relative to skeletal muscle (rectus abdominus) and myometrium	
	ii	Iso-intense relative to skeletal muscle and hypo-intense relative to myometrium	
	iii	Hyper-intense relative to skeletal muscle and hypo-intense relative to myometrium	
	iv	Hyper-intense relative to skeletal muscle and iso-intense relative to myometrium	
	v	Heterogeneous signal intensity	
	vi	Hyper-intense relative to myometrium	
	Volume in mL		
	Location within the uterus	A	Anterior Wall
		B	Posterior Wall
		C	Fundal
		D	Lateral Wall
	Position within the wall of the uterus	SM	Sub-mucosal
		IM	Intra-mural
		SS	Sub-serosal
		PSS	Pedunculated Sub-serosal

Table 8: Characteristics of uterine fibroids examined on MRI

As in Chapter 2, fibroid uteri were again described in terms of uterine category (see table 3 below).

Uterine category	Description
1	Single fibroid within the uterus
2a	2-5 fibroids with a single dominant fibroid, and additional fibroids of less than 2cm diameter
2b	2-5 fibroids with multiple fibroids of greater than 2cm diameter
3a	6-10 fibroids with a single dominant fibroid and additional fibroids less than 2cm in diameter
3b	6-10 fibroids with multiple fibroids greater than 2cm diameter
4	11-20 fibroids
5	Over 20 fibroids

Table 3(From Chapter 2)

Signal intensity refers to the relative pixel brightness as compared with surrounding structures. Most commonly in fibroids this is compared with the pixel signal intensity of skeletal muscle, or surrounding myometrium (Hricak et al., 1986). The uterine and fibroid volumes were calculated using the parallel planimetric method as outlined in chapter 2. Due to the large variation of numbers of uterine fibroids in the uteri examined, measuring the volumes of each individual fibroid in the uterus was found to be a very time-consuming process. Previous studies of fibroid growth have described measuring only the volumes of the largest fibroid within the uterus (Mavrelos et al., 2010). We measured the volumes of the three largest fibroids on MRI, in order to better describe the overall volume of fibroid tissue within the uterus.

Between October 2009 and December 2011 the notes and MR images of 370 women presenting to the fibroid clinic at St Mary's Hospital, Imperial College London NHS Healthcare Trust, were reviewed. All women were asked to complete a UFS-QOL at their first visit and following any treatments given. The UFS-QOL is a measure of the patient-reported fibroid symptoms, has been adopted as the main outcome measure in assessing response to MRgFUS (Spies et al., 2002a). The UFS-QOL uses eight questions regarding common fibroid symptoms graded on a five-point psychometric scale to assess both menstrual and pressure-related symptoms. The maximum raw score is 40 points which is transformed into a 100-point scale, or symptom severity score (SSS). Validation studies of the UFS-QOL have found that the average SSS is approximately 20, and in women with fibroids is approximately 40 (Spies et al., 2002a).

In addition to the UFS-QOL, a separate St Mary's questionnaire was completed recording details regarding fertility, previous treatments, and bowel and bladder symptoms. Details regarding age, ethnicity, body mass index (BMI), previous fibroid treatments and fertility were recorded. Details were recorded regarding the outcomes of those women undergoing MRgFUS and UAE.

Statistical tests included a test of normality of distributions and where normally distributed, student t tests were used to compare means of continuous data, and where non-parametric data was found, two-sided Mann-Whitney tests to compare patient and fibroid-related variables. Chi-squared tests were used to compare categorical variables. Continuous data that appeared skewed was converted by a logarithmic conversion and if the log scale was found to be normal, then parametric tests were used. The statistical package SPSS version 19 was used (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

Results

Between October 2009 and December 2011 the demographic details for 370 women undergoing MRI imaging of their uteri and attending the fibroid clinic at St Mary's Hospital were collected. Details of the overall demographics of this group are described in Table 9. The characteristics of the women's fibroids are described in Table 10.

The mean age at first MRI was 43.02 years (standard deviation 6.957) (Figure 32: Distribution of age).

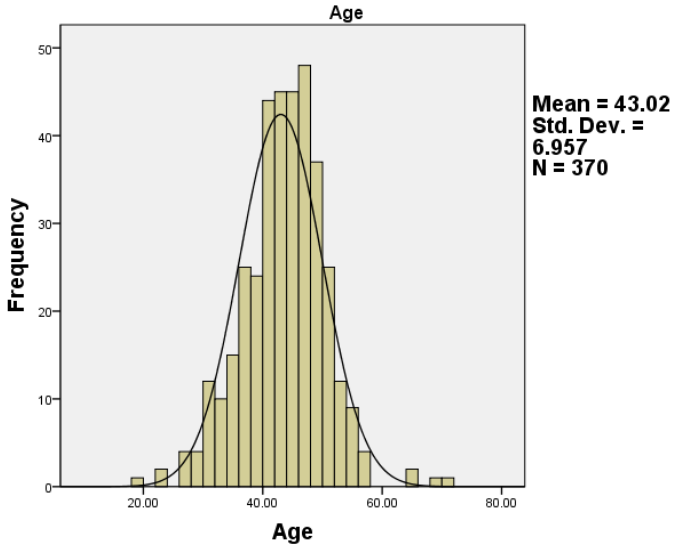
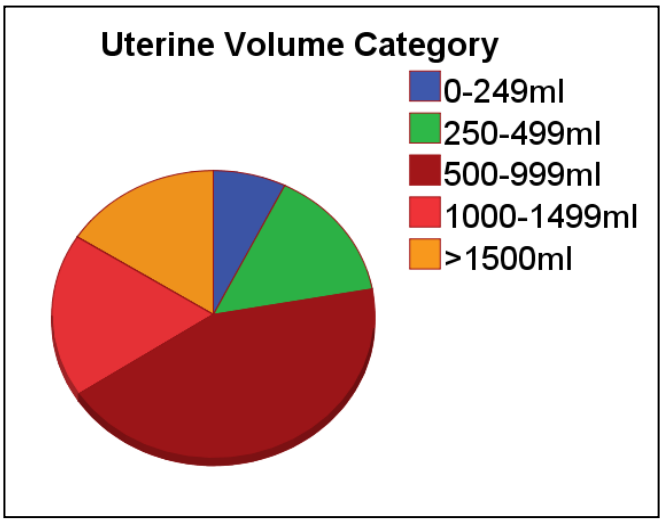
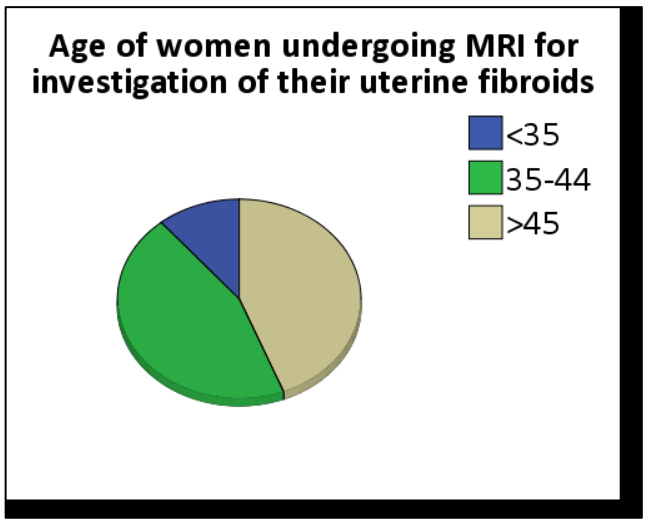


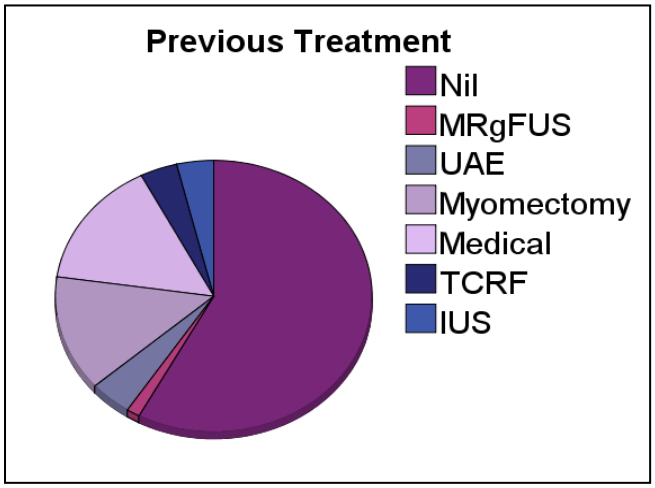
Figure 32: Distribution of age

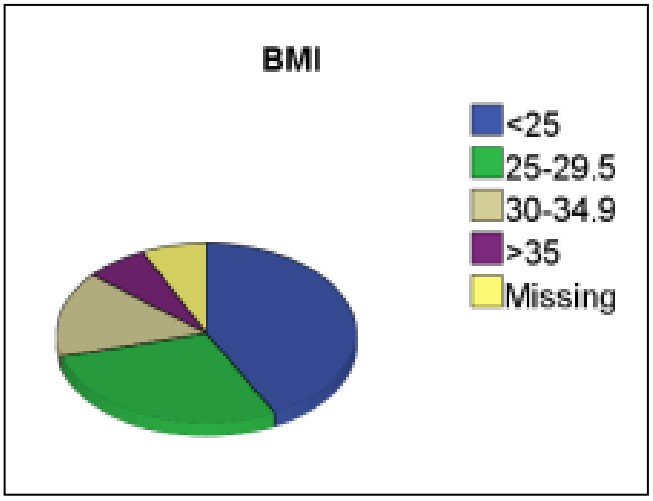
		Total Number of women	Percentage
Age	<35	41	11.1
	35-44	166	44.9
	≥45	163	44.1
Parity	0	236	63.8
	≥1	114	30.8
	Missing data	20	5.4
BMI (kg/m^2)	<25	126	34.1
	25-29.9	98	26.5
	30-34	46	12.4
	≥35	24	6.5
	Not stated ₸	76	20.5
Previous Treatment	None	214	57.8
	Myomectomy	51	13.8
	UAE	16	4.3
	TCRF	14	3.8
	IUS/Mirena Coil	14	3.8
	MRgFUS	5	1.4
Ethnicity	White	117	31.6
	Black	188	50.8
	Other	58	11.1
	Not stated	24	6.5
Primary Symptom	Menorrhagia	249	67.3
	Dysmenorrhea	11	3.0
	Pressure Symptoms	69	18.6
	Fertility Problems	9	2.4
	Urinary Symptoms	17	4.6
	Mixed	15	4.0

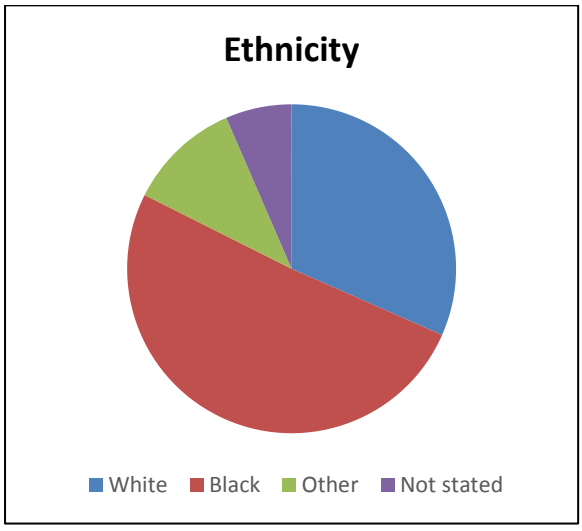
Table 9: Demographic details of women presenting to the fibroid clinic











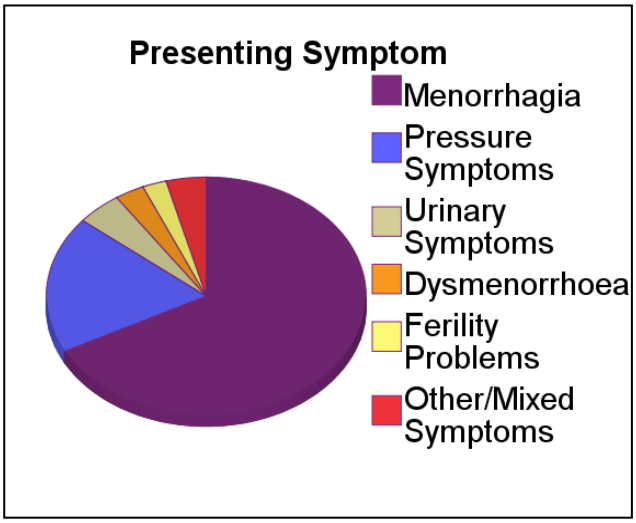


Figure 33: Pie Chart representation of study group data

		Numbers	Percentage
Number of uterine fibroids	1	60	16.2
	2-5	129	34.9
	6-10	84	21.6
	11-20	62	16.8
	≥21	24	6.5
	Adenomyosis	11	3.0
Position of largest fibroids	SM	27	7.3
	IM	270	73.0
	SS	60	16.2
	PSS	1	0.3
	Missing	12	3.2
Uterine Volume(ml)	<250ml	27	7.3
	250-499ml	55	14.9
	500-999ml	161	43.5
	1000-1499ml	68	18.4
	≥1500ml	59	15.9
Vol. of 3 largest fibroids	<49ml	26	7.0
	50-99.9ml	32	8.6
	100-199ml	52	14.1
	200-499ml	148	40.0
	≥500ml	76	20.5

Table 10: Uterine and Fibroid characteristics

The total uterine volume was calculated using the parallel planimetric method for each uterus (see Figure 16 page 72). The mean uterine volume was 955.05ml (standard deviation 656.45ml) (Figure 34). When the log of the uterine volume was used the distribution of log uterine volume was normally distributed and therefore parametric testing could be used.

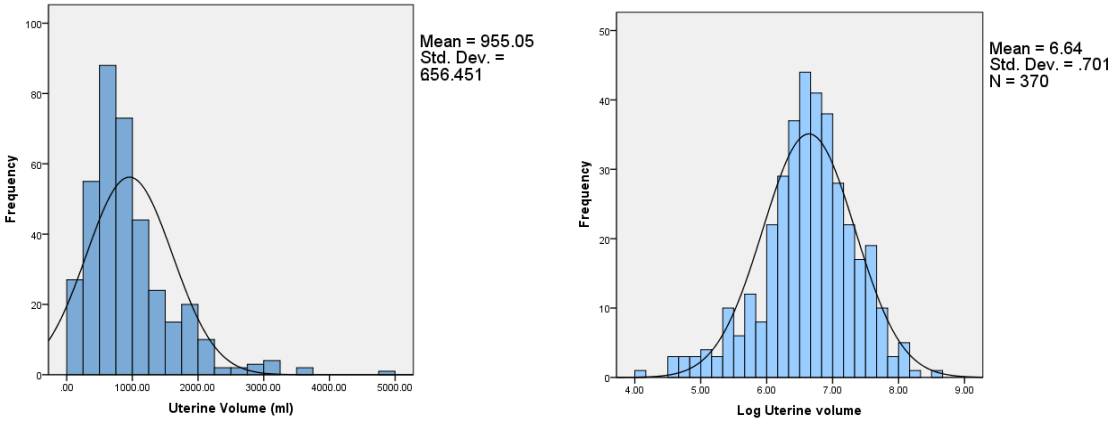


Figure 34: Distribution of measured uterine volumes (ml) and log scale of uterine volume

Uterine fibroid symptom questionnaires were completed correctly by 366 of the women. Standardised symptom severity score (SSS) was calculated from each questionnaire (Figure 35). The mean SSS was 57.80/100 (SD 21.656).

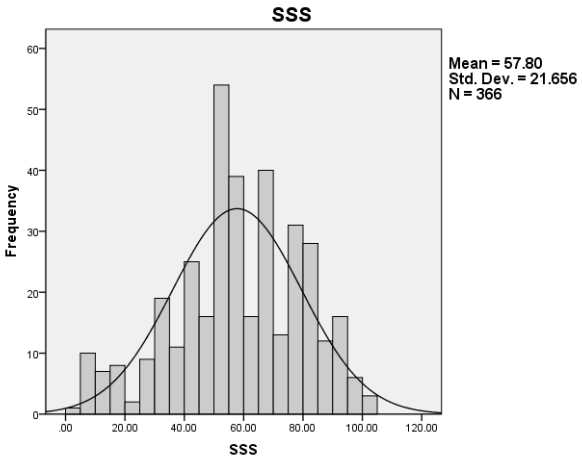


Figure 35: Distribution of SSS

The largest fibroid in each uterus was described as the dominant fibroid. The mean volume as calculated by parallel planimetric method was 326.26ml (SD 346.856). Due to the presence of adenomyosis in 11 women, the volume of the three largest fibroids was measured in 359 of the 370 subjects. The mean volume of the three largest fibroids was 416.32ml (SD 384.32). The body mass index (BMI) was calculated in 344 of the 370 women. In the remaining 26 women there was missing data either regarding height or weight of the subjects. The mean BMI was 26.68 (SD 5.625).

Results: Categorisation of fibroid uteri

The MR images of all 370 women were reviewed. Adenomyosis was found in 11 uteri. The remaining 359 subjects were assigned a uterine category as described in Table 3. The incidence of the uterine categories is shown in Table 11.

Uterine Category	Frequency n (%)	Mean Age (SD)	Mean BMI (SD)	Parity		Mean SSS (SD)	Mean Uterine volume (SD)
				P0 n (%)	≥P1 n (%)		
1 (1 dominant fibroid)	60 (16.2)	40.41 (7.82)	26.38 (7.20)	34 (61.80)	21 (38.20)	58.10 (20.75)	815.10 (626.51)
2a (2-5 UF with 1 dominant UF, and Other UF of < 2cm)	58 (15.7)	42.56 (7.28)	28.30 (6.17)	37 (66.10)	19 (33.93)	54.02 (24.07)	697.86 (435.11)
2b (2-5 UF with multiple UF of > 2cm)	71 (19.2)	44.25 (7.26)	26.79 (5.88)	44 (66.67)	22 (33.33)	60.25 (17.71)	803.83 (524.13)
3a (6-10 UF with 1 dominant UF and other UF < 2cm)	9 (2.4)	44.11 (6.94)	26.43 (4.73)	6 (75)	2 (25)	55.91 (17.43)	586.86 (160.69)
3b (6-10 fibroids with multiple UF > 2cm)	75 (20.3)	43.44 (7.29)	25.52 (4.78)	52 (72.22)	20 (27.78)	55.78 (22.99)	996.25 (704.52)
4 (11-20 UF)	62 (16.8)	44.06 (5.93)	27.10 (4.84)	36 (61.02)	23 (38.98)	55.39 (21.90)	1307.54 (725.32)
5 (>20 UF)	24 (6.5)	43.41 (6.18)	26.00 (4.86)	19 (82.61)Ŧ	4 (17.39)	69.37 (21.94)*	1491.23(726.40)
Adenomyosis	11 (3.0)	41.01 (6.44)	26.65 (4.93)	8 (72.73)	3 (27.27)	64.50 (23.32)	914.44 (454.39)

Table 11: Characteristics by uterine Category

There was no significant difference between the age and BMI between all groups. With the exception of category 5, there was no significant difference in the chances of a woman being nulliparous at presentation (Ŧ). Those women with a category 5 uterus had significantly higher baseline SSS compared to the other categories (p=0.013, student's t-test, equal variances not assumed) *.

The uterine volume by category is further illustrated by Figure 36. There was a significant difference between the uterine volumes of categories 1-3b and categories 4 and 5 (p=0.0001).

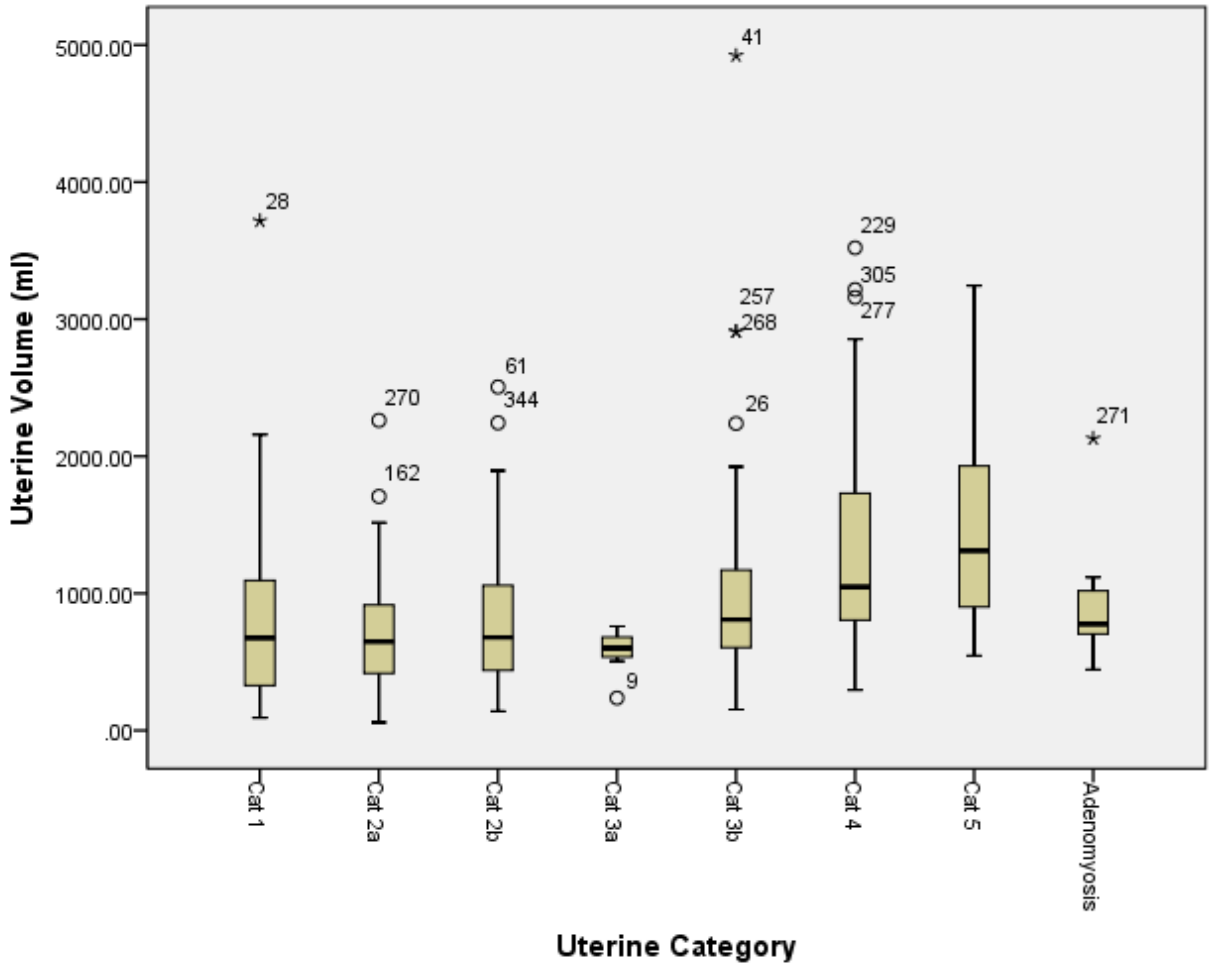
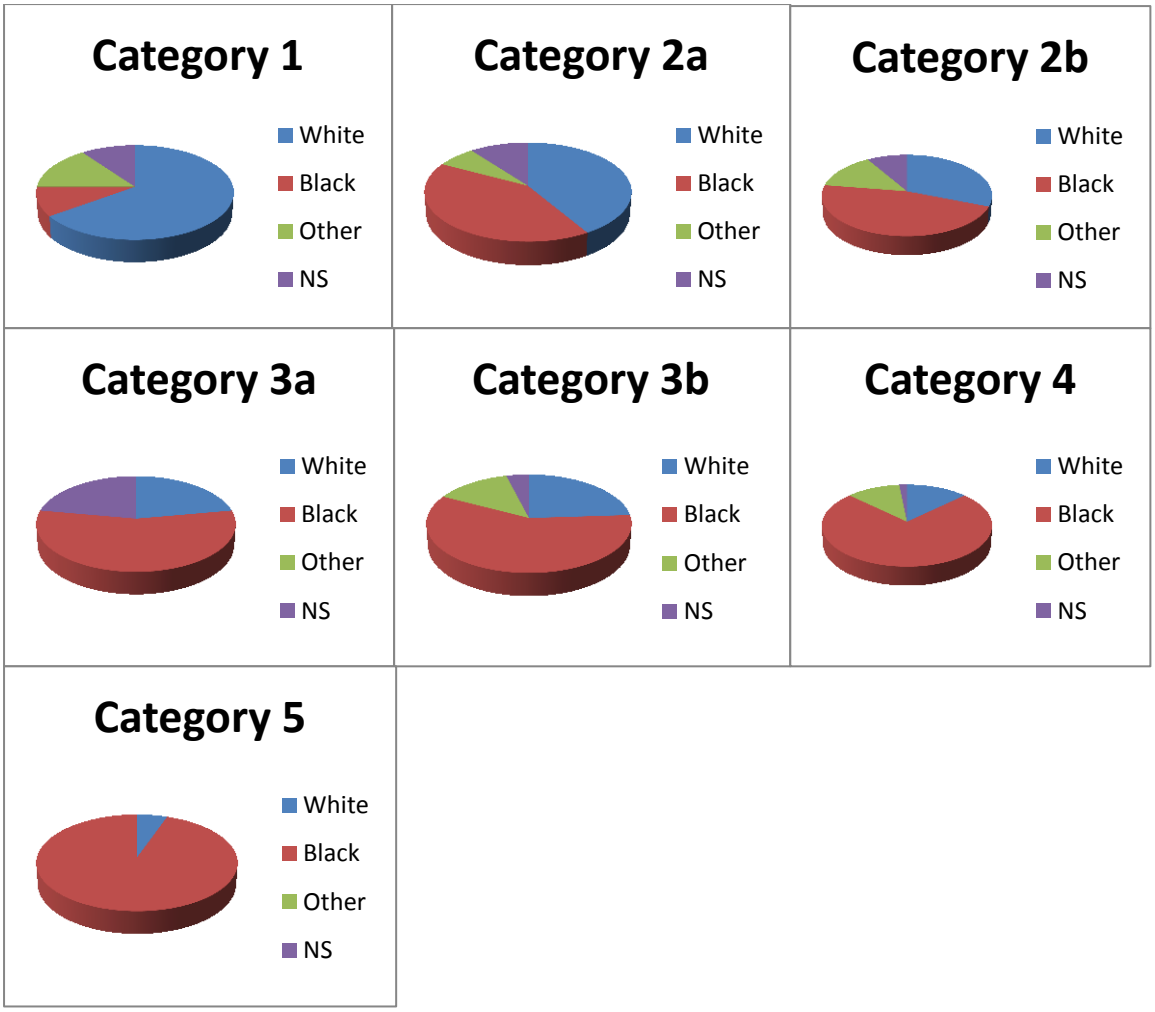


Figure 36: Uterine Volume by category

The variation in ethnicity by these categories is described in Table 12. The change from predominantly white women with category 1 fibroids through to mainly black women with category 3 or above is illustrated in the pie charts below.

Uterine Category	White n (%)	Black n (%)	Other n (%)	Not Stated n (%)
1	39 (65)	6 (10)	9 (15)	6 (10)
2a	24 (41.38)	24 (41.37)	4 (6.90)	6 (10.34)
2b	22 (30.98)	33 (46.48)	10 (14.08)	6 (8.45)
3a	2 (22.22)	5 (55.56)	0 (0)	2 (22.22)
3b	18 (24)	44 (58.67)	10 (13.33)	3 (4)
4	8 (12.9)	46 (74.19)	7 (11.29)	1 (1.61)
5	1 (4.17)	22 (91.67)	1 (4.17)	0 (0)
Adenomyosis	3 (27.27)	8 (72.73)	0 (0)	0 (0)

Table 12: Ethnicity by Uterine Category



The distribution of treatments undergone by the different uterine categories is expressed in Table 13, and Figure 37: Treatment by uterine category. The numbers and percentages of women undergoing UAE are highlighted in green, and MRgFUS are highlighted in red. This illustrates the higher percentages of women with category 1-2b undergoing MRgFUS, and higher percentages of category 2b-5 women undergoing UAE.

	Conservative n=71 (% of category)	MRgFUS N=74 (%)	UAE N=122 (%)	TCRF N=19 (%)	Myomectomy N=60 (%)	TAH N=10 (%)
Cat 1	12 (20.03)	18 (30.51)	12 (20.34)	3 (5.09)	13 (22.03)	1 (1.7)
Cat 2a	16 (27.59)	15 (25.86)	10 (17.24)	4 (6.90)	9 (15.51)	4 (6.90)
Cat 2b	9 (14.52)	17 (27.42)	30 (48.40)	4 (6.45)	10 (16.13)	1 (1.61)
Cat 3a	3 (33.33)	1 (11.11)	4 (44.44)	1 (11.11)	0 (0)	0 (0)
Cat 3b	14 (19.18)	14 (19.18)	27 (36.99)	4 (5.48)	12 (16.44)	2 (2.74)
Cat 4	14 (22.58)	5 (8.06)	26 (41.94)	3 (4.84)	12 (19.35)	2 (3.23)
Cat 5	3 (12.5)	4 (16.67)	13 (54.17)	0	4 (16.67)	0 (0)

Table 13: Treatment by uterine category

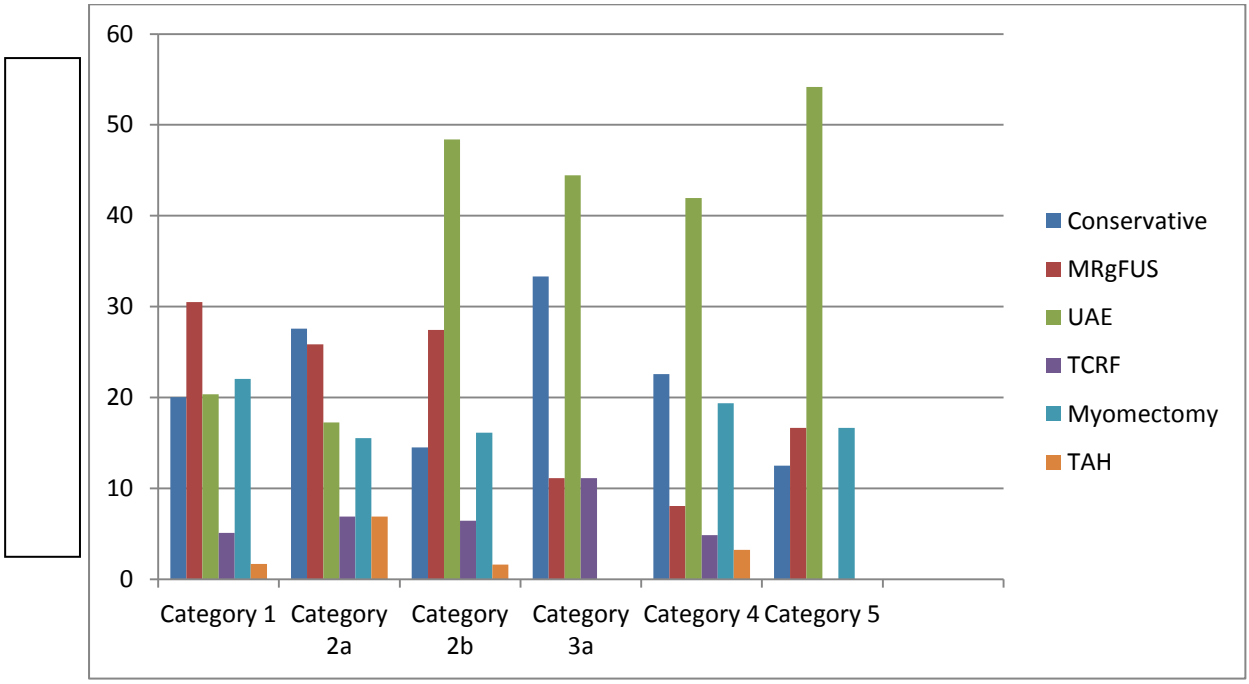


Figure 37: Treatment by uterine category

In an attempt to simplify the categorisation of these patients, uterine categories were further categorized as category I, II, or III by combining categories 1, 2a and 3a (i.e. those women with a single dominant fibroid, and other small fibroids less than 2cm in diameter), category II by combining 2b and 3b (i.e. those women with between 2-10 fibroids, with more than one fibroid larger than 2 cm in diameter), and finally category III, by combining category 4 and 5 uteri (i.e. those women with more than 10 fibroids in their uteri).

The demographic for this classification system is demonstrated by Table 14: Demographics by second categorization.

Uterine Category	Frequency n (%)	Mean Age (SD)	Mean BMI (SD)	Parity		Mean SSS (SD)	Mean Uterine volume (SD)
				P0	≥P1		
Category I	127 (35.3)	41.64 (7.60)	27.24 (6.63)	77 (64.71%)	42 (35.30%)	56.11 (22.03)	737.36 (522.64)
Category II	146 (39.5)	43.79 (6.72)	26.16 (5.12)	96 (69.57%)	42 (30.43%)	57.94 (20.66)	862.48 (536.83)
Category III	86 (23.2)	43.92 (6.22)	26.75 (4.84)	55 (67.07%)	27 (32.92%)	59.17 (22.65)	1389.57 (753.39)

Table 14: Demographics by second categorization

When further categorised in this way, there was no significant differences between age, BMI, SSS and parity between groups. As expected the mean uterine volume increased between groups.

Table 15 and Figure 38 illustrate the modes of treatment by this second form of classification. Again, percentages of women undergoing myomectomy, hysterectomy and TCRF do not vary significantly across the categories, however percentages of women undergoing MRgFUS fall and UAE increase from category I to III.

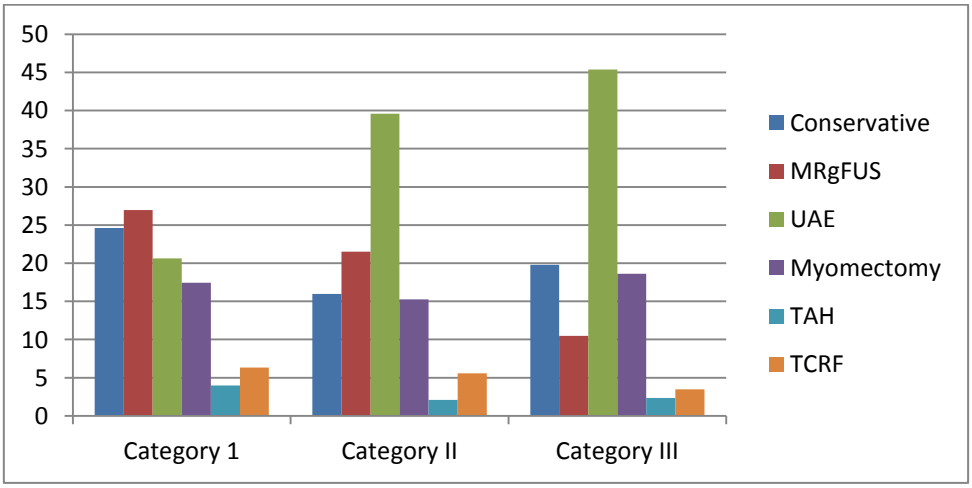


Figure 38: Treatment by category I-III

Uterine Category	Conservative n (% of category)	MRgFUS n (%)	UAE n (%)	TCRF n (%)	Myomectomy n (%)	TAH n (%)
Category I (n=127)	32 (25.2)	34 (26.8)	26 (20.5)	8 (6.3)	22 (17.3)	5 (3.9)
Category II (n= 146)	25 (17.1)	31 (21.2)	57 (37.0)	8 (5.5)	22 (15.0)	3 (2.1)
Category III (n=86)	17 (19.8)	9 (10.5)	39 (45.3)	3 (3.5)	16 (18.6)	2 (2.3)

Table 15: Modes of treatment By Category I-III

Ethnicity between groups is given in Table 16. This again demonstrates an increased incidence of multiple fibroids in black women compared with white women.

Uterine Category	White n (%)	Black n (%)	Other n (%)	Not Stated n (%)
I (n=127)	65 (51.2)	35 (27.6)	13 (10.2)	14 (11.0)
II (n=146)	40 (27.4)	77 (52.7)	20 (13.7)	9 (6.2)
III (n=86)	9 (10.5)	68 (79.1)	8 (9.3)	1 (1.2)

Table 16: Ethnicity by Category I-III

Finally in order to further simplify the categorisation, we recoded these fibroid categories into those with a single dominant fibroid with up to 10 smaller fibroids (Category A= All those fibroid uteri from Category 1, 2a and 3a), and then those uteri with multiple fibroids of greater than 2cm diameter (Category 2b, 3b 4 and 5) as Category B.

The demographic details between these final two categories are described in Table 17. There were significant differences in overall uterine volume and ethnicity between these two categories. There was no significant difference in age, BMI, parity or SSS between these last two categories. The differences in treatments undergone by these two categories are described in Table 18 and Figure 39. The only significant differences between the two categories were between MRgFUS and UAE (p=0.018 Pearson Chi-Square).

Uterine Category	Frequency n (%)	Mean Age (SD)	Mean BMI (SD)	Parity n (%)		Mean SSS (SD)	Mean Uterine volume (SD)	Ethnicity	
				P0	≥P1			White n (%)	Black n (%)
Category A (single dominant fibroid with < 10 smaller fibroids)	127 (35.4)	41.50 (7.64)	27.24 (6.63)	77 (64.71)	42 (35.29)	56.11 (22.03)	754.96 (541.58)	65 (64.3)	35 (34.7)
Category B (uteri with multiple fibroids > 2cm)	232 (64.6)	43.71 (6.27)	26.37 (5.02)	151 (69.63)	69 (31.36)	58.40 (21.38)	**1051.25 (671.00)***	**49 (25.3)Ŧ**	**145 (74.7)Ŧ**

*p= 0.000 (Student t-test of Log Volumes) Ŧ p=0.000 (Pearson Chi-Squared test)

Table 17: Demographics of Category A and B uteri

Uterine Category	Treatment					
	MRgFUS n (%)	UAE n (%)	Myomectomy n (%)	TAH n (%)	Conservative n (%)	TCRF n (%)
Category A	51 (25.9)	56 (28.4)	32 (16.2)	6 (3.0)	40 (20.3)	12 (6.1)
Category B	23 (14.5)*	66 (41.5)*	28 (17.6)	4 (3.1)	31 19.5)	7 (4.4)

*p=0.018 (Pearson Chi-Squared test)

Table 18: Treatment by Uterine category A and B

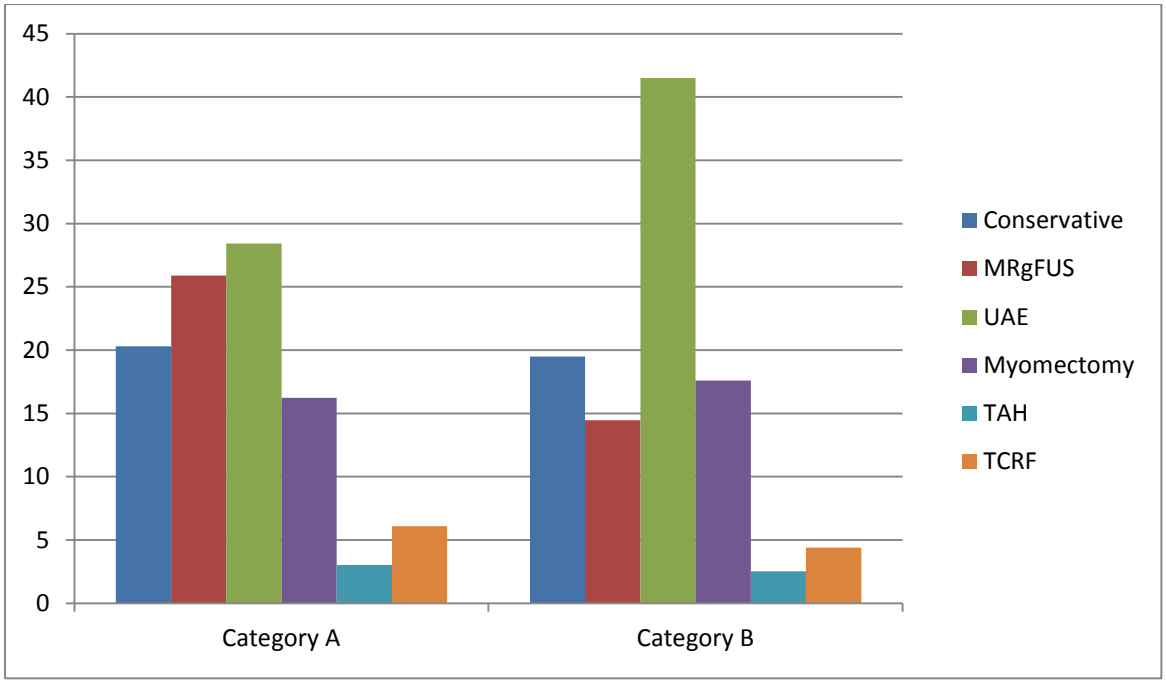


Figure 39: Treatment by category A and B

Overall, women with Category B uteri are more likely to have larger overall uterine volumes, be of black ethnicity and undergo treatment by UAE than those women with Category A uteri.

Results: Ethnicity and fibroid treatments

Of the 370 women, we had data regarding ethnicity on 346 women, 24 women did not give an answer for the question regarding ethnicity. The demographics for the 370 women are given in Table 19. Due to the relative small numbers, information regarding women reporting their ethnic origins as Arabic, Indian, Mixed, Asian, or other have been grouped together under the heading Other.

Characteristic	Total (n=370) n (%)	White (n=117) n (%)	Black (n=188) n (%)	Other (n=41) n (%)	Not stated (n=24) n (%)
Age, years					
<30 years	11 (3)	4 (3.4)	3 (1.6)	4 (9.8)	0 (0)
30-34	30 (8.1)	11 (9.4)	16 (8.5)	3 (7.3)	0 (0)
35-39	56 (15.1)	18 (15.4)	28 (14.9)	6 (14.6)	4 (16.7)
40-44	110 (29.7)	**30 (25.6)**	**64 (34.0)**	8 (19.5)	8 (33.3)
>45	163 (44.1)	54 (46.2)	77 (41.0)	20 (48.8)	12 (50)
Missing	0				
BMI					
<25	126 (34.1)	**49 (49.5)**	**57 (38.5)**	14 (41.2)	6 (46.2)
25-29	98 (26.5)	**27 (27.3)**	**56 (37.8)**	11 (32.4)	4 (30.8)
30-34	46 (12.4)	14 (14.1)	26 (17.6)	4 (11.8)	2 (15.4)
>35	24 (6.5)	9 (9.1)	9 (6.1)	5 (14.7)	1 (7.7)
Missing	76 (20.5)				
Parity					
P_0	236 (63.8)	82 (72.6)	125 (69.8)	21 (52.5)	8 (44.4)
$P_{1 \text{ or more}}$	114 (30.8)	31 (27.4)	54 (30.2)	19 (47.5)	10 (55.6)
Missing	20 (5.4)				
Primary Symptom					
Menorrhagia	249 (67.3)	**65 (56.0)**	**135 (72.2)**	31 (79.5)	18 (81.8)
Pressure Symptoms	69 (18.6)	**32 (27.6)**	**29 (15.5)**	5 (12.8)	3 (13.6)
Urinary Symptoms	17 (4.6)	6 (5.2)	8 (4.3)	2 (5.1)	3 (13.6)
Dysmenorrhoea	11 (3)	4 (3.4)	7 (3.7)	0	1 (4.5)
Infertility	6 (1.6)	2 (1.7)	4 (2.1)	0	0
Pain	9 (2.4)	7 (6.0)	2 (1.1)	0	0
Miscarriage	3 (0.8)	0	2 (1.1)	1 (2.6)	0
Missing	6 (1.6)				
Uterine Volume					
0-249ml	27 (7.3)	14 (11.9)	8 (4.3)	3 (7.3)	2 (8.3)
250-499ml	55 (14.9)	24 (20.5)	16 (8.5)	9 (22.0)	6 (25.0)
500-999ml	161 (43.5)	47 (40.2)	87 (46.3)	17 (41.5)	10 (41.7)
1000-1499ml	68 (18.4)	19 (16.2)	36 (19.1)	8 (19.5)	5 (20.8)
>1500ml	59 (15.9)	**13 (11.1)**	**41 (21.8)**	4 (9.8)	1 (4.2)

Table 19: Characteristics of women attending the fibroid clinic at St Mary's Hospital, 2009-2011

Black women with uterine fibroids were more likely to have a higher overall uterine volume (p<0.000), with 21.8% having a total uterine volume of >1500ml compared with 11.1% of white women. Black women were much more likely to complain of menorrhagia than white women (72.2% vs. 56.0 p=0.005 Pearson Chi-Square), and white women were more likely to complain of pressure related symptoms compared with black women (27.6 vs. 15.5 p=0.005) despite having smaller uterine volumes overall. Age distribution, BMI and parity were similar across all groups.

Of these 370 women, 214 (57.84%) stated that they had not undergone any form of treatment for their uterine fibroids prior to review at the St Mary's Fibroid clinic. Eighty-one (21.89%) had already undergone a myomectomy, embolization or TCRF at another hospital before their first referral to the fibroid clinic (Figure 40).

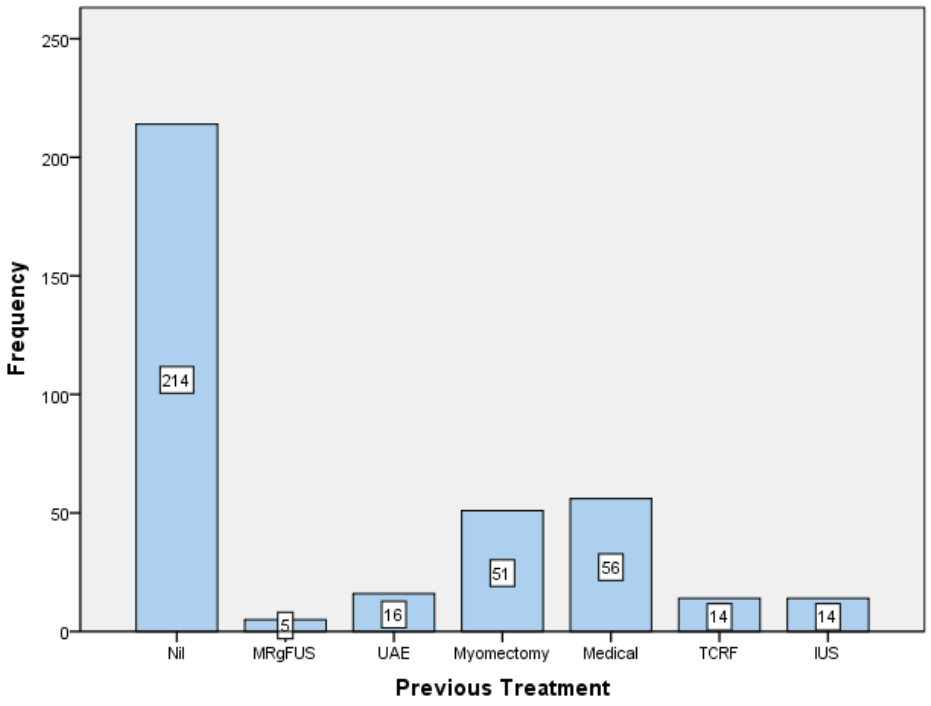


Figure 40: Previous treatment for uterine fibroids

The characteristics of the subjects undergoing differing treatment modalities are described in Table 20.

	Conservative n=71	MRgFUS n=74	UAE n=122	TCRF n=19	Myomectomy n=60	TAH N=10
Mean Age (SD)	43.24 (8.52)	43.55 (7.73)	43.71 (5.23)	43.48 (5.78)	39.61 (6.73)	46.91 (4.09)
Mean BMI (SD)	26.41 (5.69)	25.12 (5.21)	27.96 (6.45)	25.37 (3.00)	25.85 (3.69)	31.43 (5.94)
Mean SSS (SD)	**51.44[d] (24.60)**	62.20 (16.18)	59.88 (22.53)	56.82 (23.27)	56.43 (20.00)	59.09 (22.52)
Nulliparous	68.00%	74.60%	56.30%	66.67%	86.44%	40.00%
Mean Uterine volume ml(SD)	862.34 (835.1)	764.54 (397.05)	1002.70 (606.68)	799.62 (761.67)	**1235.43[b] (617.92)**	**1173.81[b] (745.31)**
Adenomyosis	5 (6.58)	0 (0)	2 (1.61)	2 (9.52)	1 (1.64)	1 (9.09)
Previous Treatment	39.5%	21.62%	54.84%	61.90%	36.07%	63.64%
HI of dominant UF	33.80%	26.38%	29.51%	25.00%	28.33%	10.00%
Mean Volume of dominant UF (SD)	312.68 (485.44)	335.93 (292.13)	267.92 (271.03)	259.68 (400.06)	379.36 (315.00)	380.37 (322.39)
Mean Volume of 3 largest UF (SD)	370.54 (545.59)	397.66 (294.33)	365.81 (300.24)	326.75 (506.08)	**606.91[c] (326.71)**	**459.27[c] (325.07)**
Primary Symptom						
Menorrhagia n (%)	**35 (46.67)[a]**	54 (73.97)	92 (76.03)	**20 (95.24)[a]**	39 (65.00)	7 (63.64)
Pressure Symptoms n (%)	20 (26.67)	14 (19.18)	16 (13.22)	0	14 (23.33)	4 (3.64)
Urinary Symptoms n (%)	6 (4.00)	3 (4.11)	5 (4.13)	0	3 (5.00)	0
Other n (%)	14 (19.18)	2 (2.74)	8 (6.61)	1 (4.76)	4 (6.67)	0

[a] p=0.001 Chi-Square test [b] p=0.002 ANOVA [c] p=0.006 ANOVA [d] p=0.01 ANOVA HI=Hyper-intensity, UF=Uterine fibroids, TCRF=Trans-cervical resection of fibroid, SD=Standard deviation, TAH= Total Abdominal Hysterectomy

Table 20: Characteristics of women undergoing treatment for their uterine fibroids

Women undergoing treatment that resulted in surgical de-bulking (Myomectomy and TAH) had larger overall uterine volumes (p=0.002) and a larger total volume of the 3 largest fibroids (p=0.006), than women from the other treatment modalities. However, the percentage of women complaining of pressure-related symptoms as a primary problem was less than that for those women opting for conservative management. The women who opted for conservative management for their fibroids were significantly less likely to complain of heavy menstrual bleeding as their primary symptom (p=0.001), and overall symptom severity scores (SSS) was significantly lower than those for other treatment groups (p=0.01). Women who received trans-cervical resection of their fibroids (TCRF) were far more likely to have heavy menstrual blood loss as their presenting symptom (p=0.0.001).

The numbers of women with children were examined against the position of the dominant fibroids (intramural (IM), sub-mucosal (SM) or sub-serosal (SS)). Position was recorded by review of the MRI images. The results in terms of number of successful pregnancies, is given in Table 21. Women with children were more likely to have SM UF than those without, however this was not significant (p=0.114 Pearson Chi-Square).

	Position of uterine fibroids			
	SM n (%)	IM	SS	Total
Parity P0	14 (6.14)	169 (74.12)	45 (19.74)	228
≥P1	13 (11.82)	83 (75.45)	14 (12.72)	110

Table 21: Parity and position of uterine fibroids

Discussion

In all, the 370 women followed over these two years had one of six possible treatment outcomes. For the purposes of this study we grouped medical treatment for uterine fibroids and no treatment together. It would be interesting to look at how many women genuinely required no treatment for their uterine fibroids, but for this study was difficult due to the number of women seeing a number of different doctors about their fibroids, and use of medications such as Tranexamic acid and Mefanamic acid were sporadic, and compliance was often poor. Such a study would require a cross-section of women in the community, and is beyond the scope of this thesis. In addition, this study included only those women undergoing MRI as part of their investigation. At present MRI is performed for all women for whom a treatment by either MRgFUS or UAE is being considered. This excluded those women with symptoms attributable to sub-mucosal uterine fibroids requiring trans-cervical resection of fibroids (TCRF) or those for whom surgical debulking by either myomectomy or hysterectomy is recommended. This presents an inherent bias to this study, However, as

study of women being considered for novel therapies for uterine fibroids it does allow comparison between those undergoing MRgFUS and those undergoing UAE.

Previous studies have reported a greater number of uterine fibroids and larger overall fibroid mass in black women compared with white women undergoing hysterectomy for fibroids (Davis et al., 2009). Black women were more likely to be anaemic and report severe or very severe pelvic pain despite diagnosis and treatment at a younger age (Kjerulff et al., 1996). Our finding of a significantly higher percentage of black women complaining of menorrhagia compared with white women would support this. The overall uterine weight at hysterectomy for black women is almost twice that of white women (Weiss et al., 2009). Overall, reporting on the racial differences within fibroid studies has been poor (Taran et al., 2010a), and although the prevalence of uterine fibroids among black women is high, the representation of black women in clinical leiomyoma studies is around 15%. Black women are known to be more likely to have multiple fibroids compared with white women, with one cross-sectional study reporting 74% and 31% incidence of multiple uterine fibroids in black and white women respectively (Day Baird et al., 2003). Our study reported 80.5% of black women had category B uteri (multiple fibroids, compared with a dominant fibroids and smaller additional uterine fibroids), compared with 43.0% of white women. At present, there remains no adequate explanation for these racial differences in fibroid presentations (Aissani et al., 2013). Even when potential confounding factors such as diet, smoking, parity and age are taken into account these significant variances remain (Day Baird et al., 2003). One explanation for these differences is that that black women have been found to have unique gene polymorphisms for oestrogen production and metabolism and mutations effecting micro-RNAs may lead to gene dysregulation in fibroid tissue, with resultant acceleration in fibroid growth (Ishikawa et al., 2009). The increase number of uterine fibroids at a similar age range compared with white women, suggest differences in the initial clonal expansion and development of fibroid tissue within the uterus. The precise initiators of fibroid growth have not as yet been identified, however certain genetic mutations have been implicated (in particular MED12 and HMGA2 mutations) (Islam et al., 2013). In addition, some hereditable changes in gene expression that are not coded in the DNA sequence (epigenetic mechanisms) including DNA methylation and histone modifications may be involved in the initiation of fibroid development (Islam et al., 2013). Perhaps once more is understood about these initial events in fibroid development and the genetic and epigenetic differences between fibroids of white and black women, these differences will be more satisfyingly explained and potential new therapeutic strategies may follow.

Our proposed categorization of fibroid uteri led to limited clinically significant findings. Interestingly there was no significant difference in mean ages between these groups. If the presence of more fibroids is related

simply to the time these fibroids have had to develop, a progressively increasing mean age would be expected from group 1 to group 5. That this is not case supports the idea that these different presentations of uterine fibroids are separate disease sub-groups that clinically behave differently in terms of their growth patterns. There was also no significant difference in BMI between any of these groups. BMI is known to be related to the presence of uterine fibroid, but not to the size of these uterine fibroids (Dandolu et al., 2010). This corresponds with our findings, and in our group BMI was not related to size or number fibroids.

As expected those women undergoing surgical de-bulking treatments for their uterine fibroids tended toward larger uterine volumes and larger overall fibroid volumes. We hoped that measuring the volumes of the three largest fibroids would allow for a meaningful assessment of fibroid load, however due to the number of women with more than 10 fibroids within their uteri this figure is not always representative of the overall fibroid burden. The approach of measuring the three largest fibroids was used because of the inherent difficulties of measuring every fibroid in the uterus. This approach to measuring fibroid load, will inevitably lead to some bias in our results.

We found no significant differences in parity between categories, with the exception of those women with greater than 20 individual uterine fibroids within their uteri having a higher incidence of nulliparity (no live births) for whom 17.39% had one or more children compared with an average of 32.86% in the other groups. Fertility outcomes are known to be significantly reduced in those women with sub-mucosal uterine fibroids, reduced by inter-mural fibroids, and sub-serosal uterine fibroids having no significant effect on pregnancy rate (Pritts, 2001). When we examined parity in relation to the position of the dominant fibroids we found that in this study, women with children were slightly more likely to have sub-mucosal fibroids than those women with no children, although this was not found to be significant ($p=0.114$). It is likely that this is a result of the way fibroid position was described. Due to the high numbers of fibroids the decision was made to describe the position of the largest fibroid. As sub-mucosal fibroids tend to be smaller than intermural and sub-serosal fibroids it is most likely that this resulted in a gross under-reporting of sub-mucosal fibroids in our study population. In any future study performed the presence of any sub-mucosal and intra-mural fibroids distorting the uterine cavity should be reported and additional information about other potential confounders (e.g. smoking, previous infection) should be reported.

The patient-reported symptom severity between groups was not significantly different with the exception of a significantly higher SSS in category 5 compared with other groups ($p=0.011$). As mentioned above, in validation studies the mean SSS for women with uterine fibroids is approximately 45 (Spies et al., 2002a). A subsequent study looked at women undergoing MRgFUS a multiple centres found a mean SSS of 61.5 (SD

14.7) (Harding et al., 2008). Overall the mean SSS in women presenting to the fibroid clinic at this tertiary referral centre is 57.8 (SD 21.7), which seems reasonable given earlier studies.

As expected overall uterine volume was significantly greater in category 4 compared with categories 1-3b, and then larger still in category 5. In terms of using uterine category to predict treatment modality, this was less successful. The uterine category in relation to treatment for uterine fibroids was described in Table 13. As Figure 37 illustrates, there is a trend towards a greater percentage undergoing UAE in the higher categories, and fewer women undergoing MRgFUS, however numbers having myomectomy, TAH or conservative management show no significant trend across the categories.

In an attempt to find a categorisation that would provide the most useful information regarding the treatment required we further categorised the fibroid uteri and category I, II and II and then again by category A and B. In the categorisation in Category I-III we could again find an increase in overall uterine volume with increasing category, however, again there was no significant differences between age, BMI, parity and SSS between these groups. Figure 38 illustrates the distribution of treatments by category I-III. Again we see an increase in treatment by UAE from Category I to III, and a decrease in the use of MRgFUS in the higher categories, with a fairly constant rate of surgery and conservative management across the groups. We finally recoded these fibroid categories into those with a single dominant fibroid with up to 10 smaller fibroids (Category A= All those fibroid uteri from Category 1, 2a and 3a), and then those uteri with multiple fibroids of greater than 2cm diameter (Category 2b, 3b 4 and 5) as Category B. Again we found significant differences in size of the uteri, significantly more black women with category B uteri, however no significant differences in age, BMI, SSS or parity across these categories. The treatments received by category A and B are described in Table 15 and Figure 38. This is of interest as the percentages of hysterectomy, myomectomy, TCRF and conservative management are equivalent across all groups. It is only UAE and MRgFUS that change significantly as expected, across the groups. All of these figures illustrate the problem with trying to form a definite pathway for women with uterine fibroids. Women with similar ages, parity, weights, symptoms and previous treatments will undergo a wide variety of different treatments. There are a number of possible explanations for this. Firstly, and possibly most importantly there is the woman's wishes and preferences. To some women the idea of living with a large benign tumour in her pelvis will be unacceptable, and surgery may seem the only desirable option. For other women, avoiding the possibility of hysterectomy at all costs is paramount, and more conservative options will be preferred. Pressures of work and home-life commitments may make the recovery following surgery or embolization unacceptable, whereas to some, the symptoms may be severe enough to warrant definitive treatment in the form of hysterectomy. When we looked at the standardised symptom severity score across the different treatments there were no significant differences in scores, with the exception of those opting of conservative management. Indeed those undergoing treatment my MRgFUS had the highest baseline SSS overall. While

guidelines regarding the types of fibroid uteri best suited to individual treatments can be made, it is the clinician in the clinic who having taken a concise history and listened to the women's wishes, and individual problems, who can best judge the mode of treatment suitable for that woman, and with the aid of a multidisciplinary team can arrange this treatment efficiently and effectively.

4. Safety and Long-term outcomes following MRgFUS

Introduction

Magnetic resonance-guided focused ultrasound (MRgFUS) uses MR imaging-guidance to direct high-intensity focused ultrasound (HiFU) into a uterine fibroid (UF) resulting in coagulative necrosis of the fibroid tissue. This treatment was first performed in 2002 (Stewart et al., 2003) and received FDA approval in 2004. Since then this treatment has been associated with favourable pregnancy outcomes (Rabinovici et al., 2006, Rabinovici et al., 2010), and encouraging short to medium term results (Funaki et al., 2009a, Taran et al., 2009, Rabinovici et al., 2007). Since its introduction, increasing experience of this treatment has led to a refinement of patient selection, with a tendency toward treating women with fewer fibroids and smaller uteri (Zaher et al., 2009). It is expected that as experience with this novel treatment increases the outcomes achieved will improve, both in terms of overall volume of fibroid ablated and reduction of adverse events (Okada et al., 2009b). At present no randomised control trials have been performed comparing MRgFUS with other treatment modalities, and the current published data regarding outcomes is from cohort studies, with a maximum follow-up of two years (Funaki et al., 2009a).

A recent systematic review of the literature regarding MRgFUS found studies of limited quality, with no randomised control trials, and found 38 prospective and retrospective studies (Gizzo et al., 2013). A summary of the studies identified is given in Table 22. The data on long term outcomes is currently very limited. Funaki et al. found a two year re-intervention rate of 21.6% for hyper-intense uterine fibroids (with a mean NPV of 36.2%) and 14.0% at two years for normo-intense fibroids (with a mean NPV of 54.35%) (Funaki et al., 2009a). This was the first study to examine how the overall percentage of fibroid tissue treated successfully as defined by the post-contrast non-perfused volume (NPV) appearance of the MR images. The authors were among the first to accurately measure NPV on each sequential slide of MRI and calculate a sum volume based on this. In addition the authors examined how the NPV and overall effectiveness related to the signal-intensity of the target fibroids. They found that in the fibroids of high signal intensity, the NPVs achieved were lower (36.2 vs. 54.35) and a greater proportion of these women went onto to require further treatments for their fibroids by two years.

A more recent prospective study found a two year re-intervention rate of 24%, with an overall NPV of 40.9% (Machtinger et al., 2012). This study also stratified outcome by signal intensity and NPV and found that

women with hyper-intense fibroids by a higher risk of requiring further treatment at two years compared with women with hypo-intense fibroids although on multivariate analysis this failed to reach significance (OR 2.87 (0.64–14.4). Treatment resulting in greater than 45% NPV had a 15% risk of re-intervention by two years, whereas treatments resulting in a 10-20% NPV had a 40% risk of re-intervention at two years (OR 5.22 (1.1-26.0) (Machtinger et al., 2012).

A recent retrospective study of women undergoing UAE and MRgFUS found a five year re-intervention rate of 66.7% at five years following a mean NPV of 36.4% (Froeling et al., 2013). In this study however the less accurate ellipsoid formula method was used to calculate the NPV, no mention of fibroid signal intensity was made and 37% of their patients were lost to follow-up.

Authors, years	Study Design	Mean Age	N	SSS at baseline	Mean Volume of Fibroids treated (mL)	NPV ratio (%)	Re-intervention
Dobrotwir and Pun, 2012	Pros	42	100	59	185.0	67.00	13.7% at 1yr
Stewart et al., 2007	Pros	44.8	109	61.7	284.7	25	--
Funaki et al., 2007	Pros	41	52	--	191.1	69.63	11.8% at 1yr
Harding et al., 2008	Pros	45	102	61.5	--	--	--
Rabinovici et al., 2010	Pros	37.2	51	--	268	40	--
Funaki et al 2009b	Pros	40.4	91	--	101.5	54.35 NI 36.2 HI	14.0% at 2yrs NI 21.6% at 2yrs HI
Okada et al., 2009b	Pros	42.5	279	--	326	46.6	8% at 1yr
Machtinger et al., 2012	Pros	45.6	81	48.82	213	40.9	24% at 2yrs
Stewart et al., 2006	Meta-analysis	45.4	155	61.7	353	38	21.9% at 1yr
Morita et al., 2008	Pros	42.6	48	--	--	60	8.3% at 6 months
LeBlang et al., 2010	Pros	46	80	--	175	55	--
Fennessy et al., 2007	Pros	45.9	64	62.1	--	25.79	--
Kim et al., 2012	Pros	44.5	27	64.8	502.5	64.2	--
Mikami et al., 2008	Pros	44	32	--	306	--	--
Voogt et al., 2012	Pros	44.8	33	--	--	21.7	--
Zhang et al., 2010	Pros	39.4	21	--	97	75	--
Park et al., 2012	Retro	39	9	30	197.8	66.9	--
Trumm et al, 2013	Retro	42	115	--	89	88	--
Gorny et al., 2011	Retro	45.6	130	--	350.3	45.4	7.4% at 1yr
Lénárd et al., 2008	Retro	45.4	66	61.5	255.5	16.3	--
Yoon et al, 2011	Pros	41.1	20	43.3	--	53.5	--
Froeling et al., 2013	Retro	36.2	36	60.7	53.2	36.4%	66.7% at 5yrs
Mean		**42.74**	**1701**	**55.92**	**232.27**	**47.38**	

HI=Hyperintense fibroid, NI=Normointense fibroid, NPV=Non-perfused volume, N=Numbers, SSS=Symptom severity score

Table 22: Collated data from previous MRgFUS Studies

The National Institute of Clinical Excellence (NICE) recently published a document outlining their advice (NICE, 2011) regarding the use of MRgFUS in the UK based on current available evidence. This guidance states that current evidence for the efficacy of MRgFUS is adequate for use in the short term, however that women should be informed that further treatment may be required and the subsequent effect on pregnancies is currently uncertain. They acknowledged the recognised complications of MRgFUS, while concluding that the evidence of safety was sufficient to support its use, provided it was subject to appropriate clinical governance and audit. NICE recommended that patient consent for MRgFUS should include information regarding the risk of recurrence of symptoms, or indeed failure of the treatment to adequately control symptoms in the first instance, and to specifically mention that further treatments may be required. Women should be aware of the uncertain effects on pregnancy and fertility, and of the risks of burns to the skin. NICE recommend that women be selected for MRgFUS or alternative treatments by a multidisciplinary team including a gynaecologist and radiologist, and that all treatments should be carried out by clinicians with specific formal training in MRgFUS. Finally, NICE encouraged further long-term research into the efficacy of MRgFUS as a treatment for uterine fibroids. They stated that this should include details regarding long-term outcomes, the need for further treatment and the incidence and outcomes of subsequent pregnancies.

MRgFUS performed at St Mary's Hospital London utilizes the ExAblate 2000 system, produced by the company InSightec. This treatment system received approval by the U.S. Food and Drug Administration (FDA) for the treatment of uterine fibroids in October 2004 (Premarket Approval (PMA) # P040003), in addition to a European licence (CE) the same year. More than 5000 ExAblate uterine fibroid procedures have been performed world-wide in commercial treatments and as follow-up in study protocols.

MRgFUS was introduced at St Mary's Hospital, Imperial College London NHS trust in 2003, and since then has been utilized as one of the therapeutic options for treating women with symptoms relating to their uterine fibroids presenting to the fibroid clinic. This chapter will describe the safety and long term effects of the ExAblate 2000 system.

The aims of chapter 4

1. Describe the incidence of adverse events following MRgFUS at St Mary's Hospital since 2003
2. Describe the re-intervention rate following MRgFUS at three and five years
3. Identify factors that affect re-intervention rate, in particular the non-perfused volume (NPV) of the UF following treatment, i.e. the amount of UF tissue treated. Other features such as the size of the

UF, signal intensity of the UF and number of UF will be examined in order of better understand which women respond favourably to this treatment modality

Methods

This was a cohort study of 280 women undergoing MRgFUS between 2003 and 2010. Data was obtained from patient records and patient questionnaires (Spies et al., 2002a). In addition, telephone consultation was attempted for all women, during which a standardized questionnaire was completed to assess their personal experience of the treatment and outcomes (Appendix B: Telephone questionnaire). Women were asked specifically about additional treatments for their uterine fibroids, and the reasons for these treatments, and the time points from their initial treatment. For those women having undergone a further intervention following MRgFUS, they were given a code depending on the number of years following their initial treatment at St Mary's; hence I was able to code for re-intervention at 1 year, 2, 3, 4 and 5 years.

In addition to re-intervention for their uterine fibroids, women were asked whether they would recommend this treatment to friend, stratifying their responses into yes, no or not sure.

The MRI images of the MRgFUS procedure were retrieved where possible, and the T1 and T2 images were reviewed. From these, uterine volume, volume of fibroids treated and non-perfused volume following contrast agent were calculated using the parallel planimetric area method (Chapter 2). These measurements were performed by two investigators, whose intra-observer agreement for the parallel planimetric method has been described in chapter 2 (JV and SQ). Fibroid uteri were described as terms of the total number of fibroids within the uterus (table 3).

Uterine category	Description
1	Single fibroid within the uterus
2a	2-5 fibroids with a single dominant fibroid, and additional fibroids of less than 2cm diameter
2b	2-5 fibroids with multiple fibroids of greater than 2cm diameter
3a	6-10 fibroids with a single dominant fibroid and additional fibroids less than 2cm in diameter
3b	6-10 fibroids with multiple fibroids greater than 2cm diameter
4	11-20 fibroids
5	Over 20 fibroids

Table 3(From Chapter 2)

The fibroids that were undergoing treatment were described in terms of their position (SM, SS, IM) and their location (anterior wall, posterior wall, fundal or lateral wall). These fibroids were also described in terms of their pixel signal intensity on T2-weighted MR imaging, in relation to both the adjacent myometrium and skeletal muscle. In addition to describing uterine fibroid signal intensity by simply hyper- or hypo-intensity in relation to the signal intensity of skeletal muscle we further divided the uterine fibroids into one of three subtypes. Type 1 were those uterine fibroids which appeared hypo-intense in relation to skeletal muscle, type 2 had signal intensity equal to that of skeletal muscle (iso-intensity) and type 3 which appeared hyper-intense in relation to skeletal muscle. This additional categorisation of signal intensity has been used in other studies examining the effects of fibroid signal intensity on MRgFUS outcomes (Funaki et al., 2009b).

Statistics

For the cohort study of those women undergoing treatment with the ExAblate 2000 system the outcomes measured was re-intervention by surgical means, embolization or repeat MRgFUS at three and five years. Treatments were stratified according to the degree of NPV achieved. 0-25% NPV for the purposes of this study was classified as a failed treatment. Treatments were then classified as 25-50% NPV and >50% NPV. In addition to NPV, the effects of age, ethnicity, parity, uterine size, numbers of fibroids and BMI on re-intervention were tested, to assess for possible confounding variables. A logistic regression model was used, and the model was tested for goodness- of-fit by Hosmer-Lemeshow test and Le Cessie-Van Houwe-Copas-Hosmer un-weighted sum of squares test (Hosmer et al., 1997). The statistical package R version 2.15.0 was used to check the fit of the logistical regression model and calculate the odds ratio of requiring further treatment at five years compared with those with less than 25% NPV. Other statistical calculations were performed used SPSS version 19 (BM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).
All distributions of continuous data were tested for normality and where the data was not normal means were compared using the Mann-Whitney test, to test the differences between medians in these two groups. For the categorical data of uterine category, the differences between those undergoing the ExAblate 2100 and 2000 systems was tested using Chi-Square testing between these two groups.

Results

Notes and patient records were reviewed for all 280 women undergoing MRgFUS between January 2003 and January 2011. Two hundred and seventeen women were successfully contacted (77.5%) for telephone consultation regarding their long-term outcomes and experiences. Two hundred and thirty nine women had

undergone their MRgFUS three or more years earlier and were therefore to contribute to the three year outcomes data, and 181 were successfully contacted (75.7%). One hundred and eighty seven women had their MRgFUS treatments over five years before the study and were therefore eligible to be included in the 5 year outcomes study. Of these 187 women, 163 were successfully contacted (87.17%).

Demographics

Details on ethnicity were available for 248 of the 280 women. Details on ethnicity are given in Table 23. Mean age was 42.2 years, mean BMI 25.3, and mean UFS-QOL was 62.4/100.

Age	**Mean (years)**	42.2		
	Range(Years)	25-84		
BMI	**Mean**	25.1		
	Standard deviation	4.6		
		Numbers	**Percentage**	**Valid percentage**
Race	**White**	135	48.2	54.4
	Black	76	27.1	30.6
	Asian	17	6.1	6.9
	Arabic	7	2.5	2.8
	Other	13	4.6	5.2
	Not stated/declined	32	11.4	
Parity	**P0**	198	70.7	
	P1	42	15.0	
	P2	35	12.5	
	>P2	5	1.8	
Previous treatment	**Nil**	202	72.1	
	Myomectomy	12	4.3	
	UFE	7	2.5	
	TCRF	12	4.3	
	IUS	47	16.8	

Table 23: Demographics of women undergoing ExAblate 2000 treatment

MRI images were successfully located for 241 of the 280 women. Mean uterine volume was 795.5cm^3 (SD 408.89), and the mean total volume of fibroid tissue attempted was 396.3cm^3 (SD 265.0). Total non-perfused volume (NPV) following contrast was 165.0cm^3 (SD 137.23). This gave a mean percentage NPV of the total fibroid volume attempted as 44.2%. The majority of fibroid uteri were category 1, 2a and 2b (Table 24).

Uterine category	Description	Numbers	Percentage
1	1 solitary fibroid	52	21.6
2a	2-5 fibroids with a single dominant fibroid and additional fibroids less than 2cm in diameter	42	17.4
2b	2-5 fibroids with multiple fibroids greater than 2cm diameter	64	26.6
3a	6-10 fibroids with a single dominant fibroid and additional fibroids less than 2cm in diameter	5	2.1
3b	6-10 fibroids with multiple fibroids greater than 2cm diameter	37	15.4
4	11-20 fibroids	28	11.6
5	Over 20 fibroids	13	5.4

Table 24: MRgFUS cases by uterine category (n=241)

Table 25 represents the changes in fibroid treatments from the initial treatments in 2003 to 2010. Over this period the mean uterine volume fell from 809.73ml (SD 445.48) to 658.38 (SD 296.37) (p=0.015), the mean %NPV increased from 41.22 (SD 25.66) to 50.49 (SD 18.23) by 2010 (p=0.038) and significantly fewer treated UF were hyper-intense on T2-weighted imaging (p=0.049). The overall volume of fibroid tissue treated increased from the initial treatments in 2003 of 333.8 ml (SD 209.75) to a peak of 544.23ml (SD 276.52) by 2005, before falling to 319.2ml (SD 241.88) by 2010. This is likely to reflect the anecdotal experience of the MRgFUS operators in terms of improving clinical outcomes, with relatively small UF being easier to treat successfully, and a shorter associated treatment time being more tolerable to patients.

Year	Mean Age (SD)	Mean BMI (SD)	Mean SSS (SD)	Mean Uterine Volume ml (SD)	Mean total fibroid volume ml (SD)	Mean %NPV (SD)	%HI Dominant Fibroid
2003	41.97 (5.44)	24.95 (4.05)	64.79 (17.47)	809.73 (445.48)	333.8 (209.75)	41.22 (25.66)	29.2
2004	43.87 (5.09)	24.11 (3.17)	62.1 (14.05)	734.20 (323.86)	387.0 (198.31)	47.33 (23.21)	29.0
2005	43.67 (8.93)	27.23 (4.14)	78.12 (16.24)	949.51 (383.33)	544.23 (276.52)	33.4 (20.34)	30.8
2006	42.74 (10.20)	25.00 (4.39)	64.1 (19.13)	958.1 (469.46)	495.1 (291.49)	39.4 (24.31)	26.9
2007	42.25 (5.93)	26.10 (5.55)	59.4 (16.93)	828.65 (446.98)	431.7 (289.64)	40.7 (22.04)	32.0
2008	43.15 (6.97)	23.75 (3.08)	64.1 (19.26)	861.90 (469.25)	445.1 (314.95)	44.54 (21.10)	36.4
2009	41.1 (6.00)	25.37 (5.97)	57.9 (19.04)	**611.01 (249.98) p=0.004a**	314.3 (195.75)	**51.53 (19.63) p=0.04a**	25.92
2010	40.4 (7.67)	25.5 (4.00)	65.46 (19.18)	**658.38 (296.37) p=0.015a**	319.2 (241.88)	**50.49 (18.23) p=0.038a**	**13.78** (p=0.049)b

[a] =Mann-Whitney test. [b] =Chi-square test. BMI= Body Mass Index. SSS=Symptom Severity score. NPV=Non-perfused Volume. HI= Hyper-intensity of treated fibroid

Table 25: ExAblate treatments 2003-2011

Safety

No adverse events and no significant pain were reported in 249 of the 280 women (88.9%). Mild to moderate pain, lasting up to five days post procedure was reported by 18 of 280 women (6.4%) in the absence of any other significant complications. Minor complications (urinary tract infection, urinary retention, vaginal bleeding, and transient buttock pain) were experienced by 11 of 280 women (3.9%). Only three women (1.1% of the total cohort) experienced severe complications. These included one case of

fibroid expulsion, one major skin burn requiring surgical repair, and one case of persistent neuropathy. No emergency hysterectomies were required following MRgFUS therapy. One woman required hospital admission for urinary retention, which resolved after three days. The percentage NPV achieved during these treatments is included on Table 26. There is no significant increase in adverse events seen with increasing NPV, however numbers are small.

Minor Complications	Numbers (%)	Mean NPV achieved	Serious complications	Numbers (%)	Mean NPV achieved
Abdominal Pain (<1 month)	18 (88.9)	49.88%	Skin burn requiring repair	1 (0.4)	27%
Back pain (<6 months)	4 (1.4)	34.62%	Fibroid expulsion	1 (0.4)	45%
UTI	4 (1.4)	35.59%	Persistent neuropathy	1 (0.4)	2.87%
Minor Skin Burns (no repair)	3 (1.1)	58.81%			
Nerve irritation (<1 month)	2 (0.7)	64.62%			
Urinary retention requiring catheter (3 days)	1 (0.4)	58.96%			
Nausea	1 (0.4)	47.50%			
Vaginal Bleeding	1 (0.4)	24.29%			

Table 26: Adverse events following treatment by ExAblate 2000 system n=280

Patient satisfaction.

223 women who were successfully contacted by telephone were asked whether they would recommend this treatment to a friend. Of these, 124 (54.2%) answered yes, they would recommend this treatment to a friend, 23 (10%) said they would not recommend this treatment, and 82 were not sure (35.8%).

Re-intervention.

Of the 239 women who had undergone their MRgFUS three or more years earlier and were therefore to contribute to the three year outcomes data and 180 were successfully contacted (75.3%). One hundred and eighty seven women had their MRgFUS treatments over five years before the study and were therefore eligible to be included in the five year outcomes study. Of these 187 women, 162 were successfully contacted (87.17%). Overall re-intervention rates by NPV are described in Table 27 and Figure 41. There is a reduction in the re-intervention rate at three years for those women achieving a greater than 25% NPV post-treatment. At five years there was a decreased re-intervention rate for those women with a greater than 50% NPV compared with those with NPV of <25%, however this was not significant (p=0.074). When the effects of NPV were adjusted for age, the NPV remained significant at three years but was not significant at five years (p=0.25).

	Re-intervention at 3 years (n=180) (%)	**Re-intervention at 5 years (n=162) (%)**
Overall	77/180 (42.8)	96/162 (59.3)
NPV of 0-25%	25/40 (62.5)	25/38 (65.8)
NPV 25-50%	31/77 (40.3)*	43/68 (63.2)
NPV >50%	22/63 (34.9)*	28/56 (50)

*p≤0.005

Table 27: Re-intervention rate by NPV category

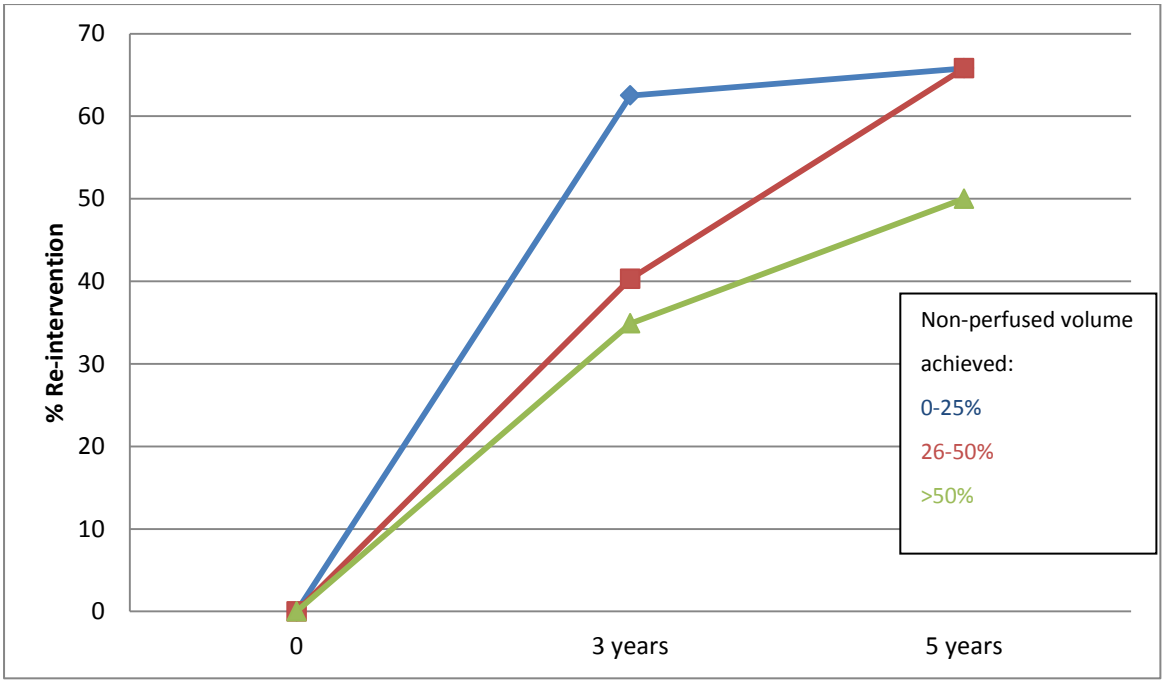


Figure 41: Re-intervention by % NPV

Step-wise, binary logistical regression modelling was used to investigate the effect of the co-variables age, ethnicity, BMI, initial SSS, uterine category and fibroid signal intensity. At 3 years signal intensity of the treated fibroids was a significant factor and at 5 years age, signal intensity and use of GnRHa were significant factors. None of the other co-variables were found to have any effects on re-intervention at three or five years.

	3 yrs	Sig.	Odds ratio (OR)	95% C.I. for OR		5 yrs	Sig.	Odds ratio (OR)	95% C.I. for OR	
				Lower	Upper				Lower	Upper
Age		0.07	--	--	--		0.02	1.08	1.01	1.16
No GnRHa use		0.21	--	--	--		0.03	2.34	1.10	4.95
Hyper-intensity of Fibroid		0.01	2.75	1.27	5.94		0.02	2.73	1.19	6.27

C.I. Confidence intervals. Sig.=Significance

Table 28: Effects of Age, GnRHa and signal intensity on re-intervention rate at 5 years following multiple regression

Further analysis the five year data found that the mean age differed between the groups, with those achieving greater than 50% NPV having a mean age of 44.0 compared with 41.4 and 41.9 in the other two groups (Table 29). Those treated achieving less than 25% NPV were more likely to be for hyper-intense fibroids, and there was no significant difference in GnRHa use across the groups.

	<25% NPV (n=38)	25-50% NPV (n=68)	>50% NPV (n=56)
Mean Age in years (SD)	41.4 (7.7)	41.9 (6.7)	44.0 (7.0)
Hyper-intensity (%)	24/38 (63.2)	16/68 (23.5)*	15/56 (26.8)*
Hypo-intense	14/38 (36.8)	52/68 (76.5)	41/56 (73.2)
GnRHa Use	16/38 (42.1)	32/68 (47.1)	22/56 (39.3)
No GnRHa use	22/38 (57.9)	36/68 (52.9)	34/56 (60.7)

***p<0.001 (Chi-Square test), NPV=Non-perfused volume, GnRHa= Gonadotrophin releasing hormone agonist, SD=Standard deviation**

Table 29: Variations between NPV categories at 5 years

The re-intervention rate by five years by fibroid type is demonstrated by Table 30. This demonstrates that type 3 fibroids have a significantly higher re-intervention rate compared with types 1 and 2 (p=0.013).

Re-intervention at 5 years	Type 1 n (%)	Type 2 n (%)	Type 3 n (%)
Yes	42/78 (53.84)	26/52 (50)	35/44 (79.54)
No	36/78 (16.15)	26/52 (50)	9/44 (20.45)

Table 30: Re-intervention by signal intensity

Discussion

These results describe our experience with MRgFUS since the introduction of this treatment modality outside of the initial safety studies in 2003. Over the last nine years there have been refinements in both the thermal ablative system and in subject selection, which we hope will further improve long-term outcomes. As our results demonstrate, MRgFUS is now being used for women with smaller overall uterine and fibroid volumes, with less hyper-intense fibroids, achieving a larger overall NPV. However, the severity of fibroid symptoms, age, BMI and background history of those women being treated has not changed significantly.

At present the evidence for the long-term effectiveness of MRgFUS in the treatment of uterine fibroid is limited. Two-year re-intervention rate from a cohort of 91 women undergoing MRgFUS in Japan was 14.0% in women with fibroids of hypo- (type 1) or iso-intensity (type 2) related to signal intensity of skeletal muscle (Funaki et al., 2009a). These women had a mean percentage NPV of 54.7 and were treating a mean fibroid volume of 129.0ml (SD 145.2ml) (Funaki et al., 2009a). The response to MRgFUS relates to the signal intensity of uterine fibroids on T2-weighted images. The NPV achieved in the type 3 fibroids is significantly lower than in type 1 and 2 fibroids. In our study we also found a clear distinction between type 3 and type 1/2 fibroids, with 79.54% of type 3 fibroids requiring some form of re-treatment by five years. This supports the idea that type 3 uterine fibroids should be avoided when using MRgFUS.

The mean age, BMI, SSS were similar in our study to other published data; however our mean uterine volume for women undergoing the ExAblate 2000 system treatment was 788 ml where as the only publication describing uterine volume had a mean volume of 595 ml (Stewart et al., 2006). It is possible that overall our unit may be treating women with larger uterine volumes than other units. The volume of fibroids treated was 337.87 mL (SD 252.75) for the ExAblate 2000 system. This is similar to the total fibroid volumes treated in the largest case series published to date (mean 358 mL, n=359) (Stewart et al., 2007), and mean fibroid volume from the 22 studies included in the review (n=1701) was 232.27ml.

It is known that incomplete uterine artery embolization procedures results in peripheral regrowth of the uterine fibroids over time (Toor et al., 2008). Even with relatively successful MRgFUS procedures the NPV is generally less than that of embolization, however the re-intervention rate is less than one would expect given the volume of fibroid tissue that remains perfused following MRgFUS. It has been suggested that the different mechanism by which MRgFUS has its effects could partially explain this (Funaki et al., 2009a). It is possible that the cells adjacent to the area of treatment may undergo apoptosis as a result of the rise in temperature, or thermal effects on small vessels resulting in partial ischemia of the tissue. As the technology develops and larger NPV are achieved it is hoped that the long term outcomes should improve. At present NPV does not appear to be related to adverse outcomes. Recent publications have reported a

54% NPV following prolonged experience with the ExAblate 2000 system (Okada et al., 2009b). A survey of MRgFUS operators found a target percentage NPV of 76% and a reported achieved NPV of 58% (Taran et al., 2010b). Our results for re-intervention at five years did not demonstrate a significant difference in re-intervention rate by degree of NPV achieved post-treatment, when age of the subject was taken into account. The five year data represent the outcomes from those women treated between 2003 and 2006. As Table 25 demonstrates, the mean uterine volume of the women treated at that time was significantly larger than the post-2006 treatments, and a higher percentage had hyper-intense uterine fibroids, with a lower mean percentage NPV achieved overall. It could be that these factors have had a negative impact on the results from this period, and with improved patient selection and increased experience the 5 year data from this more recent cohort may improve. It is possible that once the operators using the new ExAblate 2100 system gain more experience with this system, the NPV may improve further following the learning curve of prolonged experience. Overall from this data, using MRgFUS on hypo-intense uterine fibroid, following GnRHa pre-treatment and with the highest achievable NPVs is most likely to result in a positive long-term result. The data suggests that treating women with fibroids of lower signal intensity and with the use of pre-treatment GnRHa will reduce the need for re-intervention at 5 years. Once women become menopausal their reduced circulating oestrogen leads to a cessation of their periods and often a reduction in the size of their fibroids. It is likely for this reason that age has such an effect on five year re-intervention, as many of these women will be menopausal by 5 years and therefore much more likely to be asymptomatic.

Uterine category does not appear to be related to long-term outcomes in the way I had expected. It had been the initial hypothesis that the physical nature of the uterus in terms of fibroid number and ratio of fibroid size (category) would have a bearing on treatment outcome. Surveys of clinicians performing MRgFUS reported that following a symposium on focused ultrasound the majority considered the presence of five or more fibroids in the uterus to be a relative contraindication to MRgFUS (Taran et al., 2010b). When I compare outcomes at five years between those with fewer than 10 uterine fibroids and those with more than 10 fibroids, I found a trend towards a greater re-intervention in the group with more than 10 fibroids; however this failed to reach statistical significance due to relatively few women in this group (28, compared with 148).

Patient satisfaction following MRgFUS was disappointing. Ten percent claimed they would not recommend this treatment to a friend and 35.8% were not sure. It is possible that disappointment in the results of MRgFUS may be related to heightened patient expectation prior to treatment. Women seen at the Fibroid Clinic at St Mary's are often very interested in MRgFUS as a treatment option, indeed many women will have

originally requested their referral to this unit with the intention of undergoing this treatment. Many of these women will have reasons for wanting to avoid conventional surgical treatments. It has been reported that some patient groups will refuse hysterectomy even when other treatment options have been exhausted (Wuntakal and Erskine, 2009). By using the results of this study to counsel women before treatments regarding the rates of re-intervention (in line with NICE guidance) women will have more realistic expectation regarding their long-term outcomes.

When women have been previously asked about recommending MRI guided percutaneous laser myolysis to a friend, 80% claimed that they would (Hindley et al., 2002). Reports of patient satisfaction following hysterectomy have been reported as similarly high (Kjerulff et al., 2000). Compared with these figures these results of 54.2% claiming that they would recommend to a friend is lower than I had expected, however this is likely to be a result of the relatively high re-intervention rate overall. Again, more realistic pre-treatment counselling may improve this.

A major limitation of this study is the loss of patients to follow-up. While every effort was made to keep in contact with women following their treatment, 12.85% of the women with treatments over five years earlier were unable to be contacted. Many of these women came from overseas. It is not clear from their records whether their failure to attend follow-up was in relation to their being dissatisfied with the outcomes, or whether they were happy with the results and did not feel they needed to attend. The women who undergo MRgFUS are often a self-selecting group of women who may be highly motivated to avoid more conventional means of treating their fibroid-related symptoms. The only other study describing five year re-intervention data had 37% of their subjects lost to follow-up and had only 36 MRgFUS subjects by their analysis (Froeling et al., 2013). In this study the authors sought to compare MRgFUS and UAE, however described significant differences in age, uterine size and fibroid volume between the two groups. By the very nature of the selection criteria for these two treatment-modalities a direct comparison is difficult because of the differences in target patient groups.

In order to adequately assess how this treatment works in relation to conventional therapies, a randomised control trial (RCT) comparing this treatment with abdominal myomectomy should be performed. It is the author's opinion that a RCT comparing MRgFUS with UAE would be inappropriate due to the large differences in selection criteria between these two treatments.

Summary

MRgFUS is a relatively new intervention for uterine fibroids, and has been proven to be very safe in relation to other treatment modalities. Patient satisfaction and long-term results are disappointing at five years; however this may be related to the different approach to patient selection that was taken in the early years. Following improvements in the thermal ablative technology, operator experience and patient selection, it is possible that subsequent long-term results may improve. These results suggest that treating fibroids of low signal intensity with the use of GnRHa will reduce the likelihood of further re-intervention at 5 years. A robust randomised control trial of this treatment against myomectomy is recommended in order to adequately assess this treatment for uterine fibroids.

5. Initial experience of the ExAblate 2100 system

Introduction

Since January 2011 St Mary's has been using the ExAblate 2100 system (InSightec). The initial use of the ExAblate 2100 system was as a part of a phase four study conducted by the company InSightec to evaluate safety and ablation efficacy of the system when treating symptomatic uterine fibroids.

The new ExAblate 2100 system differs significantly from the previous ExAblate 2000 system. Following feedback from the various clinicians performing MRgFUS a number of alterations to the focused ultrasound transducer were made to allow for a greater volume of fibroid tissue to be treated, in a shorter time period and with the aim of improving safety to the patient.

The ExAblate 2100 system uses an automatic three-dimensional treatment planner that automatically arranges sonication spots to cover a maximum targeted fibroid volume. The system is also able to adjust the shape of the spot, the aperture of the transducer, height and tilt of the transducer thereby producing a much more fitted area of treatment energy compared with the earlier ExAblate 2000 system.

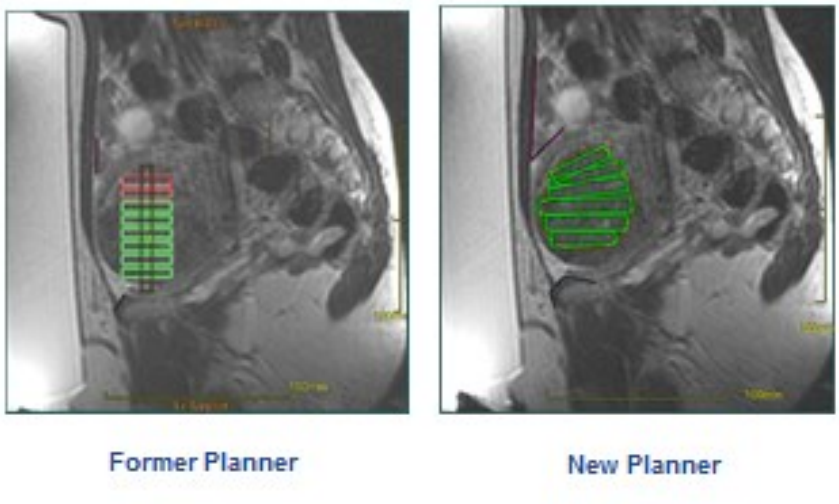


Figure 42: The former ExAblate 2000 planner and new ExAblate 2100 planner

By closing elements in the transducer the beam of high intensity focused ultrasound may be shaped in such a way as to produce a greater treatment volume. The sonication spots produced by the ExAblate 2000

system were prolate ellipsoid in shape (cigar-shaped); however the treatment planning spots were rectangular. Filling an irregularly shaped mass such as a uterine fibroid with rectangular shaped blocks resulted in areas of the fibroid not being treated. This new beam shaping allows of a more comprehensive filling of the fibroid shape (Figure 42).

The ExAblate 2000 transducer was positioned on a mechanical positioning system that could move the transducer in the plane of the MRI table. This positioning system involves the use rotary encoders that are able to convert mechanical position to a digital code, providing position feedback to ensure accurate movement. The transducer can be moved away from or closer to the patient depending on the need for focusing within the tumour, however this was limited. The ExAblate 2100 transducer is placed on a five axis robotic arm (Figure 43). This allows significantly greater control of the position of the resultant sonication spot.

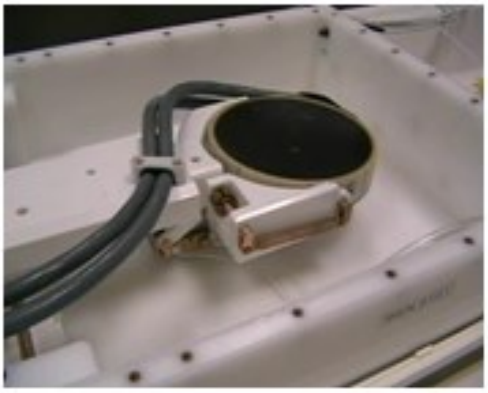


Figure 43: Five axis robotic transducers

The transducer is able to get much closer to patients` skin resulting in a reduced energy density in the beam path. The beam path will be wider at the skin and at the posterior bony structures leading to reduced energy absorption. This also allows an increased energy density to be produced safely without producing an increased energy absorption in the skin and spine. Previously fibroids with a hyper-vascular appearance were avoided as the system was unable to produce an energy density sufficient to heat the tissue to the target temperature. Hyper-vascular fibroids are able to dissipate more of the energy by conduction. The higher energy density of the ExAblate system enables these hyper-vascular fibroids to be treated more effectively.

Methods

This was a prospective cohort study of women undergoing treatment for uterine fibroids with the ExAblate 2100 MRgFUS system (n=34). Ethics committee approval was obtained (regional ethics committee ref 10/H0724/29). Results were compared with a data collected retrospectively from a cohort of women

undergoing treatment using the ExAblate 2000 system prior to January 2011 (n=238). Inclusion and exclusion criteria are described in Table 31.

Inclusion Criteria	Exclusion Criteria
1. Women age 18 or older	1. Women who are pregnant, as confirmed by serum/urine test at time of screening, or urine pregnancy test on the day of treatment
2. Women who have given written informed consent	2. Uterine size greater than 24 weeks size on abdominal palpation
3. Women who are able and willing to attend all study visits	3. Patients who are breast-feeding
4. Patient is pre or peri-menopausal (within 12 months of last menstrual period)	4. Patients with active pelvic inflammatory disease (PID)
5. Able to communicate sensations during the ExAblate procedure	5. Patients with active local or systemic infection
7. UF, which are device accessible	6. Extensive abdominal scarring in the beam path
8. Fibroid(s) clearly visible on non-contrast MRI	7. Dermoid cyst obstructing the treatment path.
9. Fibroid(s) enhances on MR contrast imaging	8. Known pelvic malignant or pre-malignant conditions
10. Symptomatic uterine fibroids defined as those resulting in scores of 21 or higher, based on patient responses to questions 1-8 of the Uterine Fibroid Symptom and Health-Related Quality of Life Questionnaire (Appendix A: UFSQOL).	9. Intrauterine device (IUD) anywhere in the treatment path 10. Contraindication for MRI Scan: I. Severe claustrophobia that would prevent completion of procedure in the MR unit II. Weight greater than 250 IBS (113Kg) III. Implanted ferromagnetic materials and/or devices contraindicated for MR scan IV. Known intolerance to MRI contrast agent (e.g. Gadolinium) V. Any other contraindication for MRI Scan

Table 31: Inclusion and Exclusion criteria

At recruitment in the fibroid clinic a complete medical history was obtained to determine the woman's general medical health, current symptoms and reasons for seeking treatment. A physical examination, MRI (T2 and T1 weighted images) and UF QOL (Appendix A: UFSQOL) were obtained.
The treatment protocol for ExAblate 2100 is outlined in Appendix E: Study Protocol for ExAblate 2100 Safety Study.

Statistics

All patients underwent standard pelvic MRI including T1 and T2 images prior to treatment. All MRI images were reviewed by two independent investigators (SQ and JV). Fibroids were graded as hypo-intense or hyper-intense relative to myometrium and rectus abdominus skeletal muscle. The non-perfused volume following both systems was calculated using the same software package utilizing the Plannimetric method (Reportcard software, GE Healthcare, Milwaukee) using the images from the sagittal T2 images (5mm slices). Volume calculation between investigators was tested for agreement using the Bland Altman method (Bland and Altman, 1986) and Interclass Correlation Coefficient.

A range of patient demographic data was recorded, including body mass index, symptom severity score, parity, ethnicity, primary symptoms and previous treatment. Prospective data was collected for those women undergoing the newer ExAblate 2100 system for one year post-treatment. All data regarding complications were recorded via review of patient notes, interview with patients at follow up, telephone and written questionnaires and direct observations of patients post procedures.

The statistical package SPSS version 19.0 (BM SPSS Statistics for Windows, Version 19.0 Armonk, NY: IBM Corp) was used to analyze differences between variables in both groups. Continuous data was tested for normal distribution by Shapiro-Wilk test. Normally distributed data was tested using student's t-test and where variables were not normally distributed Mann Whitney test was performed to investigate differences between these groups. For the categorical data of hyper-intensity, the differences between those undergoing the ExAblate 2100 and 2000 systems was tested using Chi-Square testing between these two groups.

The NPV achieved between groups was investigated for the possible effect of signal intensity differences between groups using Mann-Whitney non-parametric testing. Analysis of Covariance (ANCOVA) was used to adjust for confounding effect of different signal intensities of the fibroids treated. Differences in fibroid numbers and signal intensity between those undergoing the ExAblate 2100 and 2000 systems were

examined using Chi-Square test. A p-value less than 0.05 was taken to indicate a significant difference between groups. A univariate analysis was used to investigate differences in NPV between the treatments, using age and BMI and ethnicity as co-variants allowing for differences in these co-variants between the groups. This was done to avoid a reduction in power that would have occurred as a result of case-control matching.

Results

The MR images and treatment details from 238 women who had undergone treatment using the ExAblate 2000 system and 34 women undergoing treatment using the ExAblate 2100 system were examined. Mean follow-up from ExAblate 2000 patients was 3.81 years (SD 1.98). ExAblate 2100 patients were followed up over the first year post-treatment. The test for agreement of volume calculation between investigators using the Bland Altman method (Bland and Altman, 1986) found a bias of 5.24ml (SD 16.46) and interclass correlation coefficient of 0.998. This level of agreement was judged to be acceptable.

The demographics for the patient cohort and pre-treatment clinical features for the two groups are compared in Table 32.

		ExAblate 2000 n=238	ExAblate 2100 N=34	p-value
Age	**Mean years (Range)**	42.2 (25-8)	39.47 (25-5)	0.26[a]
BMI	**Mean (SD)**	25.1 (4.6)	23.41 (3.8)	0.04[a]
Race	**White N (%)**	135 (48.0)	14 (41.2)	0.01[b]
	Black N (%)	76 (27.0)	12 (35.3)	0.01[b]
	Asian N (%)	18 (6.4)	8 (23.5)	0.01[b]
	Arabic N (%)	7 (2.5)	0	
	Mixed N (%)	4 (1.4)	0	
Parity	**Mean (SD)**	0.51 (0.9)	0.4 (0.8)	0.33[c]
Symptom severity score	**Mean (SD)**	62.6 (17.8)	61.7 (17.9)	0.84[c]
Hyper-intense Fibroids	**N (%)**	67 (28.2)	6 (17.6)	0.19[b]
Previous Treatment	**N (%)**	43 (15.4)	5 (13.9)	0.88[b]
Number of Fibroids	**Mean (SD)**	6.43 (6.5)	4.18 (3.9)	0.04[c]
Mean Uterine Volume	**ml (SD)**	788.1 (408.0)	659.6 (259.91)	0.09[c]

Table 32: Demographics of subjects undergoing treatment with the ExAblate 2000 and 2100 systems

[a] = two tailed T-test. [b] =Chi-square test [c] =Mann-Whitney test. BMI= Body Mass Index

The percentage NPV achieved with the ExAblate 2100 system was 54.92 % (SD 19.28) compared with 43.72 % (SD 22.26) with the ExAblate 2000 system over the whole nine years, a significant difference (p=0.003) when differences in age, BMI and ethnicity are factored (Table 33). When signal intensity of the fibroids was

corrected for the differences between %NPV between the two treatments remained statistically significant (p=0.012). Of the women treated by the ExAblate 2100 system 17.6% had fibroids found to be hyper-intense on T2-weighted MRI images, compared with 28.2% of those undergoing treatment with the ExAblate 2000 system (p= 0.137). The mean NPV achieved in women with hyper-intense fibroids using the ExAblate 2100 was 43.20% compared with 57.43% in those women with hypo-intense UF. This compares favorably with the 36.33% NPV achieve in hyper-intense UF treated with the ExAblate 2000 system.

Three of the women treated with the ExAblate 2100 required a second treatment for their uterine fibroids in order to complete the treatment. These second treatments were done at 1, 2 and 7 months following the initial treatment, and were deliberately planned as the volume of fibroid tissue to be treated could not be achieved in a single session.

	ExAblate 2000 system (n=238)	**ExAblate 2100 system (n=34)**	**P value**
Mean Volume of Fibroids treated (ml) (SD)	337.78 (252.75)	305.12 (206.28)	0.04[c]
Mean Percentage NPV %	43.72	54.92	0.03[c]
% NPV in Hypo-intense fibroids	46.79	57.43	0.01[c]
% NPV in Hyper-intense fibroids	36.33	43.20	0.01[c]

[c] Analysis of variance. NPV=Non-perfused Volume

Table 33: Comparison between ExAblate 2000 and 2100 treatments

The details of the ExAblate 2000 treatments by year since 2003 are detailed in Table 34(adapted from Table 25 in the previous chapter). When compared to the NPVs initially achieved in with the ExAblate 2000 system the NPV increase and reach a statistically significant improvement by 2011 with the introduction of the ExAblate 2100 system (p= 0.007). When compared to the NPV achieved in recent years (2009 and 2010) the NPVs are greater with the ExAblate 2100 system, however this did not reach statistical significance (p=0.543)

Year	Mean Age (SD)	Mean BMI (SD)	Mean SSS (SD)	Mean Uterine Volume ml (SD)	Mean total fibroid volume ml (SD)	Mean %NPV (SD)	% HI Dominant Fibroid
2003	41.97 (5.44)	24.95 (4.05)	64.79 (17.47)	809.73 (445.48)	333.8 (209.75)	41.22 (25.66)	29.2
2004	43.87 (5.09)	24.11 (3.17)	62.1 (14.05)	734.20 (323.86)	387.0 (198.31)	47.33 (23.21)	29.0
2005	43.67 (8.93)	27.23 (4.14)	78.12 (16.24)	949.51 (383.33)	544.23 (276.52)	33.4 (20.34)	30.8
2006	42.74 (10.20)	25.00 (4.39)	64.1 (19.13)	958.1 (469.46)	495.1 (291.49)	39.4 (24.31)	26.9
2007	42.25 (5.93)	26.10 (5.55)	59.4 (16.93)	828.65 (446.98)	431.7 (289.64)	40.7 (22.04)	32.0
2008	43.15 (6.97)	23.75 (3.08)	64.1 (19.26)	861.90 (469.25)	445.1 (314.95)	44.54 (21.10)	36.4
2009	41.1 (6.00)	25.37 (5.97)	57.9 (19.04)	611.01 (249.98) p=0.004c	314.3 (195.75)	51.53 (19.63)	25.92
2010	40.4 (7.67)	25.5 (4.00)	65.46 (19.18)	658.38 (296.37) p=0.015c	319.2 (241.88)	50.49 (18.23)	13.78 (p=0.049)b
2011 (ExAblate 2100)	39.47 (6.53)	23.41 (4.25)	61.02 (17.13)	655.06 (257.49) p=0.005c	305.12 (203.43)	54.78 (19.01) (p=0.007)c	17.1

[b] =Chi-square test. [c] =Mann-Whitney test. BMI= Body Mass Index. SSS=Symptom Severity score. NPV=Non-perfused Volume. HI= Hyper-intensity of treated fibroid

Table 34: ExAblate treatments 2003-2011

Safety of the ExAblate 2100 system

Following the treatment of 34 women using the new ExAblate 2100 system there have been no recorded hospital admissions, no skins burns, and no major reported adverse events. All women were discharged from the MRgFUS center within three hours of their treatment, and only one woman required analgesia to take home (diclofenac orally for three days). There were no reported urinary tract infections and no persistent neurological problems, or other complications. For patients treated with the ExAblate 2000 system, minor complications were experienced by 4% of patients and included urinary tract infection, urinary retention, vaginal bleeding and transient buttock pain. One woman experienced vaginal fibroid expulsion, there was one skin burn requiring a small surgical intervention, and one case of a persistent neuropathy. No emergency hysterectomies were required following MRgFUS therapy in either group.

Discussion

Magnetic resonance guided focused ultrasound ablation, through controlled deposition of high acoustic energy, causes thermally-induced coagulative necrosis of leiomyoma cells. The procedure is performed using real-time MRI guidance, allowing the operator to precisely control the location, intensity and size of the focus of energy deposited. Control with MRI also allows the operator to monitor the temperature of the tissue treated. The developments of the ExAblate 2100 system have been directed toward improving this NPV, while ensuring safety. By changing the shape of the sonication spot to more accurately approximate the shape of the fibroid, the system can treat areas much closer to the serosa of the fibroid. Also, by monitoring the areas adequately treated (by measuring temperature rise within the tissue), and then allowing for a re-plan of the treatment after the initial treatment, a much more satisfactory result can be obtained. With the ExAblate 2000 system, this feature was not available, and it was not until the contrast agent had been administered at the end of the treatment that the effects of the treatment could be assessed. Following administration of the contrast agent it is not possible to perform any further treatment sonications. It is believed that there is a theoretical possibility of the gadolinium chelate dissociating and the free gadolinium becoming fixed in the tissues (Abdullah et al., 2010). If the treatment is found to be sub-optimal, the subject would be required to come back on another day for a repeat treatment, at which stage, planning exactly where to place the remaining sonications would be potentially inaccurate. The mean

percentage NPV achieved to date with the ExAblate 2100 system was 54.92 % (SD 19.28) compared with 43.72 % (SD 22.26) with the ExAblate 2000 system.

We have found an overall improvement in the NPV achieved since the introduction of MRgFUS, and the mean NPV achieved so far with the ExAblate 2100 system (54.92 %) is the highest achieve in our unit. However, when compared with the mean NPV achieved with the previous system the year before (50.49%) this was not found to be a significant overall improvement. It is possible that the year-on-year difference in NPV from 2003 to 2010 is a reflection of the greater experience with the MRgFUS procedure and treatment apparatus, and that a similar curve of improvement aided by familiarity with the technology and better patient selection will continue to evolve. This increase in experience will inform future research directions in this area. Recent publications have reported a 54% NPV following prolonged experience with the ExAblate 2000 system (Okada et al., 2009a). A survey of MRgFUS operators found a target percentage NPV of 76% and a reported achieved NPV of 58% (Taran et al., 2010b).

The year-on-year results do suggest that patient selection for MRgFUS has favored women with smaller uteri, smaller overall fibroid bulk and a tendency to treat women with fewer hyper-intense fibroids. There is a known reduced efficacy of this treatment in patients with hyper-intense fibroids on T2-weighted MR imaging (Machtinger et al., 2012). As such, a tendency towards treating single hypo-intense fibroids, rather than multiple fibroids or single hyper-intense fibroids, has become evident since the inception of the technique in 2003, whilst patient demographic data remains largely unchanged between the two groups. The higher non-perfused volumes achieved with the earlier ExAblate 2000 technology in hypo-intense fibroids in recent years would seemingly support the rationale for this shift in patient selection. The initial experience with the ExAblate 2100 system suggests that we are able to achieve greater treatment volumes even in those women with hyper-intense fibroids. However, given the overall effects on five year re-intervention rates found in chapter four of hyper-intense fibroids it may be prudent to continue to avoid treating these fibroids in order to improve long-term outcomes.

Safety of the newer ExAblate 2100 system has proven to be encouraging, with no adverse events recorded at present. There were no complications experienced using the new system. In particular, there were no skin burns or evidence of neurologic injury, which were the two most significant complications seen (albeit very rarely) using the ExAblate 2000 system. It is perhaps worth noting that the complications experienced using either the ExAblate 2000 or ExAblate 2100 were, when compared to the reported complication profile of hysterectomy or myomectomy relatively modest (Khaund and Lumsden, 2008).

As with the introduction of any new technology, a learning curve aided by experience and familiarity with the system is to be reasonably expected. We would hope that once operators using the new ExAblate 2100 system gain more experience with this system, the trajectory of NPV improvement should show further

positive improvement. A more evidence-based approach to patient selection, favoring patients with smaller, less numerous, hypo-intense fibroids may augment this anticipated process of improvement. Longer follow up of these patients is required to determine if increases in non-perfused volume in these patients translate to higher patient satisfaction and a reduced re-intervention rate.

Conclusions

Overall, the new ExAblate 2100 system has demonstrated an encouraging safety record and an improvement in the non-perfused volumes within the treated uterine fibroids achieved, especially in hyper-intense fibroids.

6. Pain and cytokine production following MRgFUS and UAE

Introduction

In recent years the emergence of non-invasive treatments for uterine fibroids such as uterine artery embolism (UAE) and MRI-guided focused ultrasound therapy (MRgFUS) have provided women with a greater choice in their management. Following UAE, women are known to experience significant pain, requiring a hospital admission and the use of high dosage opioid analgesia. In contrast, MRgFUS is a day-case procedure rarely requiring any post-treatment analgesia. Cytokines are known to have an important role in nociceptive response and inflammatory processes. The plasma levels of cytokines following - UAE and MRgFUS - have not been evaluated.

Cytokine release following tissue trauma and pain

The physiological response to trauma involves metabolic, endocrine and immune system processes that fight infection, limit further damage and initiate tissue repair. The immune response to injury involves a complex set of interactions between pro-inflammatory and anti-inflammatory cytokines. The term cytokine refers to a large family of cell-signalling molecules involved in immune processes. Cytokines include interleukins (IL), tumour necrosis factor (TNF-α, TNF-β) and interferons. Cell injury leads to an initial systemic inflammatory response, followed by a period of counter-regulatory anti-inflammatory response.

The mechanism of pain initially involves a neuronal afferent stimulus from a site of trauma, with resultant release of pro-inflammatory cytokines. This leads to the activation of cellular and humoral (antibody-mediated) immune pathways (Hall and Desborough, 1992). The cellular immune pathway involves the activation of phagocytes, cytotoxic T-lymphocytes and the release of further cytokines as a response to presenting antigens. Tissue damage (e.g. mechanical, radiation, inflammatory, thermal) leads to the release of IL-1, IL-6, TNF and Chymase by mast cells and pro-IL-1 by fibroblasts. Chymase is a serine protease which acts to change pro-IL-1 into its active form. IL-1 binds to peripheral nerve terminals causing both activation of the nerves and release of substance P. Substance P then initiates a positive feedback loop that degranulates

mast cells producing more IL-1, IL-6, TNF, and Chymase. Substance P is a neuropeptide of the tachykinin group that functions as a neurotransmitter and is expressed throughout the central and peripheral nervous systems (Harrison and Geppetti, 2001). Anti-inflammatory cytokines include IL-4 and IL-10. Il-4 is a pleiotrophic cytokine produced by activated CD4+ T cells, mast cells, eosinophils and basophils. Release of Il-4 activates and maintains the growth of B lymphocytes, stimulates the synthesis of IgG and IgE. Il-4 plays an important role in inflammation and tissue repair, promoting the activation of macrophages into repair macrophages. The activated repair macrophages release arginase, proline and TFG-β which are all involved in tissue repair. IL-4 has an effect on nociception and can induce and up-regulate the transcription of mu and delta opioid receptors within injured tissues (Börner et al., 2004). Circulating serum levels of IL-4 are reduced in patients with chronic pain, and it has been suggested that reduced opioid receptor signalling is a possible mechanism for higher pain perception in those with lower circulating Il-4 (Uçeyler et al., 2006).

Il-6 is both a pro-inflammatory and anti-inflammatory cytokine, produced by T cells, B cells, monocytes, and fibroblasts following trauma and infection. IL-6 has a major role in the response to injury and the acute phase response to inflammation and may have a role in the reactive thrombocytosis that occurs during this phase (Unsal et al., 2005). IL-6 stimulates the hepatic production of acute-phase proteins, such as C-reactive protein, and serum levels of IL-6 correlate to the extent of tissue injury (Rixen et al., 1995). Following surgical procedures serum levels of IL-6 appear to peak at 24 hours post procedure, and fall significantly by 72 hours (Høgevold et al., 2000, Yue et al., 2009) and return to normal by one week (Volk et al., 2004). Increased levels of circulating IL-6 is an early marker of tissue injury-being detectable 2-4 hours post insult. The increase in IL6 is correlated with the degree of injury (Cruickshank et al., 1990). IL-1 and IL-6 can induce peripheral and central nerve system sensitization, leading to the augmentation of the pain response (hyperalgesia) (Watkins et al., 1995). Il-6 and it's receptor gp80 and trans-membrane signal transducer gp130 are up regulated in peripheral nerves, in the dorsal root ganglia and also in the spinal cord during induced, experimental pain (De Jongh et al., 2003).

Il-1α and Il-β are produced by macrophages, monocytes, fibroblasts and dendritic cells as part of the inflammatory response to injury or infection. The hyperalgesic effects of IL-1 are caused by the production of pro-nociceptive compounds such as nitric oxide (NO), substance P, prostaglandins, nerve growth factor (NGF), or the release of calcitonin-G-related protein (CGRP) (Safieh-Garabedian et al., 1995). Systemic or central administration of exogenous pro-inflammatory cytokines leads to the enhancement of nociception and increases neuronal excitability in response to noxious stimuli (Wieseler-Frank et al., 2005). Conversely, the blockade of pro-inflammatory cytokine function (for example by using an interleukin-1 receptor

antagonist) can prevent or reverse hyperalgesia in animal models (Torres et al., 2009). Circulating plasma Il-1β is significantly raised after episodes of unstable angina (Ozeren et al., 2003).

Tissue response to UAE and MRgFUS

Embolization of the uterine arteries involves the occlusion of both uterine arteries with polyvinyl alcohol microparticles (Ravina et al., 1995). Once occlusion has occurred, there is a prolonged transient uterine ischemia; the blood within the myometrium clots and the myometrium becomes hypoxic. The myometrium is later re-perfused by collateral arteries, and the uterine clots undergo lysis. Uterine fibroids are unable to successfully lyse blood clots, and as a result the blood supply is not restored. Uterine fibroids therefore become infarcted and undergo ischemic necrosis following embolization (Burbank and Hutchins, 2000).

One of the most serious complications encountered following UAE is endometritis (uterine infection), with a reported incidence of approximately 2% (Hirst et al., 2008, Spies et al., 2002b). Severe infection can be associated with morbidity significant enough to require hysterectomy and may result in mortality. One study has investigated the inflammatory response following UAE compared with that after abdominal hysterectomy (Brøchner et al., 2009). Women undergoing embolization have a significantly reduced inflammatory response compared with those undergoing a hysterectomy. Il-6 levels were found to be significantly raised by four hours, although by a much smaller degree than for those undergoing hysterectomy.

MR-guided focused ultrasound results in the heating of fibroid tissue to greater than $55^{o}C$ at a single focal point. This results in protein denaturation and coagulative necrosis of the fibroid tissue. Necrosis refers to the histological changes as a result of irreversible damage to the cell, with resultant progressive deterioration of the cell cytoplasm organelle function (responsible for all the metabolic, synthetic and energy functions of the cell). Necrosis is associated with an increased inflammatory response. This is a result of the release of cell contents following the increased permeability of the cell membrane after injury (Majno and Joris, 1995).

MRgFUS produces multiple focal spots of coagulative necrosis within the target fibroid tissue until a significant area has been treated. During the treatment, women may experience severe lower abdominal pain usually requiring opioid analgesia. Post-procedure recover is however rapid, with little post-treatment analgesia required. Patients are able to return home on the day of treatment without the need for

prescription analgesia. Risks associated with this treatment are low, and infection extremely rare (<0.5%). The effects of focused ultrasound on circulating cytokines have as yet not been studied.

Pain-response following treatments for uterine fibroids

UAE can result in significant post-procedural pain (Goodwin et al., 1999).This has been attributed to the pathophysiological process of myometrial ischemia caused by effective embolization (Ruuskanen et al., 2009). The pain experienced following embolization increases within 3 to 6 hours of the procedure, then falling by 12 hours to a minimal level (Brøchner et al., 2009). A number of other general side effects can be experienced following UAE. These may include an often self-limiting non-purulent vaginal discharge, transient vasomotor symptoms (hot flushes) that are likely to be related to the temporary disruption of normal ovarian hormone production, constipation, cramping, spontaneous fibroid tissue expulsion, and post-embolization syndrome (PES) (Dutton et al., 2007, Edwards et al., 2007, Spies et al., 2002b). PES can occur with any embolization procedure in any solid organ as part of the immune response. PES presents with transient low-grade fever, pain, nausea, and fatigue lasting from a few hours to a few days. The transient fever is generally no higher than 38.3^{o}C and is usually associated with leucocytosis.

Pain following embolization may be managed with aggressive sustained treatment with a non-steroidal analgesic beginning 1-2 hours prior to UAE and the use of a patient-controlled analgesia pump generally for 6-12 hours afterwards. Most patients can then be transitioned to oral medications. Pain experienced following UAE persists for much longer than that experienced following MRgFUS, and usually requires larger doses of opioid analgesia.

MRI-guided focused ultrasound has been found to be relatively well tolerated by women. An initial study found pain scores following the treatment of moderate to severe in 8% of women (Hindley et al., 2002).

Aims:

I aim to investigate:

(a) The change in circulating IL-1β, IL-4 and IL-6 following UAE and MRgFUS treatments for uterine fibroids

(b) Whether there is a relationship between pain-scores and the volume of fibroid tissue treated post MRgFUS and UAE

Methods

This was a prospective study of pre- and post-treatment plasma cytokine levels following both UAE and MRgFUS for uterine fibroids. Regional and local ethics committee approval was obtained (national ethics

reference number 11/LO/0119). Subjects were recruited from the St Mary's Hospital fibroid clinic. All women were given written information regarding the study (Appendix F) and given the opportunity to ask questions about the study. If the women agreed to participate in the study, a letter was sent to their general practitioner (Appendix G) and written consent was obtained (Appendix H: Consent for Cytokine study). Inclusion and criteria are given in Table 35.

Inclusion criteria	**Exclusion Criteria**
Women due to undergo UAE or MRgFUS at St Mary's Hospital.	Presence of any other systemic disease which may contribute to circulating cytokine levels.
Willingness to have blood samples taken at intervals	Ischemic heart disease
	Acute or chronic infections
	Any form of on-going treatment for cancer
	Autoimmune disease (e.g. SLE, Rheumatoid arthritis)
	Any form of surgery three months prior to treatment
	Body mass index > 36kg/m^2
	Cerebral vascular accident
	Pregnant or lactating

Table 35: Inclusion and exclusion criteria for Cytokine Study

Blood samples were collected from subjects in the two groups by S. Quinn (Table 36).

UAE patients (n=20):	MRgFUS patients (n=20)
1. Pre-treatment	1. Pre-treatment
2. 2 hours post treatment	2. immediately post treatment
3. 4 hours post treatment	3. 2 hours post treatment
4. 6 hours post treatment	
5. 24 hours post treatment	

Table 36: Protocol for Collection of plasma samples before and after treatment

Peripheral venous blood was collected after application of a tourniquet for less than two minutes into heparinised plasma separator tube samples and left to clot for 30 minutes before undergoing centrifugation for 15 minutes at 2000 revolutions per minute (RPM). Plasma was separated by pipette and stored at -80°C, until the time of analysis.

In addition to prior to collection of each blood sample, pain-scores were recorded using a visual analogue scale (VAS) (Figure 44: Visual analogue scale (VAS) for pain). Trial documentation is shown in Appendix I: Cytokine Study trial documentation. Following UAE and MRgFUS, the total uterine volume and non-perfused volume of the fibroids were calculated using the parallel planimetric area method (Figure 16, page 72).

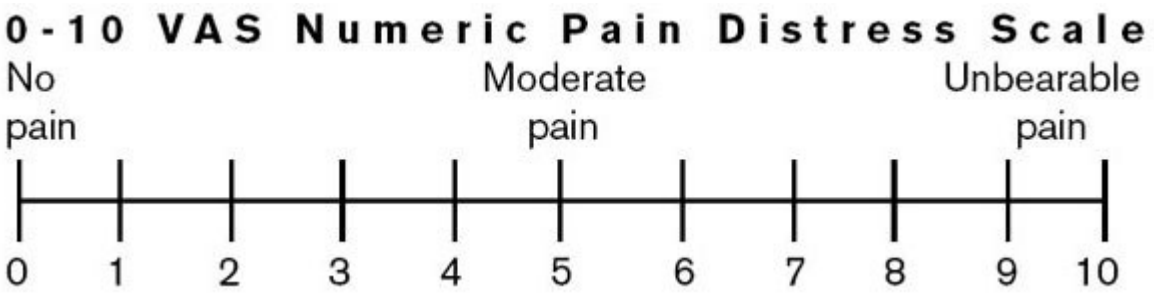


Figure 44: Visual analogue scale (VAS) for pain

Sample Processing

Prior to assay, samples were thawed and centrifuged to remove any clotted material. All samples were analysed following the first thaw. Samples were assayed using the Meso Scale Discovery (MSD®) 96-well multi-Array custom cytokine kit for IL-1β, IL-4 and IL-6 (Meso Scale Diagnostics, Rockville, MD). Manufacturer's guidelines were used. These assays use an immunoassay preparation in which capture-antibodies (for specific cytokines) are coated on a specific spot within the wells of a Multi-Spot® plate (Figure 45: MSD electrode (Meso Scale Discovery™)). Prior to placing the calibrator cytokine mixtures or plasma samples in the wells, the plates are prepared with a buffer solution provided by the company MSD™. Twenty-five micrograms of the buffer was added to each individual well by pipetting to the bottom of each assay well. The capture plate was then sealed and incubated for 30 minutes with rapid shaking (300-1000 rpm) at room temperature. Following this, 25µl of each calibrator or sample was dispensed into the bottom of each well. Duplicate wells of calibrator and sample were used to reduce handling error. Calibrator samples were produced by preparing a combined working stock solution of each cytokine. IL-1β, IL-4 and IL-6 calibrators were provided at 50µg/ml stock concentration. To 470µl of diluent buffer solution we added 10µl of each 50µg/ml cytokine calibrator to produce a final volume of 500µl. this combined working stock had a concentration of 1000000pg/ml. This was further diluted by a further 4-fold to produce 7 individual calibrators. The eighth calibrator was a buffer solution alone (0pg/ml concentration). The calibrator standards are show in Table 37: Calibration samples for cytokine study.

Standard	Concentration (pg/ml)	Dilution Factor
Combined working stock	1000000	
Diluted stock calibrator	10000	100
Standard 1	2500	4
Standard 2	625	4
Standard 3	156	4
Standard 4	39	4
Standard 5	9.8	4
Standard 6	2.4	4
Standard 7	0.61	4
Standard 8	0	n/a

Table 37: Calibration samples for cytokine study

Electrochemiluminescence

Electrochemiluminescence is a form of light energy that is produced during electrochemical reactions in solution, not resulting in heat. Electrochemically generated intermediates at electrode surfaces undergo electron-transfer reactions to form excited states that emit light (Forster et al., 2009a, Richter, 2004). The reactions involved require three constituents to be aligned in order to occur. A precursor molecule must first be allowed to diffuse into an electrode surface in order to be activated. Following this a Ruthenium complex ($Ru(bpy)_3^{2+}$) reacts with Tripropylamine (TPA) to release a photon and electron. The wavelength of the emitted photon of light corresponds to the energy gap between the two states, an excited state and a lower-level state. By labelling biological molecules, this light emitted is used to identify different molecules at very low concentrations (Forster et al., 2009b) (Figure 45).

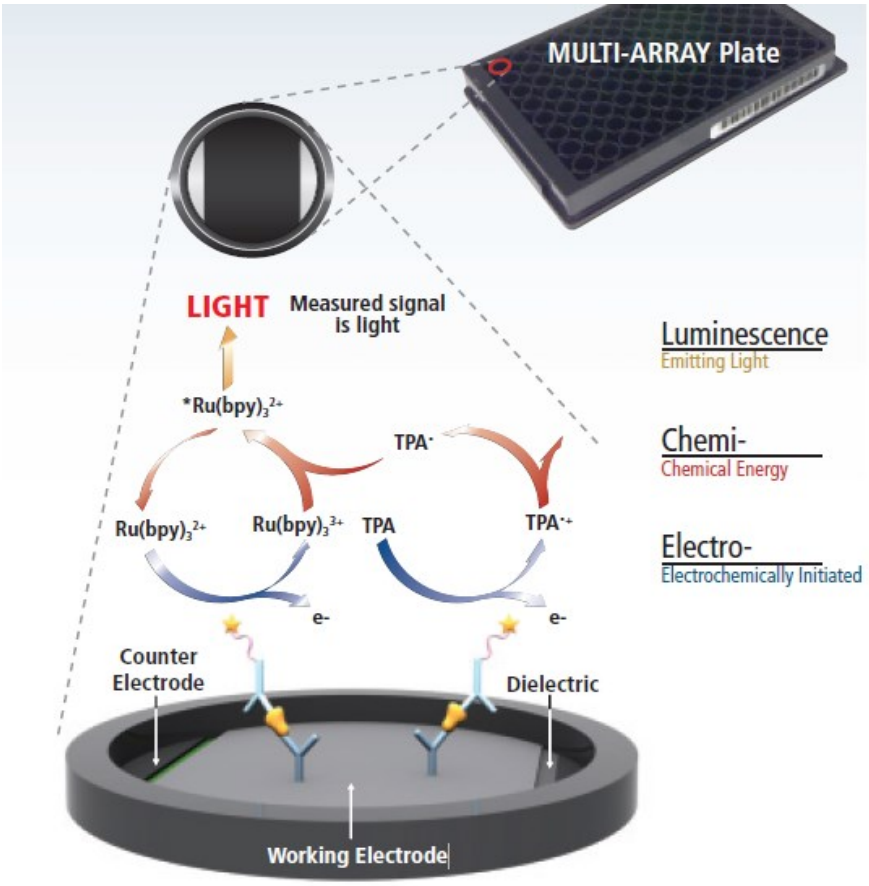


Figure 45: MSD electrode (Meso Scale Discovery™)

Once the calibrator standards and samples were added to the plates, the cytokine plates were sealed and incubated for two hours with rapid shaking (300-1000rpm) at room temperature. These plates were then irrigated three times with a diluted detergent solution (0.05% Tween-20 in phosphate buffer solution (PBS)). The plates were dried and then 25µl of the detection antibody solution was dispensed into the bottom of each well. The plates were again sealed and incubated for two hours with rapid shaking (300-1000rpm) at room temperature. The plates were irrigated again three times with detergent solution, dried and finally 150µl of read buffer solution was added to each well, while ensuring that no air bubbles were present in the wells (air bubbles found in the assay wells can interfere with the reliability of the cytokine readings). A reverse pipetting technique (expelling any air prior to aspiration of sample) was used to ensure that air bubbles were not created during pipetting the read buffer. The plates were then transferred to the flow cytometry labs for immediate reading by the SECTOR® imager. Following reading by the imager the results were analysed using the values obtained by the calibrator standards. The analysis software used by the MSD DISCOVERY WORKBENCH® uses a four parameter logistic model (or sigmoidal dose-response model). By running the calibrators in two duplicates the software produces a standard curve based on the mean of the two values produced (Figure 46). The analysis software produces a report including information regarding the concentration (pg/ml), signal produced by the well, the mean signal between the two wells, the coefficient of variation between the two wells, the calculated concentrations for the cytokine in question (pg/ml) and the mean calculated concentration between the two wells, and again the coefficient of variation between the two calculated concentrations.

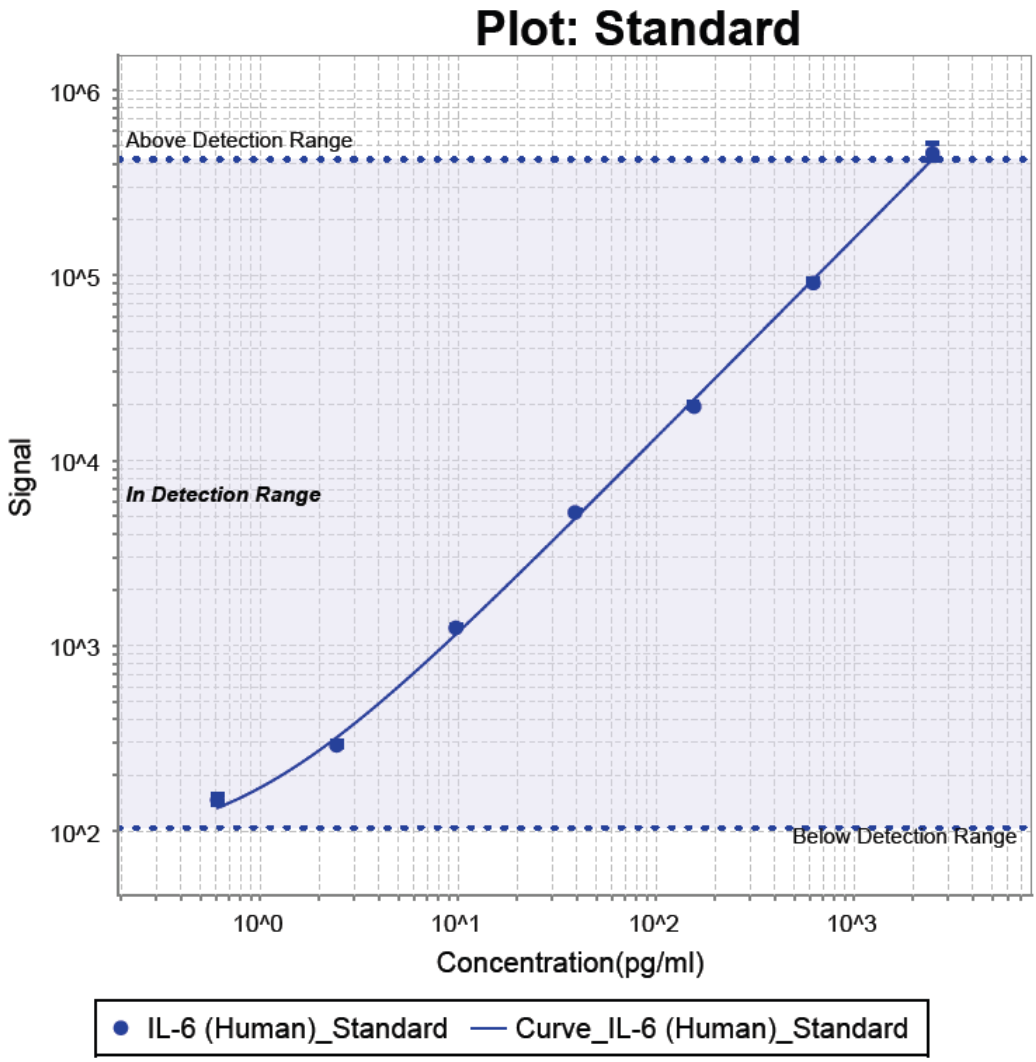


Figure 46: Detection plot produced for IL-6

Between July 2011 and January 2012, 20 subjects undergoing MRgFUS and 20 women undergoing UAE were recruited to this study from the tertiary fibroid clinic at St Mary's Hospital, Imperial College Healthcare NHS Trust (Figure 47).

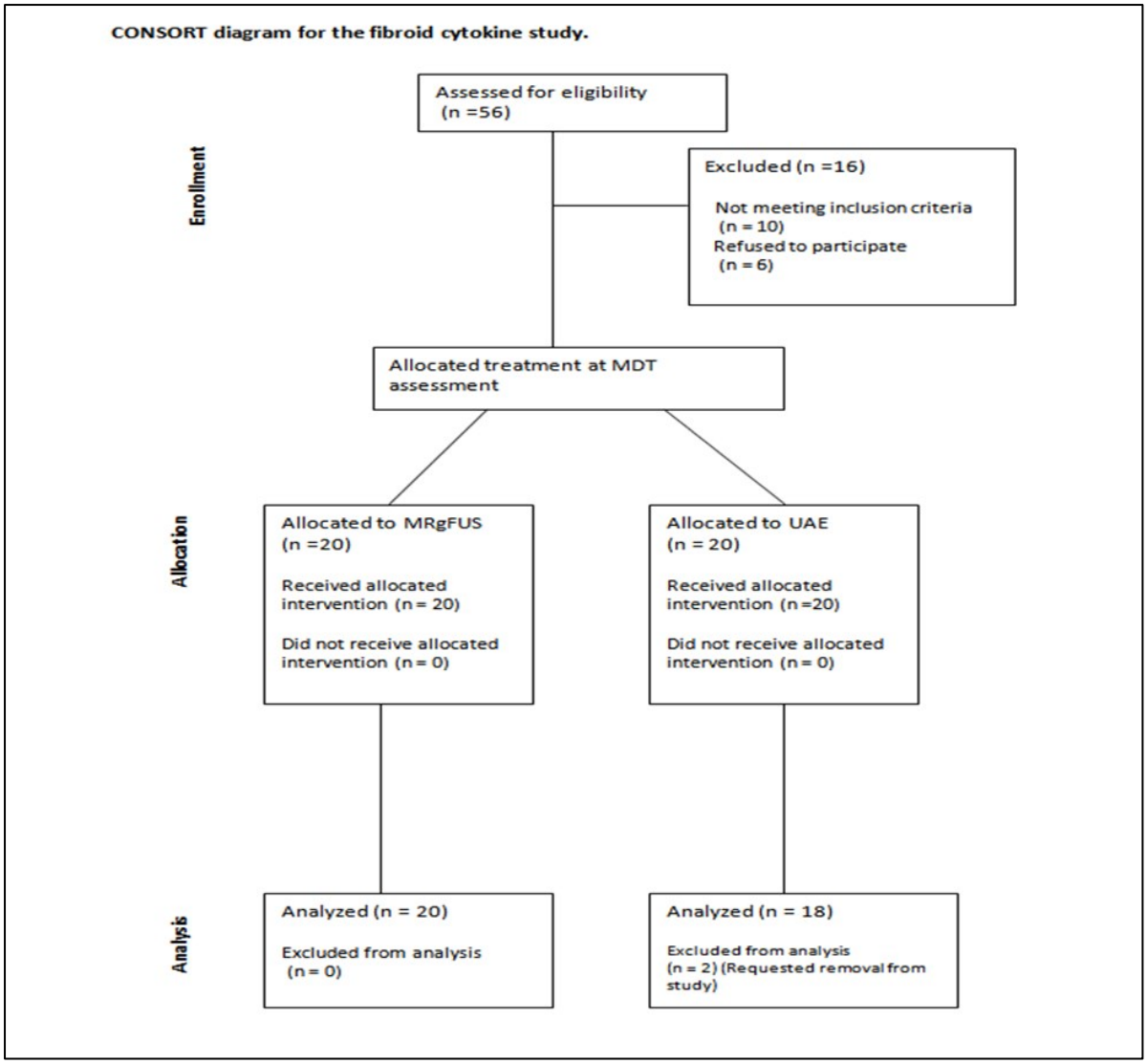


Figure 47: Consort diagram for the fibroid cytokine study

As I was unsure of the degree of change in circulating cytokine levels to expect, it was agreed that as a pilot study 20 participants in each arm would be reasonable, and that further study could be arranged following our initial analysis, if warranted. 2 women in the UAE arm of the study requested to be withdrawn from the study following their treatments. 5 women refused blood collection at 24 hours in the UAE group. Pre-treatment MRI images were unavailable on two of the UAE subjects. Demographics of these 38 women are given below in Table 38.

	MRgFUS (n=20) Mean (SD)	UAE (n=18 Mean (SD)	P Value
Age (Years)	38.9 (6.5)	43.3 (6.1)**	0.02*
BMI (kg/m^2)	21.8 (2.6)	30.2 (7.9)*	0.00 ∓
SSS (Symptom severity score)	67.4 (22.6)	62.9 (22.0)	0.56*
Uterine Volume (ml)	666.8 (279.6)	906.6 (550.2)	0.64 ∓
Volume of three largest fibroids (ml)	308.2 (240.6)	306.2 (226.1)	0.80 ∓

*Student T Test ∓ Mann-Whitney U test

Table 38: Demographics of subjects enrolled in the cytokine study

Between the two groups there was a significant difference in the mean BMI (p=0.001) and ages of the subjects (p=0.016). None of the other variables were statistically different (for uterine volume p=0.64).

Statistics

Distribution of values for circulating cytokine results were tested for normal distribution. Where data was normally distributed student's t-test was used to test for significance. Non-parametric data was tested using Wilcoxon signed rank test. All statistical analysis was performed using SPSS version 19 (BM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

Results

Following MRgFUS, mean VAS pain score immediately post treatment was 4.58 (SD 2.27) and by two hours was 2.1 (SD 1.52) (Figure 48).

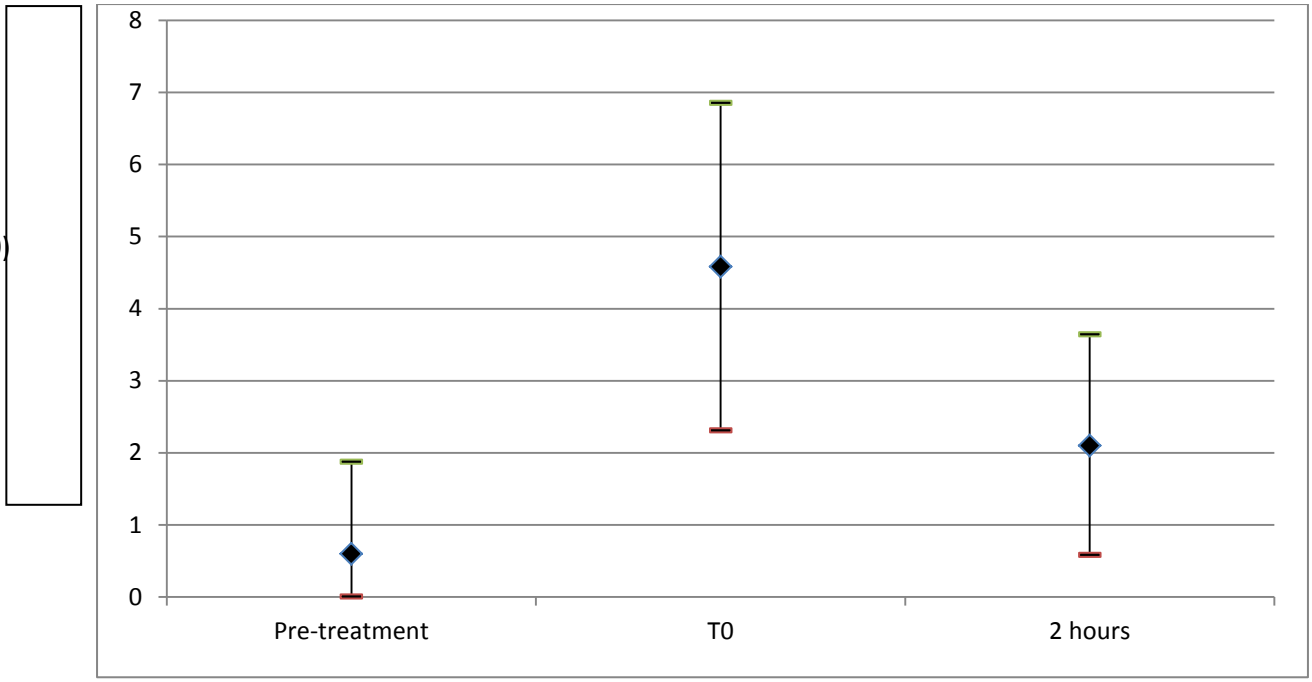


Figure 48: Visual analogue pain score (VAS) post MRgFUS (mean and SD)

Immediately following UAE, the mean VAS was 7.80 (SD 2.39), falling to 5.80 (SD 2.12) at two hours, increasing to 6.40 (SD 1.47) at four hours and then falling to 4.31 (SD 1.88) by 24 hours (Figure 49).

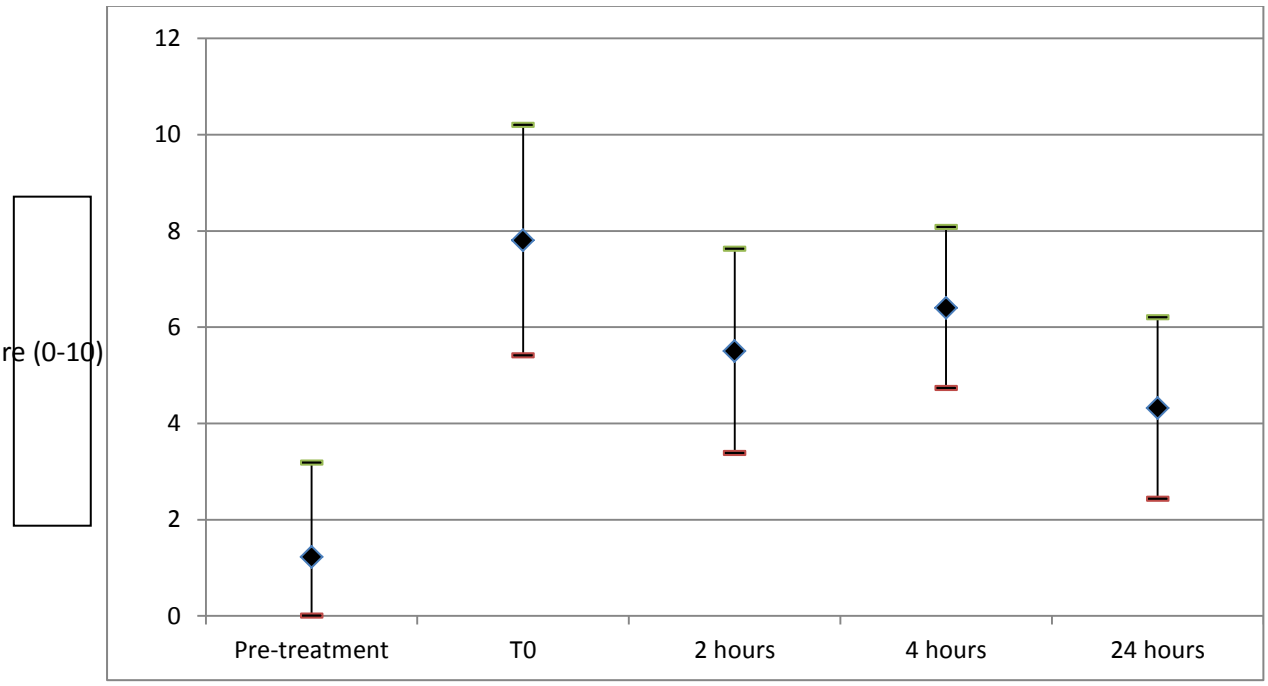


Figure 49: VAS post UAE (mean and SD)

Mean VAS pain scores immediately following UAE and MRgFUS and at two hours were significantly different between to two groups (7.80 vs. 4.65 p= 0.01 and 2.2 vs. 5.5 p= 0.04 respectively). Women undergoing MRgFUS were discharged by three hours, so we did not record VAS score at that time. No patient undergoing MRgFUS required a longer admission time, and none required take home analgesia. Following UAE there was a non-significant trend towards a lower post treatment VAS score and increasing uterine volume (see Figure 50).

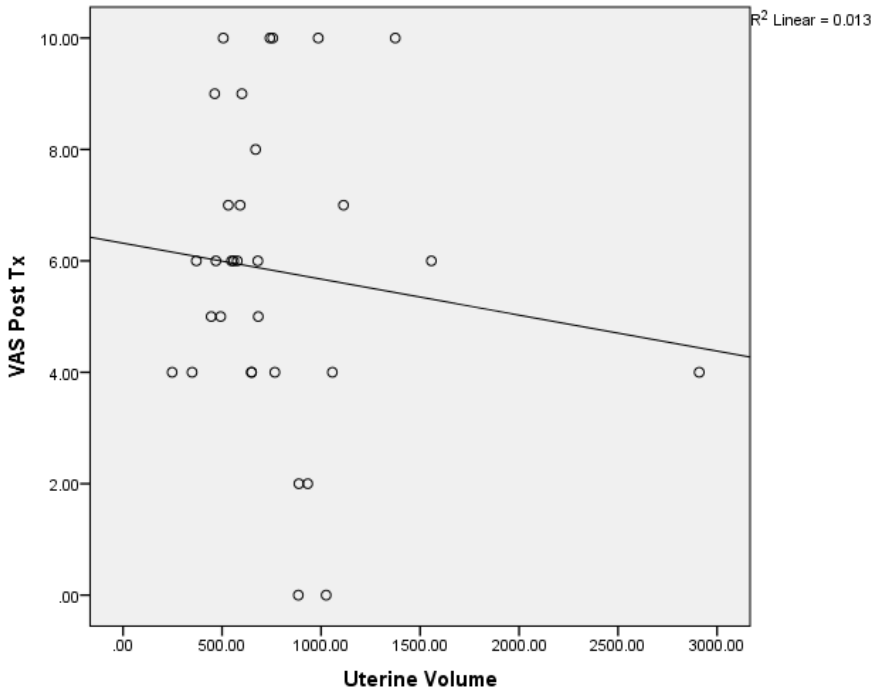


Figure 50: VAS following UAE and total uterine volume

This trend was similar when VAS was measured against total fibroid volume of the three largest fibroids (Figure 51).

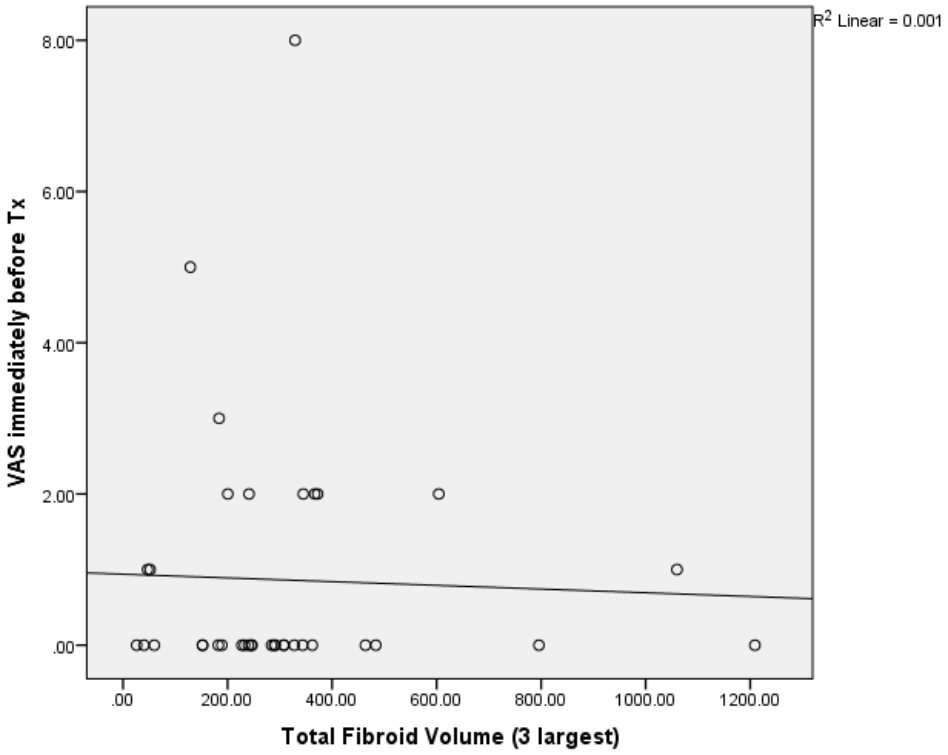


Figure 51: VAS scores post UAE and Fibroid volume

When VAS at 24 hours was plotted against total uterine volume there was no significant relationship or trend (Figure 52).

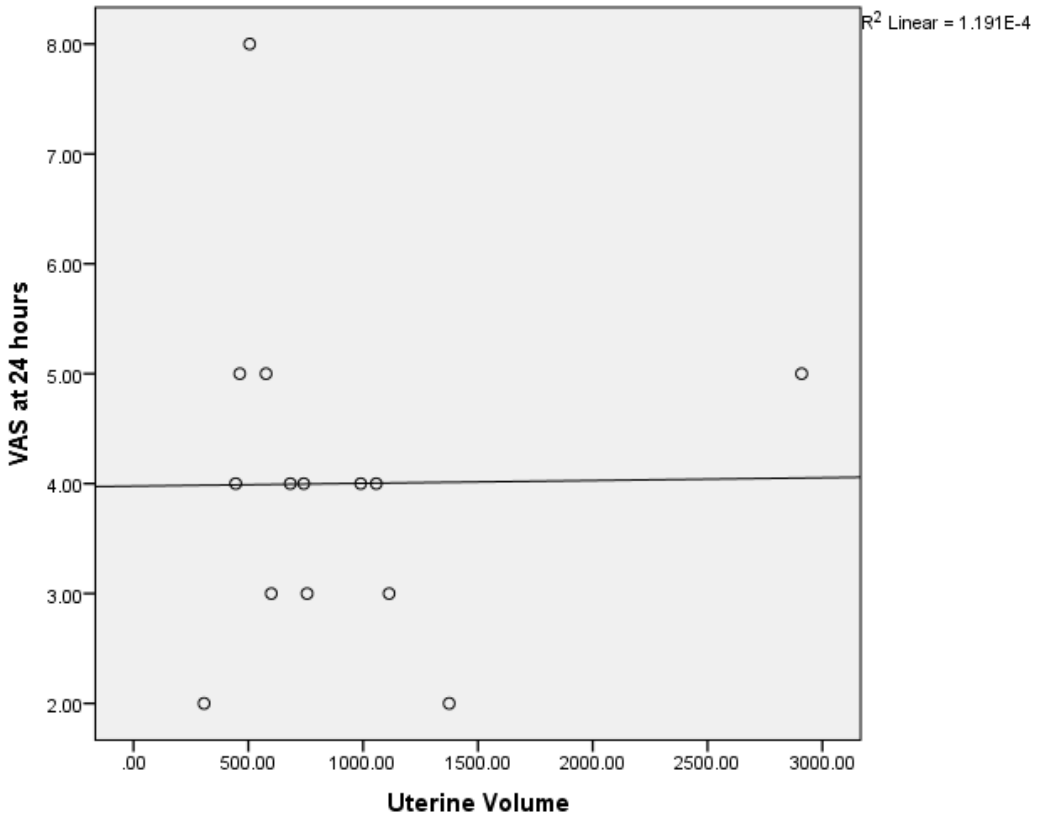


Figure 52: VAS at 24 hours and Total Uterine Volume

Pain scores at 24 hours plotted against volume of the three largest fibroids found a positive, but non-significant increase (Figure 53). Overall, we found no significant relationship between reported VAS and uterine or fibroid size.

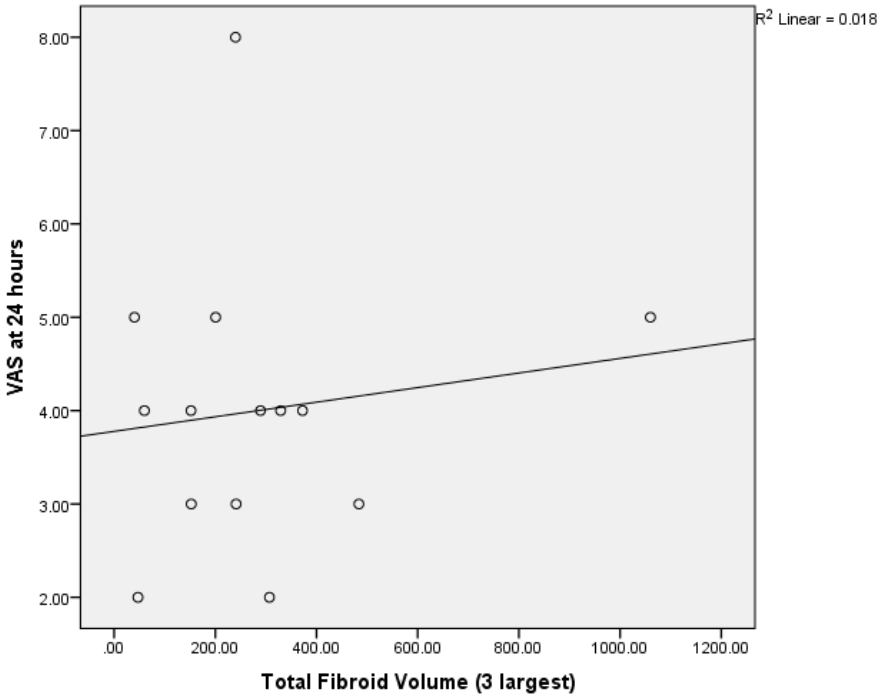


Figure 53: VAS scores at 24 hours and fibroid volume

The MSD plates were unable to detect a significant signal in 23 (60.5%) of the duplicate samples for IL-1β, and in none of the samples for IL-4. Of the results obtained, the mean IL-1β at baseline was 0.22 SD 0.091 (n=15), and IL-6 was 3.97 SD 5.96 (n=32) at baseline. Detectable circulating IL-1β and IL-6 levels are described in Table 39, Figure 54 and Table 40 and Figure 56. Baseline Il-6 was higher in the UAE group (mean 4.23pg/ml vs. 3.62 pg/ml) however this was not statistically significant (Wilcoxon signed rank 0.91).

Cytokine	Pre-MRgFUS Mean (SD)	Immediately post MRgFUS Mean (SD)	2 hours post MRgFUS Mean (SD)
IL-1β (pg/ml)	0.24 (0.13)	0.21 (0.09)	0.22 (0.03)
IL-6 (pg/ml)	3.62 (5.22)	5.30 (3.62)	9.90 (6.22)ŧ

¥ p=0.007 ₸ p=0.005

Table 39: Cytokine concentration following MRgFUS

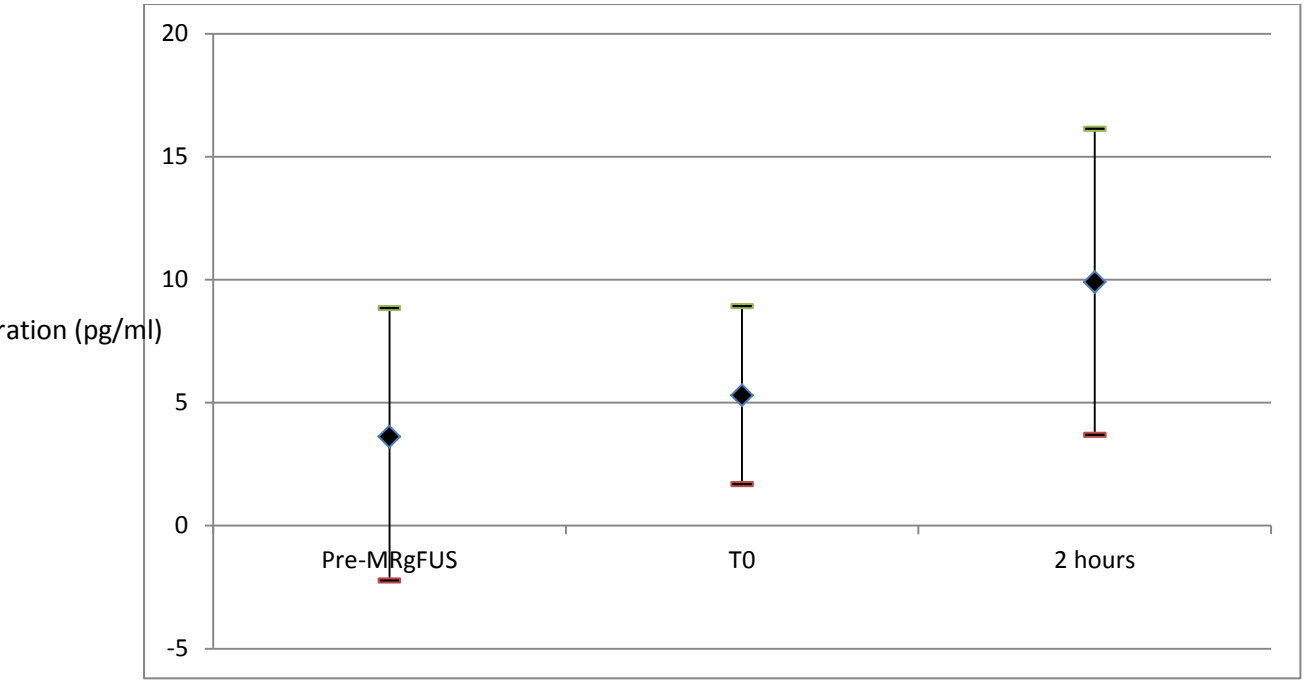


Figure 54: Circulating IL-6 concentration post-MRgFUS (Mean and SD)

The longitudinal changes in IL-6 following MRgFUS are described on Figure 55.

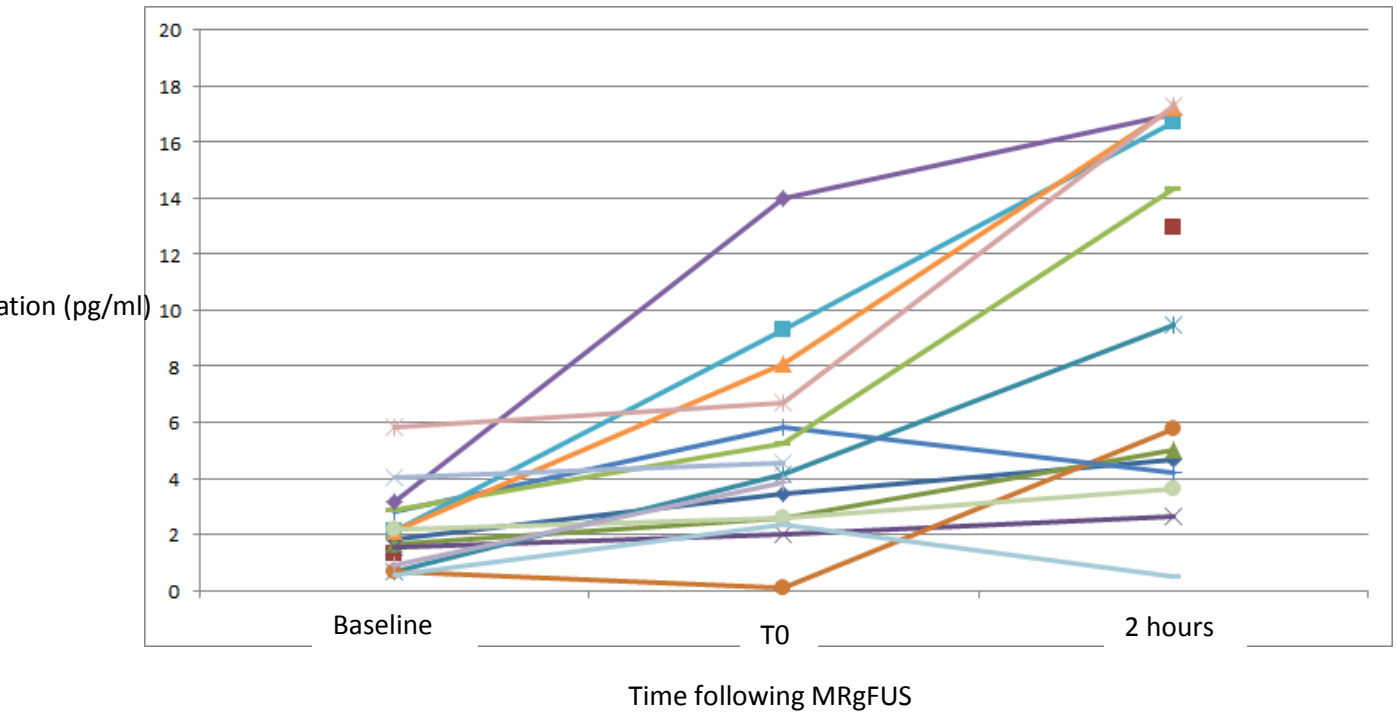


Figure 55: Longitudinal change in IL-6 following MRgFUS

Circulating IL-6 was significantly raised immediately following MRgFUS (p=0.007), and also at two hours post treatment (p=0.005).

The concentration in Il-1β was raised immediately post treatment in the case of UAE although this failed to reach significance (p=0.225, Wilcoxon signed rank test). By twenty four hours the baseline was still elevated although not significantly so (p=0.465).

Cytokine	Pre-UAE Mean (SD)	Immediately post treatment Mean (SD)	4 hours Mean (SD)	24 hours Mean (SD)
IL-1β (pg/ml)	0.22 (0.04)	0.26 (0.1)	n/a	0.24 (0.06)
IL-6 (pg/ml)	3.74 (5.86)	5.16 (5.63)	6.66 (3.43)**	18.54 (14.62)***

**p=0.03 ***p<0.00

Table 40: Cytokines following UAE

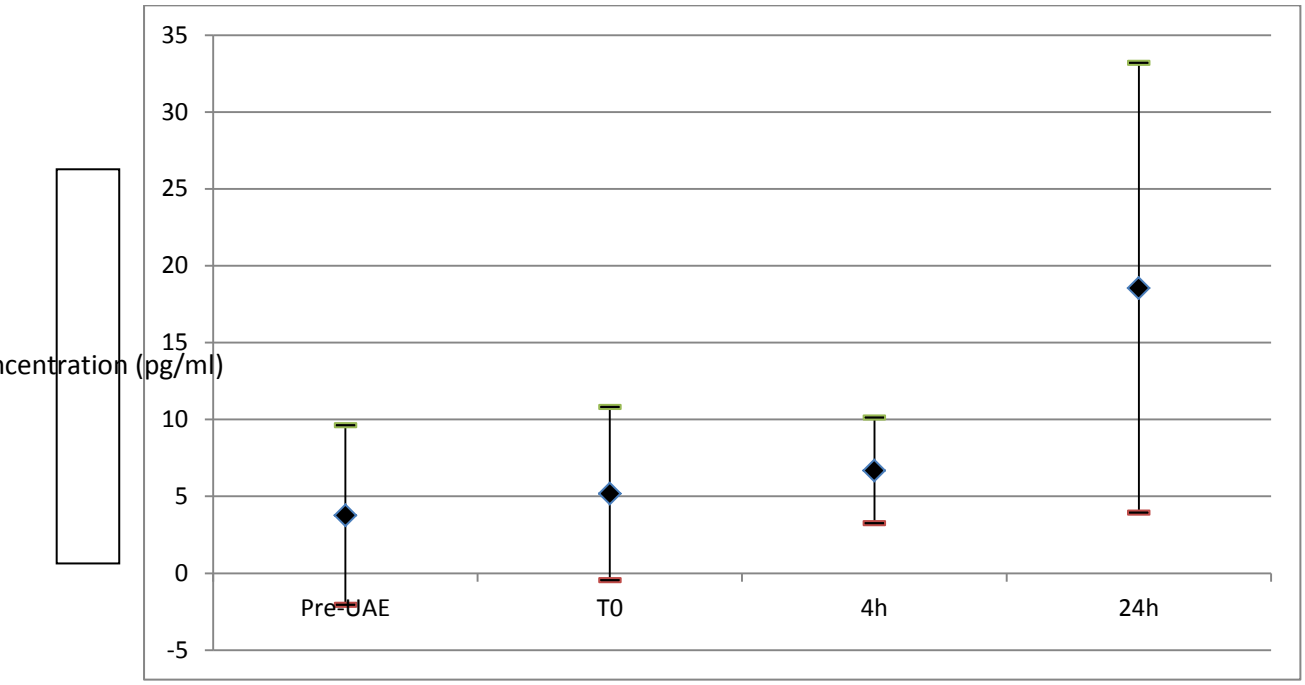


Figure 56: Circulating IL-6 concentration post-UAE (mean and SD)

The longitudinal hanges in IL-6 post UAE are described in Figure 57.

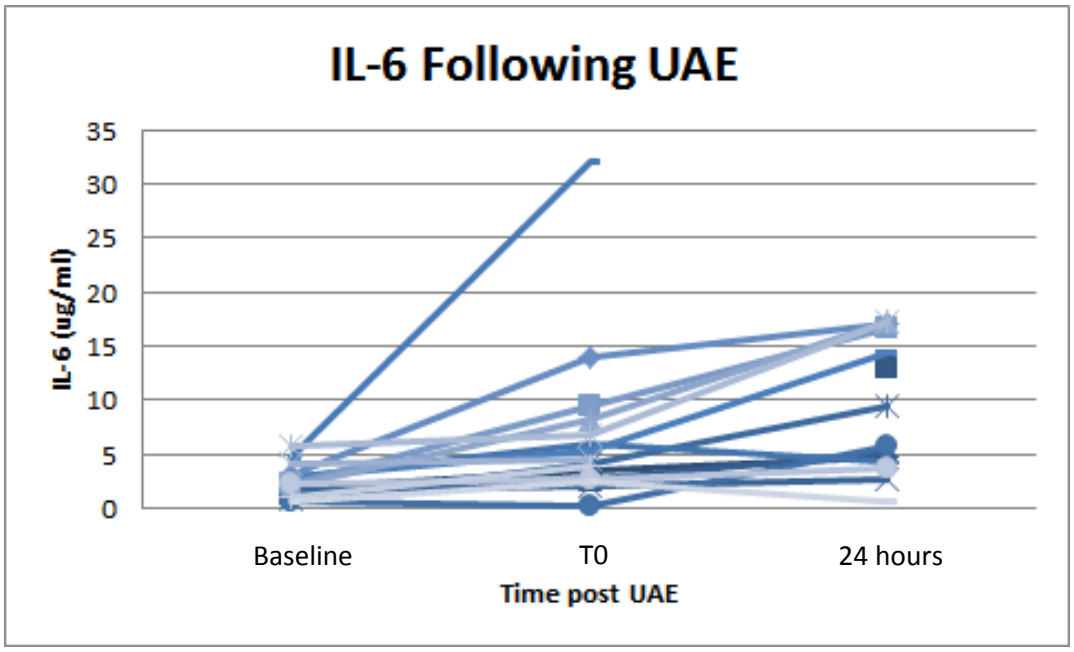


Figure 57: IL-6 following UAE

The circulating plasma levels of IL-6 were significantly raised at four hours following UAE ($p=0.028$) and at 24 hours following UAE ($p<0.001$). There was no relationship between IL-6 level 24 hours post UAE treatment and the volume of the fibroid uterus (Figure 58).

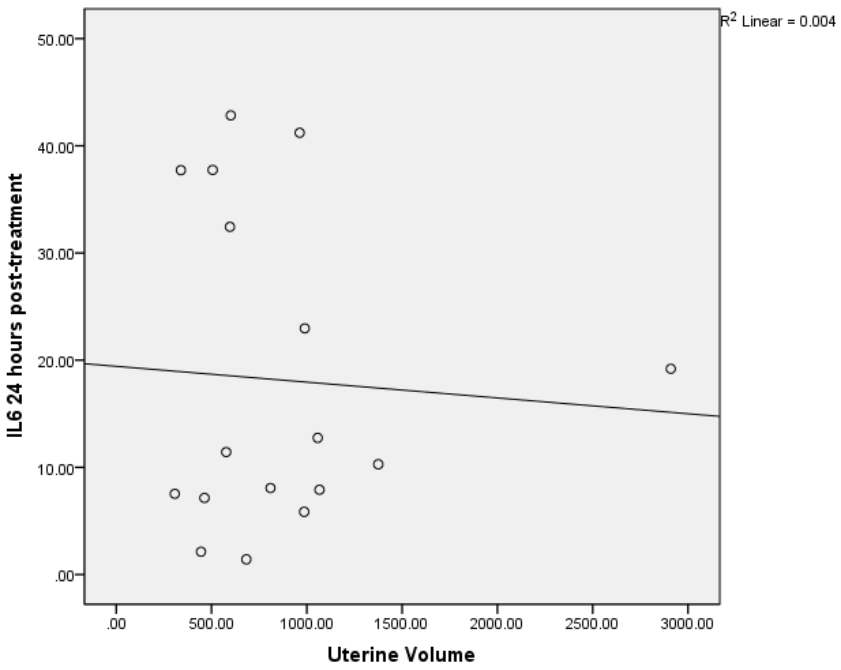


Figure 58: IL-6 at 24 hours post UAE and total uterine volume

I used the calculated non-perfused volume (NPV) following MRgFUS as an indicator of fibroid volume treated and correlated this with peak IL-6 (at two hours), however this again showed no significant correlation (r=0.281 p=0.43) (see Figure 59).

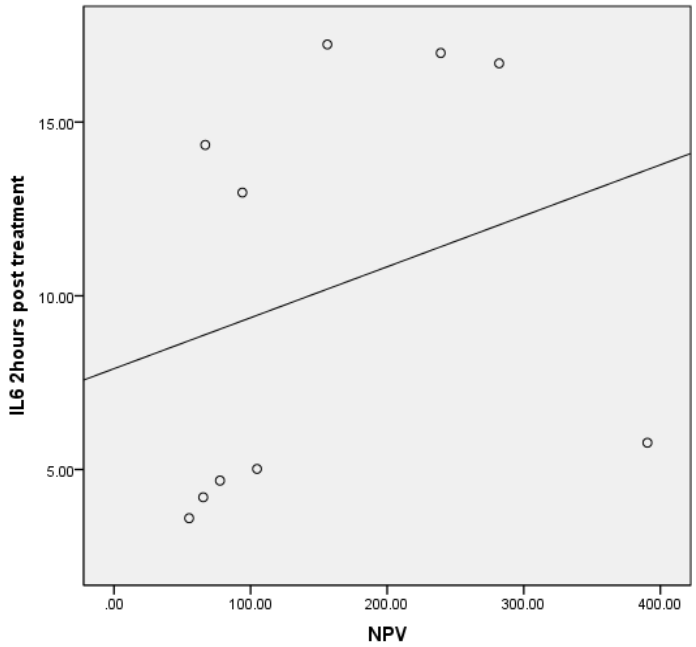


Figure 59:IL6 and the NPV achieved at MRgFUS

In order to investigation any relationship between maximum pain scores and maximum IL-6 levels these were plotted (Figure 60), and Pearson Correlation was 0.002 (p=0.995).

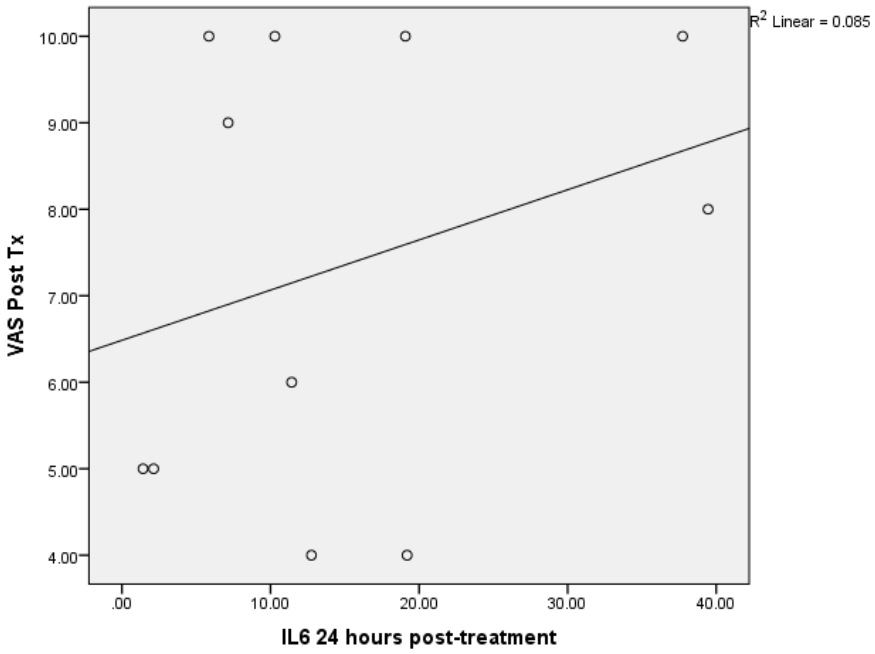


Figure 60: VAS verses peak IL-6 post UAE

Similarly, peak IL-6 levels (at two hours) were plotted against maximum pain scores post MRgFUS with no significant correlation, Pearson correlation -0.384 p=0.243 (Figure 61).

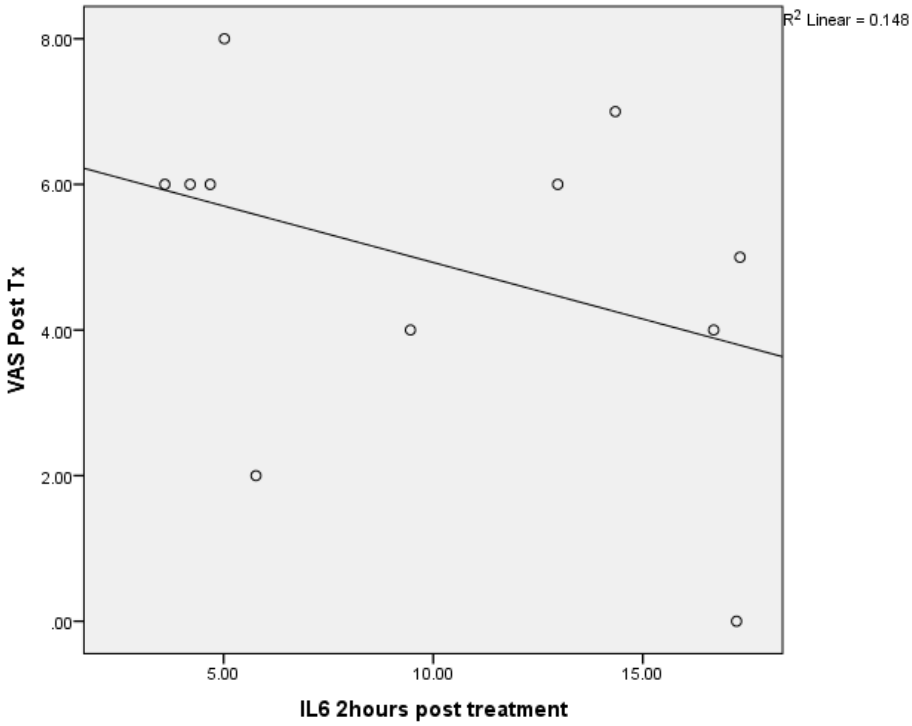


Figure 61: Peak Il-6 against maximum VAS post-MRgFUS

Discussion

At present women requiring treatment for their UF symptoms are reviewed at our fibroid multidisciplinary team (MDT) meeting following their MR imaging. Women are then stratified to myomectomy, hysterectomy, UAE or MRgFUS according to their symptoms, fertility, and number of fibroids and volume of fibroids age and BMI. Because of this stratification there is, as expected, a significant difference in between the UAE and MRgFUS groups. This has the potential to confound the results of this study. Circulating levels of inflammatory cytokines including IL-6 are known to be increased in women with increased adiposity (Browning et al., 2008). The UAE group had a mean BMI of 30.19kg/m^2 compared with 21.89kg/m^2 in the MRgFUS group (p=0.001), and also had a higher baseline circulating Il-6 of 3.74pg/mL vs. 3.38pg/mL, however this was not found to be statistically significant. The UAE group had higher mean uterine volumes and volumes of treated fibroids compared with MRgFUS, in addition to having a higher mean age of 43.43 years (CI 40.06-46.80) verses 38.09 years (CI 33.59-42.59) for MRgFUS. The mean standardised symptom severity score for those undergoing UAE was less than for those undergoing MRgFUS (62.50 vs. 69.31) however this was not statistically significant. This study was designed to answer questions about how circulating cytokines change following these treatments rather than any direct comparison between these two treatment modalities. However, when two different treatments are examined in a study the temptation is to infer comparisons based on the data. In this case in particular it is important that this is not done, as the two groups of women undergoing these different treatments were significantly different.

We found lower VAS pain scores following MRgFUS compared with UAE, as well as a much greater drop in VAS scores by two hours post treatment post treatment. This correlates with the reduced hospital stay following MRgFUS compared with UAE, and reduced requirement for post-treatment analgesia. During the MRgFUS treatment the majority of women will require up to 100mg of intravenous pethidine, 1g of intravenous paracetamol, in addition to 100mg of rectal diclofenac (NSAID) prior to commencing the treatment. In our experience several women have requested that the focused ultrasound be stopped prior to completion because of the severity of pain experienced. The very high levels of pain experienced during the treatment is then followed by a rapid fall in pain scores, with all of the subjects being discharged without take-home analgesia at 2-3 hours post-treatment. Following UAE all women were given a patient-controlled analgesia (PCA) device containing morphine, which is used for up to 12 hours after the treatment. All subjects undergoing UAE were discharged after 24 hours, with prescription analgesia to take home. In an earlier study, the mean VAS following UAE was three in the first 24 hours, and 4.9 during the first week post-treatment (Bruno et al., 2004), however responses to the treatment seem to vary considerably, with 20% of women describing VAS of greater than seven in the first week.

We expected the pain experienced with UAE and MRgFUS to be associated with a corresponding rise in circulating pro-inflammatory cytokines. In particular Il-1β, which although has a short circulating half-life is closely associated with pain (Pillarisetti, 2011), we expected to see raised at the peak of pain scores, however this was not the case. The reason no significant change in Il-1β was detected is likely to reflect its very short half-life, and also potentially affected by the small numbers of successfully analysed samples. In other studies, attempts at measuring circulating Il-1β post-operatively have experienced difficulty due to low levels detectable in the plasma (Høgevold et al., 2000).

The greater initial rise in IL-6 immediately following UAE compared with MRgFUS post-treatment is likely to reflect the pain experienced during the treatment. In any further work, continued monitoring of IL-6 at 6, 12, 24 and 48 hours and beyond following MRgFUS in order to detect the peak concentration would be useful.
There appears to be little short-term correlation between VAS pain scores and Il-6 levels. Certainly from our small study we could find no obvious correlation. One study of synovial fluid Il-6 and knee pain, found a relationship between Il-6 and joint function, but not with joint pain (Orita et al., 2011). Any tissue injury brings about an immediate, localised pain, which is sustained after the injury, suggesting that substances are being produced in order to maintain this pain (De Jongh et al., 2003). Il-6 is produced in large quantities at the site of injury (Holzheimer and Steinmetz, 2000) and then enters the circulation, the concentration depending on the degree of injury. The circulating levels of IL-6 fall to pre-trauma levels by 24-36 hours, and behave in a similar way to pain, with an initial surge and gradual fall (De Jongh et al., 2003). When IL-6 inhibitors are given before surgery the pain experienced is significantly reduced (Wordliczek et al., 2000). The prolonged peak of IL-6 following UAE may reflect the on-going immunological mechanisms following ischaemic injury.

The MRgFUS treatments took between two to four hours to complete, which means that by the time the immediate post-treatment sample was taken (on exiting the MRI) the process of coagulative necrosis would have been underway within areas of the fibroid tissue for up to four hours. Following embolization, levels of cortisol rise by four hours, before returning to baseline by 24 hours (Brøchner et al., 2009). Compared with myomectomy, there is a relatively muted inflammatory response following UAE with Il-6 concentration following surgery reaching over 200pg/ml, compared with less than 100pg/ml following UAE (Brøchner et al., 2009). Because the final samples following MRgFUS were taken at two hours, and 24 hours following UAE, we cannot confidently state how high the Il-6 concentrations would have continued to rise. As we know the extent of this rise in IL-6 is related to tissue injury we expected the rise in IL-6 to correspond to the volume

of fibroid tissue treated. From this limited study we did not find that to be the case. Further studies should be carried out in order to examine the effects of these treatments on immune function by measuring circulating C-reactive protein (CRP) myeloperoxidase (MPO), tumour necrosis factor α (TNFα), white blood cell count and cortisol levels.

Ischaemic injury to tissue caused by UAE results from the cessation of blood flow, and hence reduction of the oxygen supply to the fibroid tissue. This cessation is immediate and involves the whole fibroid tissue and much of the surrounding myometrium, with the remaining uterine tissue re-perfusing by 48-72 hours, leaving the fibroid tissue irreversibly infarcted (Scheurig-Muenkler et al., 2010). Infarction of a significant volume of tissue is associated with a significant inflammatory response, and associated pain. In addition, a degenerating fibroid caused by ischemia during pregnancy is associated with similar, severe pain. Circulating plasma IL-6 levels are elevated following an ischaemic stroke, with circulatory levels corresponding with CT brain infarct volume, stroke severity and degree of recovery (Smith et al., 2004). Ischaemic stroke is associated with a rise in IL-6 levels from day one with a peak around day three before falling to pre-stroke levels by day 14 (Perini et al., 2001). Circulating IL-6 levels are also raised following acute coronary syndrome, however to a lesser degree than in those with stable angina (Sarrafzadegan et al., 2012). Following ischemia of a potentially large volume of fibroid tissue in UAE the observed rise in circulating IL-6 is expected. We were unable to find a correlation between this rise in IL-6 and the volume of tissue embolized. This may partly be due to our not measuring IL-6 beyond 24 hours. The actual peak concentration of circulating IL-6 may be somewhat higher that the values we have for 24 hours. Also, knowing what volume of tissue has been treated is problematic. For MRgFUS this was relatively straightforward as all women undergoing treatment have an immediate post-procedure contrast-enhanced MRI and from these images an accurate NPV was calculated using the parallel planimetric method. For those undergoing UAE, a contrast-enhanced MRI is not performed until six months following treatment, and due to possible re-perfusion of the fibroid and uterus this may not accurately reflect the volume of tissue becoming ischaemic on the day of treatment. It is now known that large portions of the myometrium and endometrium become ischaemic immediately following UAE, with re-perfusion within 24-72 hours (Scheurig-Muenkler et al., 2010).

This is the first study to examine the effects of focused ultrasound on circulating pro-inflammatory cytokines. Focused ultrasound results in coagulative necrosis of fibroid tissue; a process that involves the preservation of much of the cellular outline and tissue architecture. The injury results in denaturation of structural cell proteins in addition to lysosomal enzymes, therefore reducing the breakdown of proteins within the damaged cells. This process occurs solely within the fibroid tissue surrounded by the pseudo-capsule of the

fibroid. It is possible that this preservation of cellular outline reduces the systemic release of pro-inflammatory cytokines. As circulating IL-6 was not measured beyond two hours post-treatment we cannot state how high this level would have become, however from the pattern of the pain experienced following MRgFUS we would expect a more rapid fall in the IL-6 in line with the pain experienced. Future research in to the effects of MRgFUS should aim to further measure how long the IL-6 remains raised and also the effect on other inflammatory markers.

The MSD plates were unable to detect a significant signal in 23 (60.5%) of the duplicate samples for IL-1β. There are a number of potential problems with measuring circulating cytokines including the rate of degradation during storage, the effects of freeze-thaw cycles, and the effects of inter-assay variation (de Jager et al., 2009). To avoid this all our assays were performed in duplicate, freeze-thaw cycles were avoided and samples were stored at -80°C for no longer than three months from initial storage. However despite these measures we were unable to detect significant levels of IL-1β in many samples, and none of the assays for IL-4 were successful. Circulating IL-4 levels have been successfully detected previously (de Jager et al., 2009), however in future studies it may be sensible to measure alternative cytokines such as Il-10 and TNF-α.

Summary

Uterine artery embolization and MRgFUS treat different groups of women with different size and number of uterine fibroids; therefore it is not unexpected that they experience different pain intensity post-treatment. While this study has suggested some changes in circulating cytokines post treatments, no correlation between change in cytokine levels and fibroid size or pain experienced was found.

7. Growth Factor Production Following MRgFUS and UAE

Introduction

Both Magnetic Resonance guided Focused Ultrasound (MRgFUS) and Uterine Artery Embolization (UAE) are currently used to treat uterine fibroids, by non-invasive or minimally invasive techniques respectively. Following each of these treatments there is the possibility of re-vascularisation and subsequent regrowth of the fibroids, often resulting in surgical re-intervention. As discussed in Chapter 4, we found that following MRgFUS treatment resulting in over 50% non-perfused volume (NPV) of the fibroids almost 40% of women will require re-intervention by five years. Long-term studies of UAE outcomes describe a five year re-intervention of between 19.8 and 32% (Moss et al., 2011, Hirst et al., 2008). Although the desired end-result of these treatment modalities is the same - a significant non-perfused volume of the fibroid tissue - the mechanisms by which they achieve this are different. Coagulative necrosis secondary to thermal damage to tissues is the mechanism by which MRgFUS has its effect, whereas UAE results in ischaemic necrosis following the embolization of vessels supplying the fibroid. Vascular endothelial growth factor (VEGF) is one of the key promoters of angiogenesis, and production of which increases in response to cell hypoxia (Jelkmann, 2001b).

As discussed in earlier chapters, blood flow through uterine fibroids has been examined, and there is significantly reduced flow when compared to the surrounding myometrium (deSouza and Williams, 2002b). Using angiography, smaller uterine fibroids have demonstrably less dense vasculature compared myometrium, (Fleischer et al., 2008). Larger fibroids however, appear to have increased vascularity, especially at the peri-fibroid tissue around the edges, compared to the surrounding myometrium. These findings supported a hypothesis that initial regression of the vasculature within smaller fibroids is followed by an increase in new vessel production at the periphery, this being the source of new vessel growth as the fibroid enlarges (Walocha et al., 2003).

Angiogenesis is a stepwise process, each step under the control of various promoters and inhibitors. It is the fine balance of these promoters and inhibitors that results in either growth or regression of new blood vessels. Vascular endothelial growth factor is an important promoter of angiogenesis. Following ischaemic stroke, VEGF is involved in neurovascular remodelling (Ma et al., 2012). Levels of VEGF within uterine fibroid tissue are greater than in the surrounding myometrium (Gentry et al., 2001). It is not clear whether the increased VEGF-expression within fibroids is indicative of increased VEGF activity or simply a case of biologically inactive VEGF contained within the extracellular matrix (ECM) of the uterine fibroid tissue (Fleischer et al., 2008). In addition to VEGF, other heparin-binding growth factors such as basic fibroblast growth factor (bFGF) (another promoter of angiogenesis)demonstrate increased immunohistochemical staining compared with matched myometrium, possibly due to the higher ECM component of uterine fibroids (Mangrulkar et al., 1995). This large store of heparin-binding growth factors may in part account for the increased growth of uterine fibroids when compared with surrounding myometrium.

In the plasma, VEGF is stored within platelets and bound to the plasma proteins alpha-2-macroglobulin and fms-like tyrosine kinase-1 (sFlt-1), the VEGF-binding capacity of which increases greatly during pregnancy (Jelkmann, 2001a). VEGF is a multifunctional glycoprotein that increases the permeability of vessels within the fibroid tissue and stimulates endothelial growth. Additional functions of VEGF are the induction of capillary tube formation, the induction of endothelial cell proliferation, promotion of cell migration and acting as a chemo-attractant for endothelial cells (Sanci et al., 2011). VEGF has also been referred to as vascular permeability factor (VPF) or vasculotropin in the literature. Circulating plasma VEGF levels are significantly higher in women with uterine fibroids (331pg/ml) compared with women with no gynaecological pathology (39pg/mL) (Huang et al., 2004). Plasma VEGF levels have been examined following UAE for up to 30 days, and appear to rise by day one, followed by short fall, and then subsequent secondary rise by day 30 (Takeda et al., 2005). An inverse correlation between uterine artery pulsility index (PI) and the level of VEGF on day 30 has been found, suggesting that raised levels of VEGF may be related to a less successful embolization result (Takeda et al., 2005). It was suggested that the restoration of blood flow within the uterus following embolization is due to a possible compensatory mechanism reliant on VEGF production (Takeda et al., 2005). The circulating plasma levels of VEGF following MRgFUS have not been measured in humans. Murine models however, report a fall in VEGF by day seven following the use of high-intensity focused ultrasound (HIFU) (Yang et al., 2004). There were initial concerns regarding the use of MRgFUS for cancer and the risk of metastasis; those concerns were related to the potential increase in angiogenesis and metastasis that a rise in circulating VEGF may produce. As it transpired, following focused ultrasound, staining for VEGF within the target tissue and concentration in the circulating serum is reduced compared to controls. This effect appears to persist for longer than seven days (Yang et al., 2004). Other

studies have reported a transient increase in circulating serum VEGF following surgery and a relationship between severity of surgery and level of rise in VEGF (McDonnell et al., 2001, Bondestam et al., 2000). These studies found that following minor procedures there was no significant rise in serum VEGF levels, whereas cases that involved a much larger degree of surgical trauma were associated with a rise in circulating VEGF (Bondestam et al., 2000). No studies have measured VEGF beyond one month of treatment.

Aim

In this study I compare levels of circulating plasma VEGF following UAE and MRgFUS treatments for uterine fibroids.

Methods

This was a prospective cohort study of circulating plasma VEGF following UAE and MRgFUS for the treatment of uterine fibroids. Local and regional ethics committee approval was obtained (national ethics committee reference number 11/LO/0118). Subjects were recruited to this study from the St Mary's fibroid clinic. Inclusion and exclusion criteria are described on Table 41. Written participant information leaflets were given to the women (page 234) and written consent (page 245) was taken prior to treatment. Trial documentation is given in Appendix L (page 247).

Inclusion criteria	Exclusion Criteria
Women undergoing UAE or MRgFUS at St Mary's Hospital.	Presence of any other systemic disease which may contribute to circulating cytokine levels.
Willingness to have blood samples taken at intervals	Ischemic heart disease
	Acute or chronic infections
	Any form of on-going treatment for cancer
	Autoimmune disease (e.g. SLE, Rheumatoid arthritis)
	Any form of surgery 3 months prior to treatment
	Cerebral vascular accident
	Body mass index > 36kg/m^2
	Pregnancy or lactating

Table 41: Inclusion and exclusion criteria for VEGF Study

Procedures and measurements

Prior to treatment, all subjects underwent MR imaging of their uteri, and total uterine volume and fibroid volume was calculated using the parallel planimetric method (Figure 16 page 72). In the case of MRgFUS subjects we could easily describe the volume of fibroid treated as a gadolinium contrast agent was administered at the end of each treatment and the non-perfused volume (NPV) of each fibroid was calculated by the parallel planimetric method. In the case of UAE however, it is significantly harder to adequately assess the volume of treated fibroid tissue. All women undergo a contrast-enhanced MRI at 6 months post-treatment, however while this is a good indicator to the overall success of the treatment, it does not necessarily reflect the NPV at the end of the treatment as it is known that there may be significant re-perfusion following the initial embolization. Indeed, much of the myometrium and endometrium appears non-enhancing post-contrast in the short term and re-perfusion of the uterus may not occur for several days (Scheurig-Muenkler et al., 2010). For this reason, the volume of the entire uterine volume was calculated by parallel planimetric method and the volumes of the three largest fibroids were also calculated by this method.

Circulating VEGF was measured from plasma samples. VEGF is contained with platelets and released on coagulation (Jelkmann, 2001b); it was for this reason plasma samples were used. Blood samples were collected in EDTA coated collection bottles prior to treatment, allowed 30-60 minutes to clot and then samples were centrifuged at 2000 revolutions per minute (rpm) at 0-2^{o}C for 15 minutes. Following this, plasma was extracted by pipetting. The plasma samples obtained were stored at -80^{o}C in the laboratory in the Mint Wing, until time of analysis. Participants were asked to attend for follow-up samples at one week, one month and three months post treatment.

Prior to assay, samples were thawed, and centrifuged to remove any clotted material. All samples were analysed following the first thaw. Samples were assayed using the Meso Scale Discovery (MSD®) 96-well Multi-Array custom cytokine assay for VEGF was performed by the same methods described in Chapter 6 (page 147) and will not be repeated here. The calibrator standards are shown in Table 42.

Standard	Concentration (pg/ml)	Dilution Factor
Combined working stock	1000000	
Diluted stock calibrator	10000	100
Standard 1	2500	4
Standard 2	625	4
Standard 3	156	4
Standard 4	39	4
Standard 5	9.8	4
Standard 6	2.4	4
Standard 7	0.61	4
Standard 8	0	n/a

Table 42: Calibration samples for VEGF study

Calibrator dilutions were prepared using dilutions of 100000, 25000, 6250, 1563, 391, 98 and 24pg/ml of VEGF. The final calibrator was diluent alone (i.e. zero VEGF concentration). In a similar method to the cytokine study, by running the calibrators in two duplicates the software produced a standard curve based on the mean of the two values produced (Figure 62). The analysis software produced a report including information regarding the concentration (pg/ml), signal produced by the well, the mean signal between the

two wells, the coefficient of variation between the two wells, the calculated concentrations for VEGF (pg/ml) and the mean calculated concentration between the two wells, and again the coefficient of variation between the two calculated concentrations.

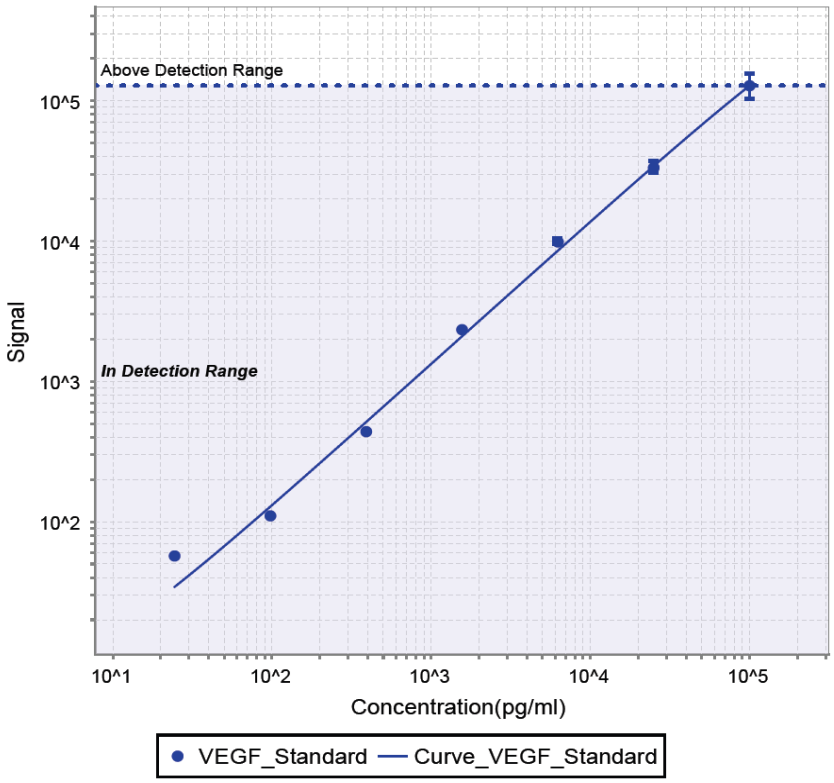


Figure 62: Standard plot for VEGF

Forty-five women were recruited to the study, and seven women were excluded due to failure to detect a VEGF reading on their plasma samples (Figure 63). Overall, there were 21 women in the MRgFUS group and 17 women in the UAE group. The characteristics of the enrolled subjects are described on Table 43. Figure 63 illustrates the recruitment of women to this study from the fibroid clinic at St Marys Hospital, Imperial College NHS Healthcare Trust.

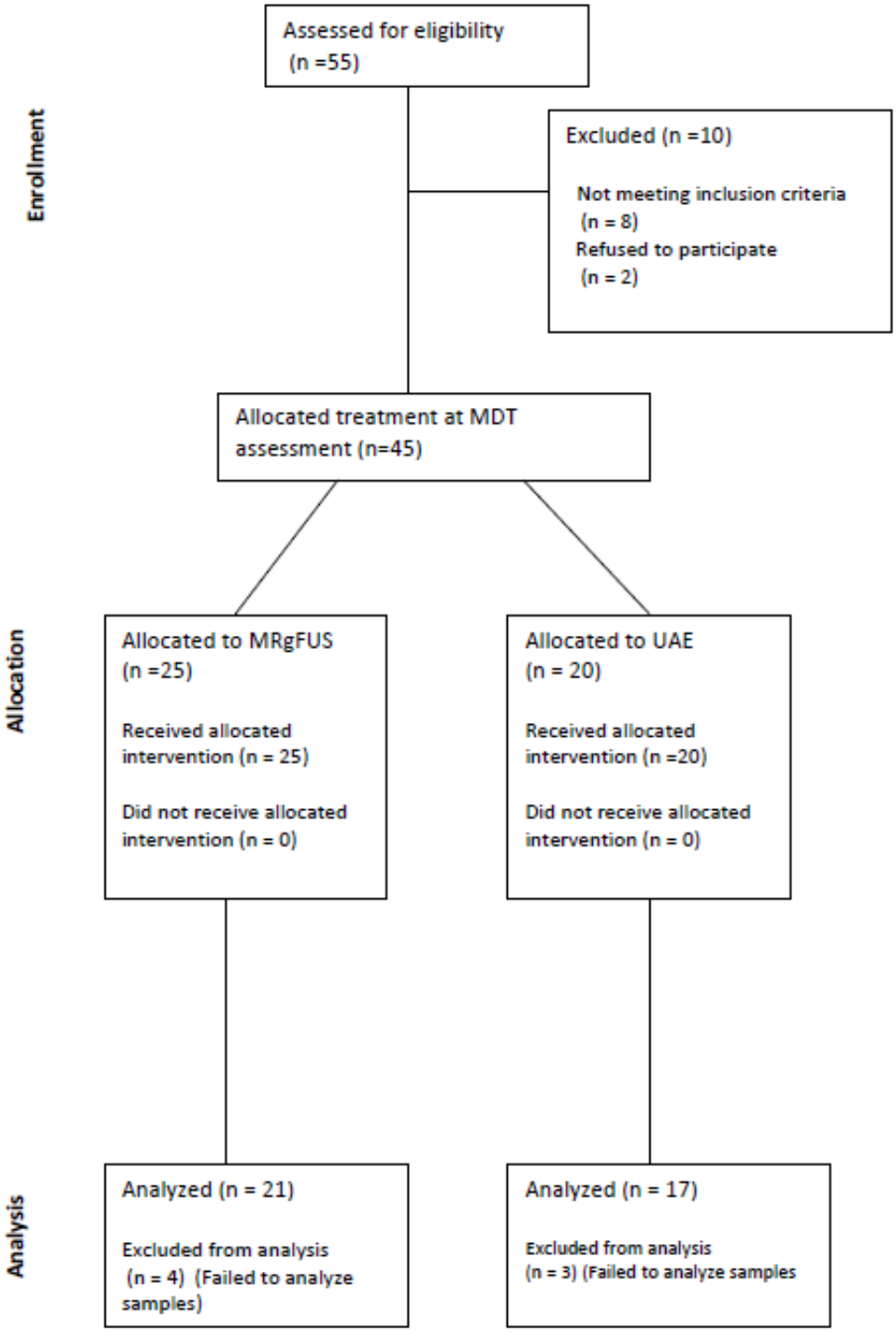


Figure 63: Consort diagram for Fibroid VEGF study

	MRgFUS n=21 Mean (SD)	UAE n=17 Mean (SD)	P value
Age (years)	38.2 (6.9)	42.1 (3.6)	0.01*
BMI (kg/m$^{2)}$	21.8 (2.6)	31.8 (8.8)	0.0Ŧ
SSS (Symptom severity score)	67.4 (22.6)	62.5 (25.9)	0.60*
Uterine Volume (ml)	667.7 (286.5)	882.15 (578.9)	0.13Ŧ
Volume of fibroid treated (ml)	313.87 (245.0)	299.56 (214.7)	0.58Ŧ

*Student T-test, Ŧ Mann- Whitney U test, SD= Standard deviation

Table 43: MRgFUS and UAE characteristics

The UAE group were older than the MRgFUS group (p=0.007), with a higher BMI (31.76kg/m^2 verses 21.80kg/m^2 p=0.001). There was no significant difference in uterine volume or SSS between these two groups. The MRgFUS group had an overall higher mean SSS (Table 43) however again this was not statistically significant.

Data Analysis

SPSS version 19 (BM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp) was used to analyse the data. Data sets were examined for normal distribution and where a normal distribution was found (parametric data) student t-test was used to test for significance between the means in the alternate groups. Non-parametric data was tested used Wilcoxon signed rank test. Correlation was tested by non-parametric ANOVA; coefficient of determination (R^2) was described. P values of less than 0.05 were considered to indicate a statistically significant difference.

Results

The mean NPV as a percentage of the total target fibroid tissue was 52.21% (SD 22.13) following MRgFUS. The overall base line circulating plasma VEGF levels was 133.56 pg/mL (SD 79.39).

Figure 64 illustrates the absence of any relationship between the volume of fibroids within the uterus and the baseline circulating VEGF levels at baseline. Likewise there was no significant relationship between total uterine volume and baseline VEGF (Figure 65). Here the coefficient of determination (R^2) value was 0.023 (p=0.365).

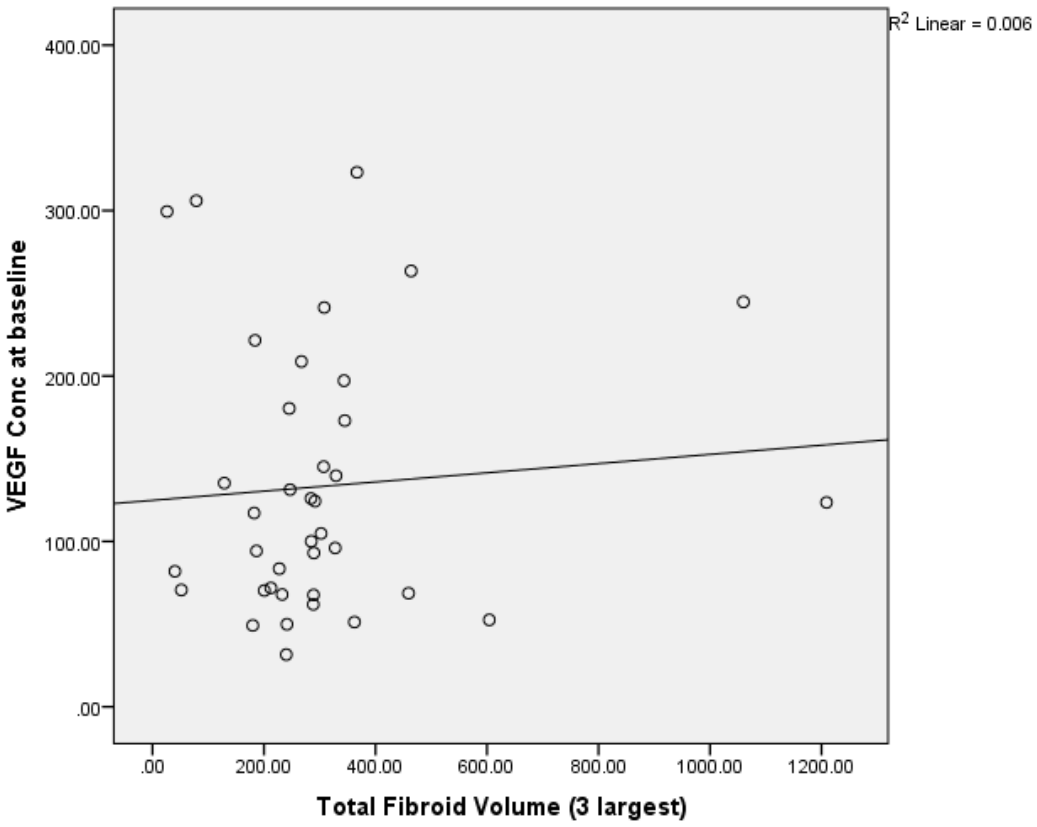


Figure 64: VEGF at baseline and Fibroid volume- for UAE and MRgFUS (pg/ml)

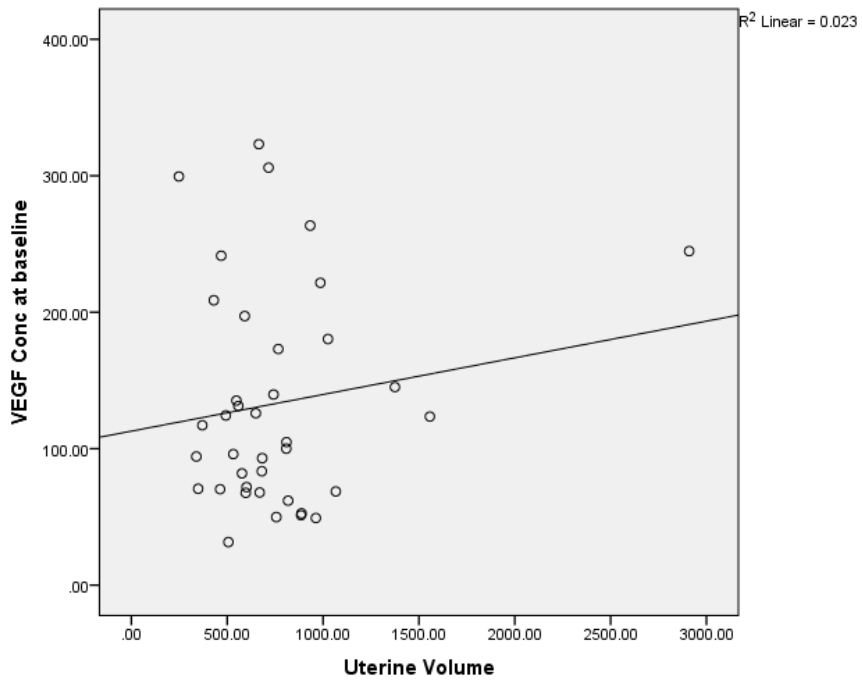


Figure 65: Baseline VEGF and overall uterine volume (pg/ml)

The circulating VEGF levels following MRgFUS are described in Table 44 and Figure 66.

	MRgFUS n=21 (Mean pg/mL and SD)
Pre-treatment Mean (SD)	150.73 (86.82)
Immediately post treatment Mean (SD)	120.95 (64.48)
2 hours Mean (SD)	129.68 (85.06)
24 hours Mean (SD)	n/a
1 week Mean (SD)	129.30 (55.97)
1 month Mean (SD)	113.31(47.99)
3 months Mean (SD)	109.39 (57.94)

*p=0.008 (Wilcoxon Signed Rank Test), SD=Standard deviation

Table 44: VEGF following MRgFUS

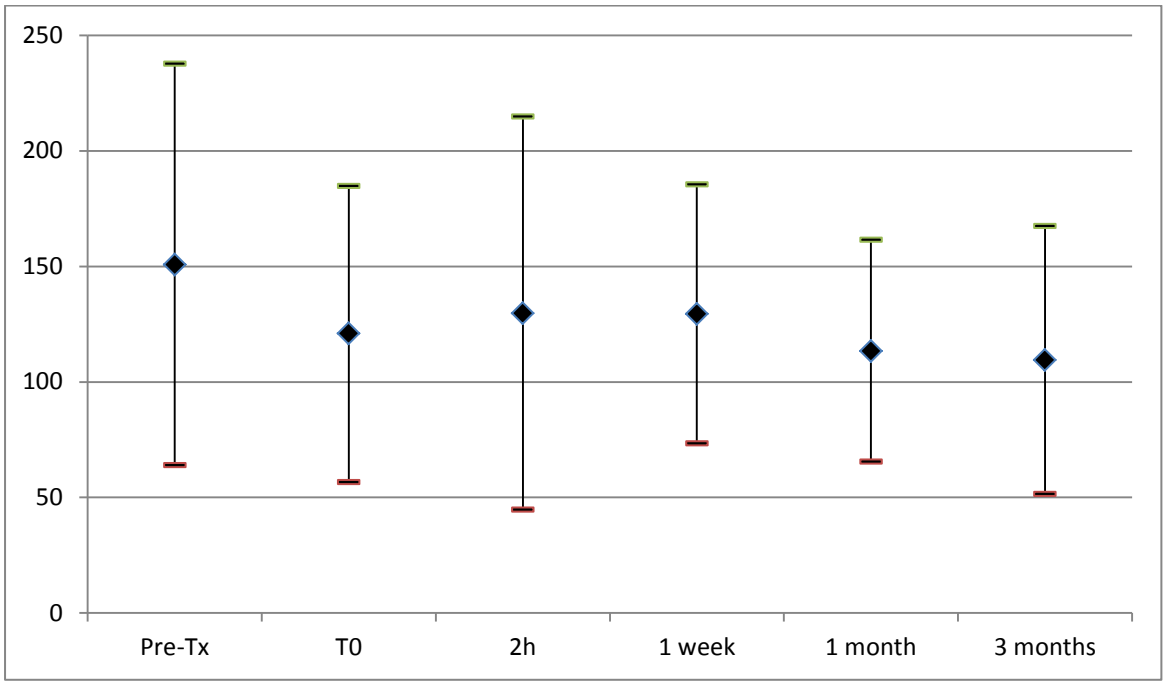


Figure 66: Circulating Plasma VEGF post-MRgFUS (mean with SD)

Longitudinal change in VEGF post MRgFUS is described in figure 67, which suggests an overall downward trend in VEGF by one to three months post-treatment, although again this is not significant.

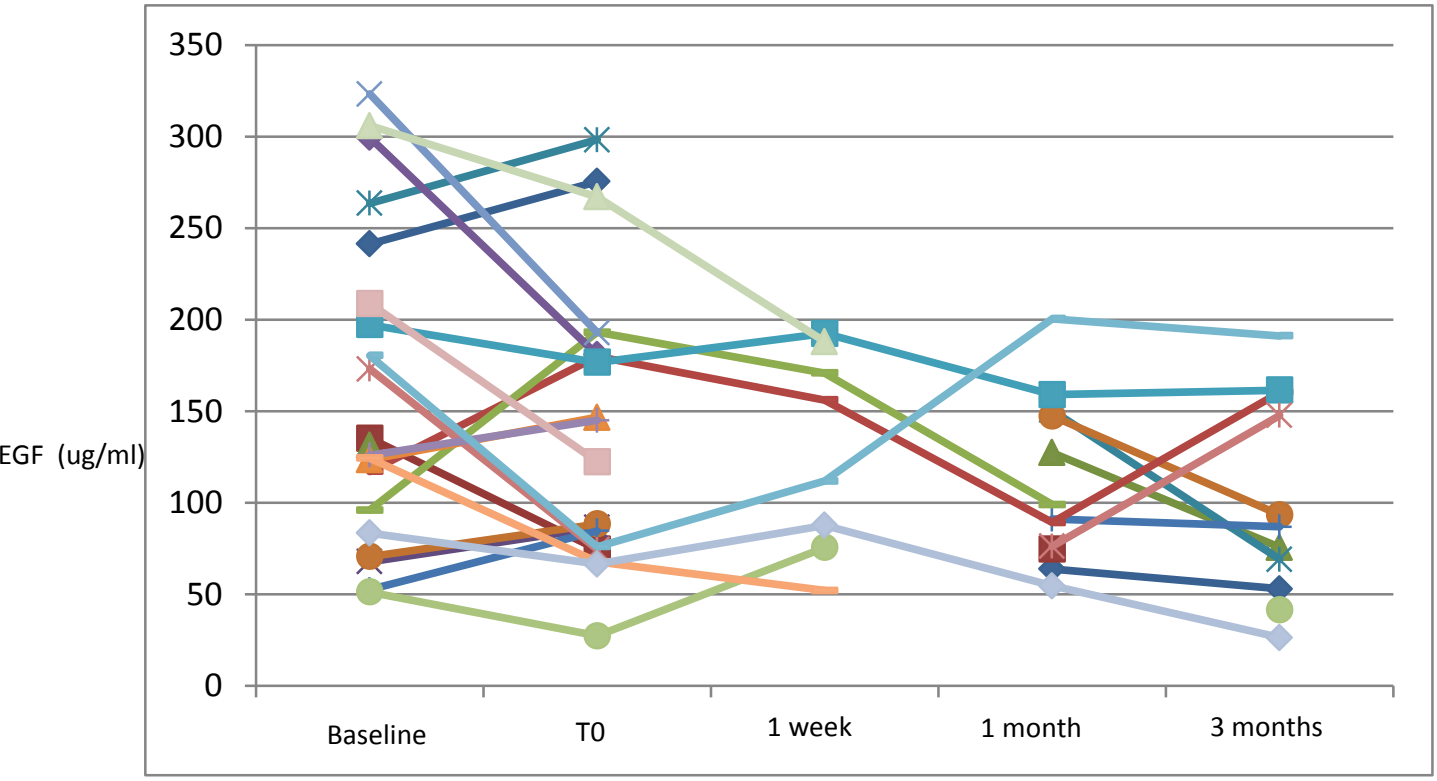


Figure 67: Longitudinal change in VEGF following MRgFUS

Although there was a downward trend of circulating VEGF following MRgFUS, this failed to reach statistical significance (Wilcoxon signed rank test between baseline and three months, p=0.155). I then further examined those women with a fall in their plasma VEGF levels immediately following MRgFUS and those with an increase in VEGF post-treatment. I also examined those women with an increase in VEGF at one week and those with a decrease in VEGF at one week (table 46). From these figures I could find no statistically significant effect on the change of VEGF.

	Mean Age years (SD)	Mean BMI (SD)	Mean Uterine Volume (ml) (SD)	Mean Volume of Fibroids treated (mL)
↑VEGF at T0	35.3 (6.4)	21.8 (2.6)	697.3 (359.3)	400.1 (320.7)
↓VEGF at T0	36.6 (9.9)	22.6 (3.0)	640.7 (225.4)	343.8 (110.7)
↑VEGF at 1 week	36.5 (6.4)	19.6 (1.7)	616.8 (219.2)	274.9 (84.1)
↓VEGF at 1 week	42 (5.0)	22 (2.2)	706.3 (231.6)	239.6 (114.7)

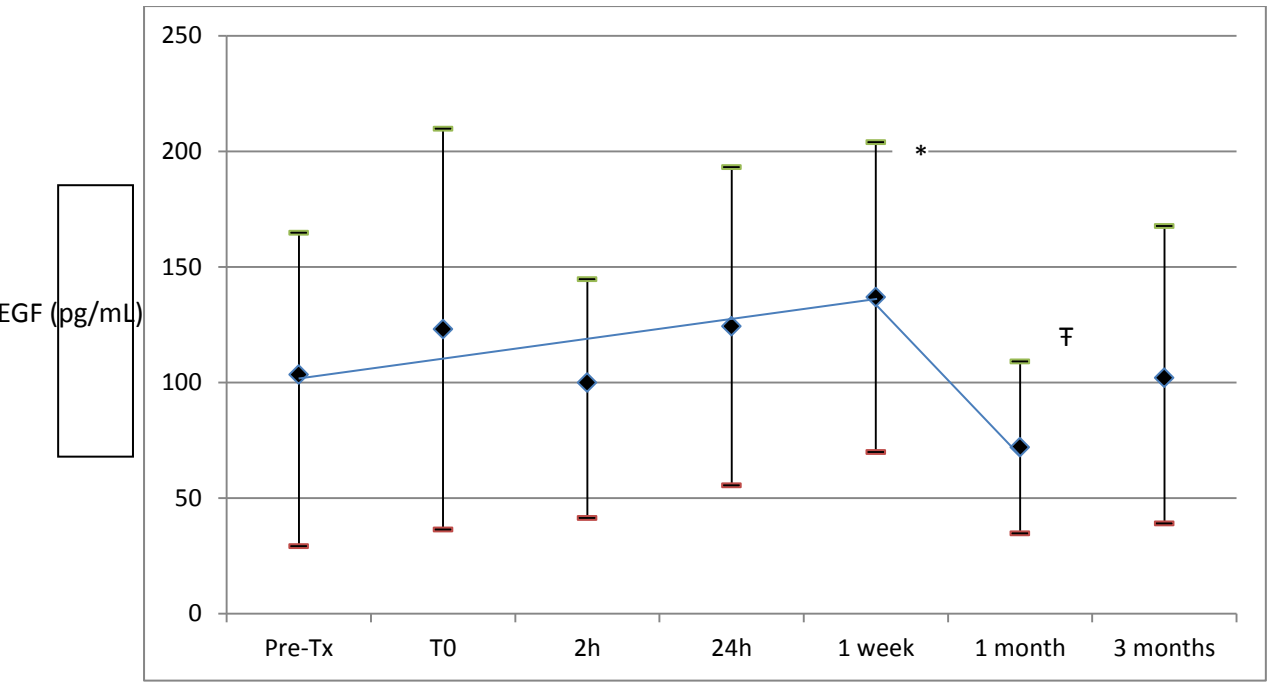
Table 45: Details of age, BMI, uterine and fibroid sizes for subjects with different changes in VEGF levels post MRgFUS

Table 46 and Figure 68 illustrate the changes in VEGF following UAE.

	UAE n=17 (Mean pg/mL and SD)
Pre-treatment Mean (SD)	103.70 (61.31)*
Immediately post treatment Mean (SD)	123.09 (86.65)
2 hours Mean (SD)	99.89 (44.74)
24 hours Mean (SD)	124.33 (68.81)
1 week Mean (SD)	136.93 (67.04)*
1 month Mean (SD)	71.90 (37.12)
3 months Mean (SD)	101.97 (62.90)

*p=0.008 (Wilcoxon Signed Rank Test)

Table 46: VEGF following UAE



*p=0.008 Ŧp=0.043

Figure 68: Circulating VEGF following UAE (mean with SD)

Using Wilcoxon sign rank testing we could demonstrate a significant change in the circulating VEGF levels at one week (p=0.008 Wilcoxon signed rank test) compared with the baseline level. Between one week and one month following UAE there was a significant fall in the mean circulating VEGF levels (p= 0.043 Wilcoxon signed rank test), with a return to the baseline seen by three months (see Figure 68). The longitudinal changes in VEGF following UAE are described in figure 69.

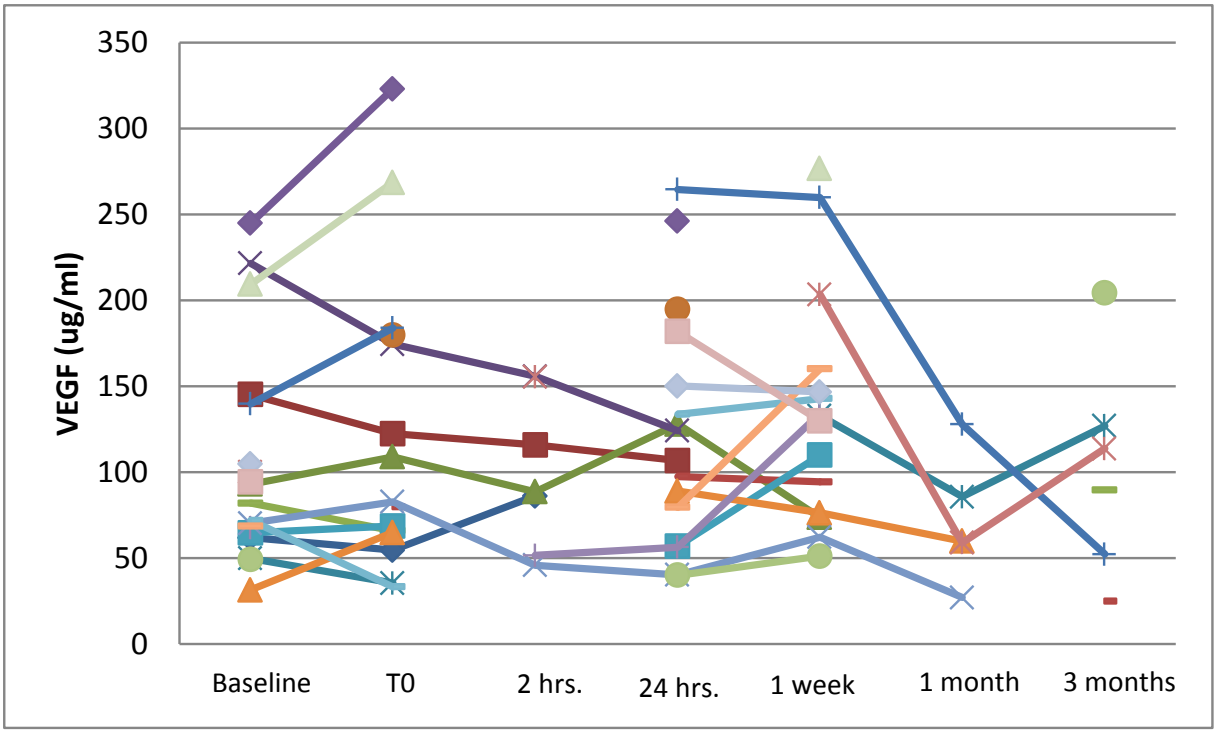


Figure 69: Longitudinal change in VEGF following UAE

Of those cases where the VEGF rises at T0 (immediately post-UAE) those subjects with a rise in their plasma VEGF tended towards a higher age, lower BMI, and higher uterine and fibroid volumes. These trends however failed to reach statistical significance. At one week post UAE, those subjects with an increase in detectable plasma VEGF levels tended towards younger women, again with a lower BMI, but with little difference in their uterine or fibroid volumes. Again, any differences failed to reach statistical significance.

	Mean Age years (SD)	Mean BMI (SD)	Mean Uterine Volume (ml) (SD)	Mean Volume of Fibroids treated (mL)
↑VEGF at T0	45.0 (6.4)	31.6 (11.2)	1060.6 (930.4)	423.7 (321.0)
↓VEGF at T0	40.7 (3.6)	33.1 (7.5)	852.0 (296.8)	212.1 (96.0)
↑VEGF at 1 week	42.7 (4.7)	30.2 (8.2)	730.8 (221.6)	251.8 (111.9)
↓VEGF at 1 week	47 (5.6)	33.7 (6.0)	702.6 (250.3)	223.2 (58.2)

Table 47: Details of age, BMI, uterine and fibroid sizes for subjects with different changes in VEGF levels post UAE

Examining the change in circulating VEGF levels in relation to the NPV achieved at MRgFUS, immediately following treatment we see a trend toward an overall decrease in VEGF level, however this fails to reach statistical significance (R^2 =0.099, p=0.204) (Figure 70). By two hours, the change in VEGF levels from the baseline demonstrated no correlation with NPV (Figure 71). Likewise, at one week, one month and three months, there are no significant changes in VEGF from the baseline.

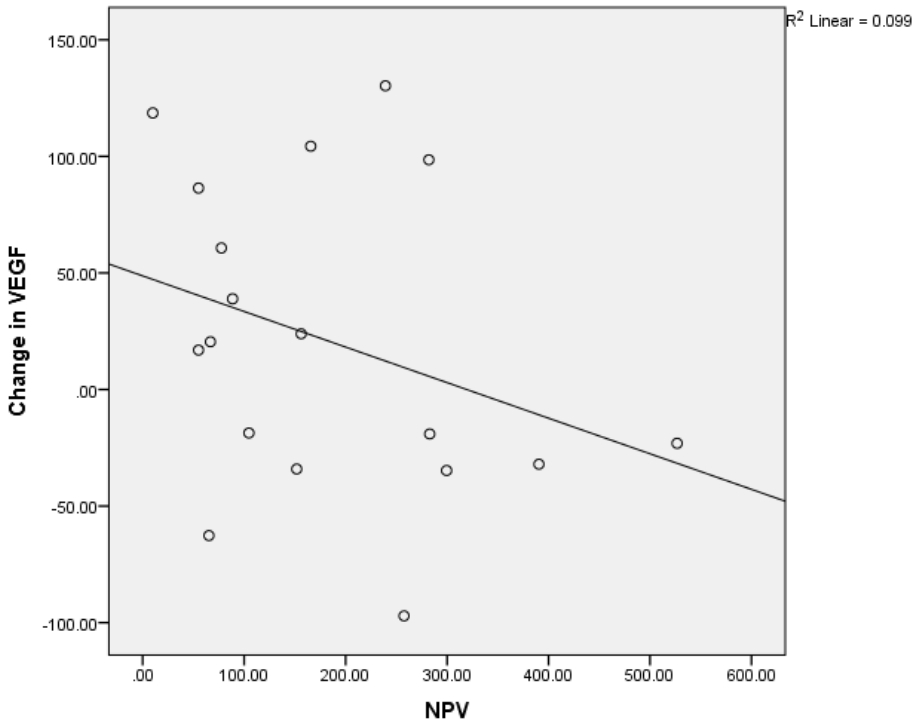


Figure 70: Change in VEGF immediately following MRgFUS (pg/ml)

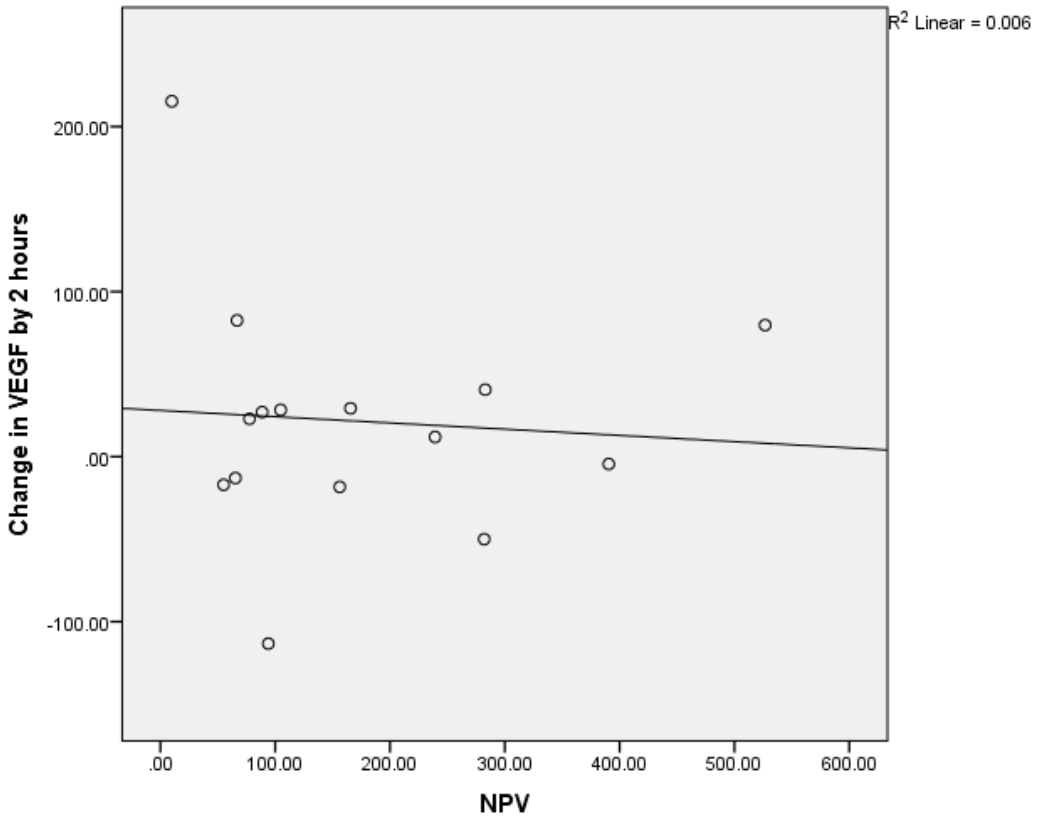


Figure 71: Change in VEGF at 2 hours post MRgFUS (pg/ml)

The actual VEGF values, ages, and uterine volumes for those women undergoing MRgFUS, and UAE are described in Appendix M. Two of these women had a history of reactions to intravenous contrast following earlier MRI scans and therefore it was not possible to give the contrast agent required to calculate their NPV. These tables illustrate one of the major problems with this study, that of compliance with follow-up. The percentage return for follow-up bloods at one week, one month and three months was 38.1%, 52.4% and 47.6% respectively.

Follow-up following UAE was similar, with five women refused to have their bloods taken at 24 hours post treatment, and compliance at one week, one month and three months was 64.7%, 29.4% and 29.4% respectively.

The women who had uterine fibroids that appeared hypo-intense on T2 imaging had a mean concentration of plasma VEGF of 135.88 pg/mL (SD 76.79), and those that appeared hyper-intense had a baseline of

127.25pg/mL (SD 90.23) (p=0.53). There was also no significant difference in the degree of VEGF concentration change for each of time points between the hyper-intense and hypo-intense uterine fibroids.

Discussion

Women with uterine fibroids have a significantly higher level of circulating VEGF compared with women without fibroids (Huang et al., 2004). Circulating plasma VEGF levels have previously been reported as between 0-124pg/mL (mean 44 pg/mL) prior to embolization in earlier studies (Takeda et al., 2005). Our results found a higher mean baseline VEGF level of 133.56pg/mL, however other studies found a baseline VEGF level of 311.1pg/ml in women with uterine fibroids (Huang et al., 2004), over double that of our findings. The Electrochemiluminescence method that we used to calculate the circulating plasma VEGF levels resulted in a very large distribution for our results, which made the interpretation of these results very limited. Indeed, at baseline there was a greater difference between the VEGF results between these treatment modalities than following the treatments themselves. It is recommended that EDTA, heparin or citrate-prepared blood collection samples should be prepared and frozen within one hour of venepuncture; lest the platelets start to coagulate and result in VEGF release (Banks et al., 1998). In our study the average time from collection to processing was one hour and it is unlikely that delays in processing samples will have affected our results.

Given the increase circulating VEGF levels in those women with uterine fibroids, we had expected to find a relationship between the overall fibroid volume and VEGF concentrations. However, in this study we found no such correlation.

Earlier study have found that VEGF levels rise rapidly following UAE (Takeda et al., 2005), and it was suggested that the resultant hypoxia following UAE led to an up regulation of VEGF expression by the fibroid. A biphasic change in VEGF was described, with an initial fall in VEGF by seven days, with a rise again by one month (Takeda et al., 2005). Our study has found a similar increase in VEGF by 24 hours following UAE that appeared to be maintained up to one week, with a fall at one month, before returning to pre-treatment levels by three months. The numbers of participants and ages of the participants in our study and the earlier studies are not significantly different (15 participants, aged 37-47 in the Takeda group). The Takeda group examined the pulsility index (PI) of the uterine arteries before and after UAE and found that the blood flow

within the uterine artery was rapidly reduced after embolization, but that this had recovered by one month. They suggested VGEF may play a role in a compensatory mechanism restoring impaired uterine artery blood flow, possibly by promotion of collateral vessel formation and increased supply to the fibroids and myometrium following embolization. High levels of VEGF following UAE may be an indicator of a higher likelihood of treatment failure. The significant rise in VEGF following UAE that peaked at one week that we found corresponds with the re-perfusion of the myometrium and remaining non-fibroid uterine tissue described elsewhere (deSouza and Williams, 2002a). It could be that that as MRgFUS does not involve this injury and subsequent repair of the myometrial vasculature, the VEGF levels are not raised as we had first expected. Certainly our initial hypothesis that circulating VEGF levels would rise following MRgFUS and that this might be linked to reperfusion of the fibroid has not turned out to be correct. Other circulating factors may have a role in predicting the success or otherwise of MRgFUS, but from our results VEGF does not.

The effect on circulating VEGF levels has been examined in women with malignancies treated with MRgFUS. One study found a significant decrease in circulating VEGF at seven days following high intensity focused ultrasound in cancer patients with non-metastatic disease, however no significant change in those women with metastatic disease (Zhou et al., 2008). Animal models have demonstrated an increase in the tissue expression of VEGF by 24 hours following focused ultrasound to benign muscle (Burks et al., 2011).

Plasma VEGF levels rise during the first few days after major surgery, and it is suggested that this is in relation to tissue healing (Bondestam et al., 2000). In addition, haemolysis following surgical trauma may account for this rise as VEGF is transported within the peripheral blood cells and platelets (Salven et al., 1999). In animal models using tumours with an increased background level of VEGF, a decrease in circulating VEGF levels is seen following both focused ultrasound and surgery (Yang et al., 2004). This model demonstrated a greater decrease in VEGF following focused ultrasound compared with surgery, with no return to the baseline levels by seven days post-surgery. Prior to the focused ultrasound they found positive staining for VEGF within the melanoma tissue, which was then absent after the treatment. In control groups with no tumours, initially low VEGF levels are then followed by high increase on the first day post-MRgFUS followed by a slow decline over seven days. We found a trend toward a decrease in the plasma VEGF levels which appeared to continue to fall to three months, although this failed to reach a statistically significant difference from the baseline. Our mean percentage NPV following MRgFUS was 52.21%. We were unable to detect a significant degree of change in VEGF in relation to the percentage NPV achieved. One of our cases achieved a 99.58% NPV following MRgFUS, and this was associated with an initial rise immediately following the treatment followed by a sharp fall by two hours. Unfortunately this subject did not attend for follow-up. The next two highest NPV achieved were 81.79% and 78.63%. The former was associated with a sharp fall in

VEGF immediately post-treatment, and the latter with a sharp rise. Both cases involved predominantly hypo-intense uterine fibroids, and there was no difference in age of the women or uterine volumes. This anecdotal effect on circulating VEGF levels and the relatively small numbers studied makes drawing any firm conclusions regarding the effect of MRgFUS on circulating VEGF difficult.

I examined the possibility of a confounding effect of signal intensity of the uterine fibroids on circulating VEGF levels, and change in EGF over time, and found no significant effect. The signal intensity of T2 weighted MR images are related to the cellularity of the fibroid tissue. Uterine fibroids with relatively higher signal intensity on T2 are often composed of compact smooth muscle cells with little or no collagen (Murase et al., 1999). We hypothesised that this degree of cellularity may affect the circulating levels of VEGF; however from our results this does not appear to be the case. I also examined the potential effects of age, body mass index, uterine volume and total fibroid volume on the change of circulating VEGF and found no significant effects, however the numbers included were low, and it is possible that a larger study may find a different result.

Circulating VEGF levels have been used a biomarker of neovascularisation and vascular re-modelling (Valipour et al., 2008). Circulating VEGF levels are known to rise during periods of tissue hypoxia following coronary heart disease, with a peak around seven days following the initial ischaemic event (Soeki et al., 2000). In severe sepsis circulating VEGF levels are significantly raised by day one, before normalising by day 28 (van der Flier et al., 2005). During recovery from an acute respiratory infection VEGF levels may decrease in relation to the immunosuppression following administration of systemic steroids or antibiotics (Valipour et al., 2008). We found no significant change in circulating VEGF levels following MRgFUS, however a there was a downward trend in VEGF levels. It is possible that this decrease in VEGF post-MRgFUS may be related to a reduced volume of surviving fibroid tissue post-ablation. However, as we were unable to find a significant correlation between VEGF and fibroid volume, this is difficult to prove, and the wide confidence intervals of our VEGF values further reduces the certainty of our findings.

There are several limitations of this study including the poor compliance with follow-up of the participants. It is possible that further investigation of post-treatment VEGF levels the downward trend may become significant. Future work in this area should investigate more local effects on growth factors, by obtaining biopsy tissue samples from the fibroid post-treatment. When measuring circulating plasma VEGF every effort should be made to reduce any potential deterioration of the samples, and optimising of sample storage and processing.

8. Discussion

Uterine fibroids are a singular form of tumour. Benign in nature, they may be solitary or numerous; they may cause a great deal of distress for their women, or may lie unnoticed for decades without causing a single attributable symptom. Their sizes may range from a few millimetres to over 20 centimetres in diameter, and their shapes can be irregular in nature. Whether they originate in the sub-mucosal, intramural or sub-serosal part of the uterine wall will have a clinically significant effect on the resultant symptoms experienced. A woman with numerous large sub-serosal and intermural fibroids maybe relatively asymptomatic, whereas a woman with a solitary one centimetre sub-mucosal fibroid may experience menorrhagia that may result in hospitalisation due to the resultant anaemia. The problems caused by uterine fibroids range from the characteristic heavy menstrual blood loss to pressure effects on bladder and bowel function, a wide range of fertility-related problems, and psychological effects, the type of which may be observed in chronic conditions. It is because of this superfluity of clinical presentations that I have attempted in chapter two and three of this thesis to refine the measurement and description of uterine fibroids and to establish a pattern of treatment depending on the features of these fibroids. In this effort I have been only partially successful. In chapter two identified the parallel Plannimetric method as being the most accurate way of defining fibroid volume. It could be argued that the clinical need for such accuracy may be limited, as it is the patient's symptoms that inform the clinician whether a treatment has been successful. However, in any research scenario involving the assessment of fibroid volume and treatments that do not involve the surgical removal of fibroid tissue, accuracy of fibroid volume measurement is paramount. As many of the modern therapies for uterine fibroids aim to reduce the volume of uterine fibroids in addition to relieving clinical symptoms, accuracy of this measurement is imperative and as such parallel Plannimetric method should be used in any studies investigating the effectiveness of these treatments. In addition I attempted to classify uteri containing fibroids according to the number and pattern of fibroid distribution. Anecdotally, we had observed from our weekly fibroid multidisciplinary team meetings that women with fibroid uteri often fall in to two broad categories; those with one to three large, dominant fibroids, and any number of smaller fibroids scattered throughout the uterus, or uteri with a great many fibroids all of varying sizes, but with no one fibroid being particularly larger than the others. From a surgical prospective this observation was of relevance as at myomectomy it is rarely feasible to remove all the fibroids; to do so would increase the risks of bleeding, potentially further compromise fertility and increase the likelihood of hysterectomy. The surgeon will usually make a judgement about which fibroids are likely to be contributing most to the patient`s problems (usually the largest fibroids, or the sub-mucosal fibroids). However in a uterus with over 20 fibroids all of similar size, surgical removal of all these fibroid may not be feasible due to the risks of

hysterectomy, and difficulties in repairing the uterus following multiple incisions. This will depend to a certain extent on the skills of the individual surgeon, however for many women with multiple fibroids, uterine artery embolization or hysterectomy may be more suitable depending on their fertility status. Overall, I found that rates of myomectomy and hysterectomy throughout the different uterine categories remained fairly constant. The greatest change was the decision to use MRgFUS or UAE as the preferred treatment; those women with fewer fibroids being more likely to be treated by MRgFUS and those with multiple fibroids being treated more commonly by UAE. This represents the judgements made by the fibroid multidisciplinary team meeting (MDT) based on anecdotal evidence that women with fewer fibroid responds more favourably to MRgFUS. This opinion appears to be shared by other practitioners of MRgFUS internationally where the average numbers of fibroids treated is 3.7 (range 2-8) (Taran et al., 2010b).

In chapter three I described the wide variety of fibroid uteri encountered, from the solitary fibroid measuring over 20 centimetres in diameter, to the uterus with more than 30 small fibroid scattered throughout. Again, these different types of fibroid uteri will require different therapeutic approaches. This study was limited by the very selection bias that women undergoing MRI for their uterine fibroids will be subject to, however I believe can still provide useful information about types of women presenting with symptoms relating to their fibroid uteri. I had hoped to provide an algorithm to allow patient pathways to be put in place to facilitate management of these women. This proved more difficult than I had first thought. When I attempted to categorise fibroid uteri by the size and number of fibroids contained I found that with the exception of UAE and MRgFUS, the rates of myomectomy, hysterectomy, TCRF and conservative management were similar in all groups. I found no significant differences in the symptom severity, ages, BMI and parity between categories, with the exception of those women with greater than 20 individual uterine fibroids within their uteri having a higher incidence of nulliparity (no live births); and for whom 17.39% had one or more children compared with an average of 32.86% in the other groups. While it is not entirely surprising that women with this number of fibroids within their uteri would have difficulty carrying a pregnancy to term, it is interesting that those women with 10 to 20 fibroids were no less likely to have a child than those women with five or fewer uterine fibroids. Fertility outcomes are known to be significantly poorer in those women with sub-mucosal uterine fibroids and possibly reduced by intramural fibroids with sub-serosal uterine fibroids having no significant effect (Pritts, 2001). I examined the parity of these women in relation to the position of the dominant fibroids and found that in this study, parous women were slightly more likely to have sub-mucosal fibroids than nulliparous women, although this was not found to be significant ($p=0.114$).

The categorisation of uterine fibroids in terms of numbers and sizes of fibroids has a potential usefulness in standardising the descriptions of fibroid uteri, and in determining whether a patient is more suitable for

MRgFUS or UAE, with categories 1-2 being better suited to MRgFUS and categories 3-5 being more appropriately treated by UAE. Categorisation, in addition to the clinical history and wishes of the patient should inform management.

MRgFUS is an attractive treatment modality for women with uterine fibroids. Compared with UAE and surgical treatments it is safe, with a rapid recovery period and a short stay in hospital. Previous studies have demonstrated that MRgFUS may be cost-effective in terms in cost per quality-adjusted life-year (QALY) gained (Zowall et al., 2008). The cost-effectiveness however will be significantly affected by the re-intervention rate which we found to be relatively high (50% at five years for those achieving >50% NPV and 34.9% at three years). This finding fits with the results of the only published five year data which found a re-intervention rate of 66.7% with an overall NPV of 36.4% (Froeling et al., 2013). The five year re-intervention rate following uterine artery embolization (UAE) ranges from 28-32% (van der Kooij et al., 2010, Moss et al., 2011); however the risks for UAE are significantly higher. While the re-intervention rate following MRgFUS is high, the relatively low risk nature of MRgFUS, combined with the rapid return to normal activities of daily living still makes this treatment an attractive option.

Patient selection in our unit appears to be improving; however this does not appear to be having a significant impact on outcomes in terms of re-intervention. Since 2003 we are treating similarly aged women with similar weights, however with smaller uteri, and overall volume of uterine fibroids. We are achieving a much higher percentage NPV (41.22 in 2003 to 54.92 in 2011), with fewer hyper-intense fibroids. It is hoped that improved patient selection and treatment results (in terms of percentage fibroid treated) will yield better results when prospective data is collected from the women being treated currently. I found that NPV achieved at MRgFUS did not significantly affect the re-intervention rate at five years. In this study I grouped all the women with NPV of greater than 50% together, due to the relatively small numbers involved. It could be that as treatment planning and technology improves MRgFUS treatments will be able to more regularly reach NPV of closer to the greater than 80% achieved at UAE. Numerous improvements to the technology involved in MRgFUS have improved the safety and effectiveness of this treatment. The innovations in treatment effectiveness are aimed at improving the non-perfused volume (NPV) achieved; i.e. the overall percentage of the fibroid treated. However, our figures do not demonstrate a direct relationship between NPV and improved re-intervention rate. It is possible that there may be a “threshold” NPV, beyond which the clinical outcomes improve, but from this data it is not apparent. As improvements continue, the mean NPV will inevitably increase, and further prospective data may support the hypothesis of increased NPV being associated with better outcomes, however at present the data does not support this. The initial results with the new ExAblate2100 system are encouraging, however the mean NPV achieved in the pilot study is

54.92%, versus 43.72% from the earlier ExAblate 2000 system (p=0.007). There is still much more to be done to achieve the higher NPV that may further improve long-term outcomes.

Patient satisfaction was disappointing following MRgFUS with 54.2% answering yes, they would recommend this treatment to a friend, 10% saying they would not recommend this treatment, and 35.8% who were not sure. As I mentioned in the earlier discussion, this relatively low patient satisfaction may be related to the high expectations of patients undergoing a "novel" treatment. In our future practice, a much more realistic conversation about patient expectations and requirements should be undertaken in the clinic prior to arranging treatment. The figures produced in this study will better inform these discussions in both in terms of re-intervention rates and possible side-effects.

The new ExAblate 2100 system is safe and results in a higher NPV within the fibroid compared with the previous system. This is very encouraging, however development in this area needs to continue to increase the volume of fibroid treated. A relatively low number of women undergoing MRgFUS received pre-treatment GnRHa, despite the evidence of enhanced tissue effects (Smart et al., 2006). The use of GnRHa should become a more routine part of MRgFUS treatment for uterine fibroids, although the side effects of these often make their use unpopular with patients. The recent introduction of selective progesterone receptor modulators (SPRMs) for treatment of uterine fibroids initially in the short term is very encouraging and may be an important next step in improving results following MRgFUS. As yet, SPRMs have not been used in any studies prior to MRgFUS, but their effects of reducing fibroid size and vasculature pre-treatment may be similar to GnRHa, without the significant side-effects. Ulipristal acetate down-regulates the expression of angiogenic growth factors, including VEGF, and increases the expression of matrix metalloproteinases and decreases the expression of tissue inhibitor of metalloproteinases (TIMPs) and collagens in uterine fibroid cells (Spitz, 2009). This may reduce collagen deposition in fibroid tissue, impairing tissue integrity and this change in tissue structure may change the thermal effects of high-intensity focused ultrasound. A study of the effects of SPRMs prior to MRgFUS may produce interesting results.

In chapter six I demonstrated that UAE is significantly more painful than MRgFUS for women, with significantly higher VAS pain scores at all time-points post-treatment and significantly longer duration in hospital. There was a significant rise of circulating IL-6 following both MRgFUS and UAE, but no detectable change in IL-4 and IL-1β. I found a more rapid increase in circulating IL-6 following MRgFUS, but was unable

to detect the peak of circulating IL-6 due to constraints on subject participation. Although IL-6 is known to augment the pain response following injury (Watkins et al., 1995), I found no correlation in this study between reported VAS scores and circulating IL-6 levels. Neither the NPV achieved nor the overall size of the treated fibroid had a significant effect on circulating IL-6 post-treatment. This was perhaps a reflection of the relatively small numbers studied. Previous studies have found circulating IL-6 to be related to the degree of tissue injury following myocardial ischemia (Cruickshank et al., 1990). It is possible that due to the effects of embolization on the uterine vasculature there is less of a release of pro-inflammatory cytokines into the peripheral circulation. During MRgFUS the treatment is contained entirely within the fibroid pseudo-capsule; this may also explain the lack of correlation between uterine size and IL-6 change. Further work is required to chart the rise and fall of circulating inflammatory cytokines following both these treatments, including the addition of measurement of CRP, IL-10 and TNF-α among others to better assess the effects on the immune response and possible recovery mechanisms.

Our study of circulating plasma VEGF levels following UAE and MRgFUS resulted in a wide range of VEGF levels that may reflect an error in adequately detecting VEGF in our samples, or may simply be a reflection of a naturally wide range of VEGF values. While we did see a downward trend following MRgFUS this failed to reach statistical significance. There was a significant pattern following UAE with a rise in VEGF at one week and subsequent fall at one month post treatment. This may reflect the changes in uterine vasculature following embolization, with the resultant hypoxia following embolization leading to an up regulation of VEGF expression by the fibroid. It has been suggested that VEGF may play a role in restoring uterine artery flow by the promotion of collateral blood vessel formation following UAE (Takeda et al., 2005). High circulating levels of VEGF post-embolization may be a predictor of treatment failure, as re-vascularisation of the fibroids is an indicator of UAE failure. My finding of a peak in VEGF at one week post-UAE corresponds with the timing of the return to normal blood flow within the uterus (deSouza and Williams, 2002a). As MRgFUS does not affect the uterine vasculature this may explain the absence of a discernible change in circulating VEGF. From the re-perfusion of uterine fibroids often seen as the six month follow-up contrast-enhanced MRI, I had initially hypothesised that circulating VEGF might rise following MRgFUS, and that the degree of increase might be related to treatment failure and re-perfusion of the fibroid. Other circulating factors may have a role in predicting the success or otherwise of MRgFUS, but from our results VEGF does not.

MRgFUS is emerging as an alternative to surgery or UAE in managing women with symptomatic uterine fibroids, however as with all treatment modalities, care needs to be taken to ensure that the correct women are receiving this treatment, with correctly managed expectations, safety, and follow-up. As advances in the technology continue and costs to the health providers hopefully fall, MRgFUS may become a more established treatment option for women with symptomatic uterine fibroids, however at present there remains insufficient evidence to support its routine use. Once the volume of fibroid that can be successfully treated is increased to levels similar to that achieved at UAE, a randomised control trial of the use of MRgFUS should answer the clinical question of whether MRgFUS is a viable alternative treatment for women with symptomatic uterine fibroids.

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Appendix A: UFSQOL

Pt. Initials: _____ **Pt. ID:** _____

Date: _____

UTERINE FIBROID SYMPTOM AND HEALTH-RELATED QUALITY OF LIFE QUESTIONNAIRE (UFS-QOL)

Listed below are symptoms experienced by women who have uterine fibroids. Please consider each symptom as it relates to your uterine fibroids or menstrual cycle. Each question asks how much distress you have experienced from each symptom during the previous 3 months.

There are no right or wrong answers. Please be sure to answer every question by checking (✓) the most appropriate box. If a question does not apply to you, please mark "not at all" as a response.

During the previous 3 months, how distressed were you by...	Not at all	A little bit	Some-what	A great deal	A very great deal
1. Heavy bleeding during your menstrual period	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2. Passing blood clots during your menstrual period	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3. Fluctuation in the duration of your menstrual period compared to your previous cycle	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
4. Fluctuation in the length of your monthly cycle compared to your previous cycles	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
5. Feeling tightness or pressure in your pelvic area	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
6. Frequent urination during the daytime hours	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
7. Frequent nighttime urination	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
8. Feeling fatigued	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

The following questions ask about your feelings and experiences regarding the impact of uterine fibroid symptoms on your life. Please consider each question as it relates to your experiences with uterine fibroids during the previous 3 months.

There are no right or wrong answers. Please be sure to answer every question by checking (✓) the most appropriate box. If the question does not apply to you, please check "none of the time" as your option.

During the previous 3 months, how often have your symptoms related to uterine fibroids...	None of the time	A little of the time	Some of the time	Most of the time	All of the time
9. Made you feel anxious about the unpredictable onset or duration of your periods?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
10. Made you anxious about traveling?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
11. Interfered with your physical activities?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
12. Caused you to feel tired or worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
13. Made you decrease the amount of time you spent on exercise or other physical activities?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
14. Made you feel as if you are not in control of your life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
15. Made you concerned about soiling underclothes?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
16. Made you feel less productive?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
17. Caused you to feel drowsy or sleepy during the day?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
18. Made you feel self-conscious of weight gain?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
19. Made you feel that it was difficult to carry out your usual activities?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
20. Interfered with your social activities?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
21. Made you feel conscious about the size and appearance of your stomach?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
22. Made you concerned about soiling bed linen?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

During the previous 3 months, how often have your symptoms related to uterine fibroids...	None of the time	A little of the time	Some of the time	Most of the time	All of the time
23. Made you feel sad, discouraged, or hopeless?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
24. Made you feel down hearted and blue?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
25. Made you feel wiped out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
26. Caused you to be concerned or worried about your health?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
27. Caused you to plan activities more carefully?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
28. Made you feel inconvenienced about always carrying extra pads, tampons, and clothing to avoid accidents?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
29. Caused you embarrassment?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
30. Made you feel uncertain about your future?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
31. Made you feel irritable?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
32. Made you concerned about soiling outer clothes?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
33. Affected the size of clothing you wear during your periods?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
34. Made you feel that you are not in control of your health?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
35. Made you feel weak as if energy was drained from your body?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
36. Diminished your sexual desire?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
37. Caused you to avoid sexual relations?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

To calculate a symptom score for symptom severity, create a summed score from the items listed below and then use the formula below the table to transform the value. This will provide symptom scores where higher score values are indicative of greater symptom severity or bother and lower scores will indicate minimal symptom severity (high scores = bad).

Scale	Sum Item Values	Lowest and Highest Possible Raw Scores	Possible Raw Score Range
Symptom Severity	Sum 1 – 8	8, 40	32

Transformation for Symptom Severity raw scores ONLY:

$$\text{Transformed Score} = \frac{(\textit{Actual}\text{ raw score} - \textit{lowest}\text{ possible raw score})}{\textit{Possible raw score range}} \times 100$$

For the HRQL subscales (concern, activities, energy/mood, control, self-conscious, and sexual function), create summed scores of the items listed below for each individual subscale. To calculate the HRQL total score, sum the value of each individual subscale (do not sum individual items). Use the formula below the table to transform all values. Higher scores will be indicative of better HRQL (high = good).

Scale	Sum Item Values	Lowest and Highest Possible Raw Scores	Possible Raw Score Range
Concern	9+15+22+28+32	5, 25	20
Activities	10+11+13+19+20+27+29	7, 35	28
Energy/mood	12+17+23+24+25+31+35	7, 35	28
Control	14+16+26+30+34	5, 25	20
Self-conscious	18+21+33	3, 15	12
Sexual function	36+37	2, 10	8
HRQL TOTAL	Sum of 6 Subscale Scores	29, 145	116

Formula for transformation of HRQL raw scores ONLY:

$$\text{Transformed Score} = \frac{(\textit{Highest}\text{ possible score} - \textit{Actual raw}\text{ score})}{\textit{Possible raw score range}} \times 100$$

Missing Items

For the subscale analyses, if < 50% of the scale items are missing, the scale should be retained with the mean scale score of the items present used to impute a score for the missing items. If ≥ 50% of the items are missing, no scale score should be calculated, the subscale score should be considered missing. If a subscale score is missing, the HRQL total cannot be calculated.

Appendix B: Telephone questionnaire

Imperial College London

MRgFUS long-term follow-up study 2011

Dr Stephen Quinn, Professor Lesley Regan, Professor W Gedroyc

Date: **Date of MRgFUS:**

Patient Number:

Did you have any adverse events/side-effects following treatment?

N/Y Skin Burns Pain UTI Fibroid Expulsion Back Pain Nerve Irritation

Have you had any further treatment for your fibroids following your treatment?

Myomectomy UAE Hysterectomy TCRF

Overall would you describe your symptoms/QoL as:

Better Same Worse

Over all would you describe your bleeding as:

Better Same Worse

Overall would you describe your bladder symptoms as:

Better Same Worse

Have you gone through the menopause since your treatment?

Yes No Not sure

Do you feel your bladder symptoms have changed since you r treatment?

Better Same Worse Not sure

Have you had a pregnancy since your treatment?

Yes No Number

If so, what was the outcome?

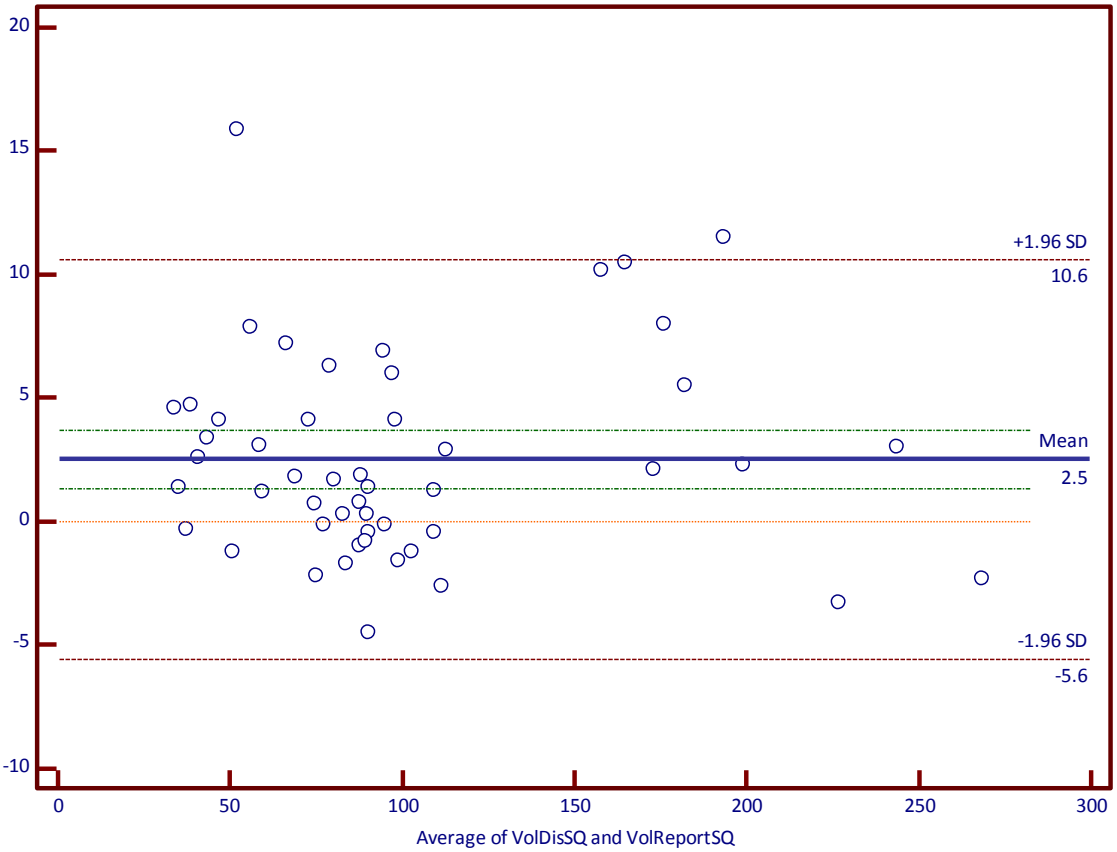
SVD CS Forceps MC<12/40 MC>12/40

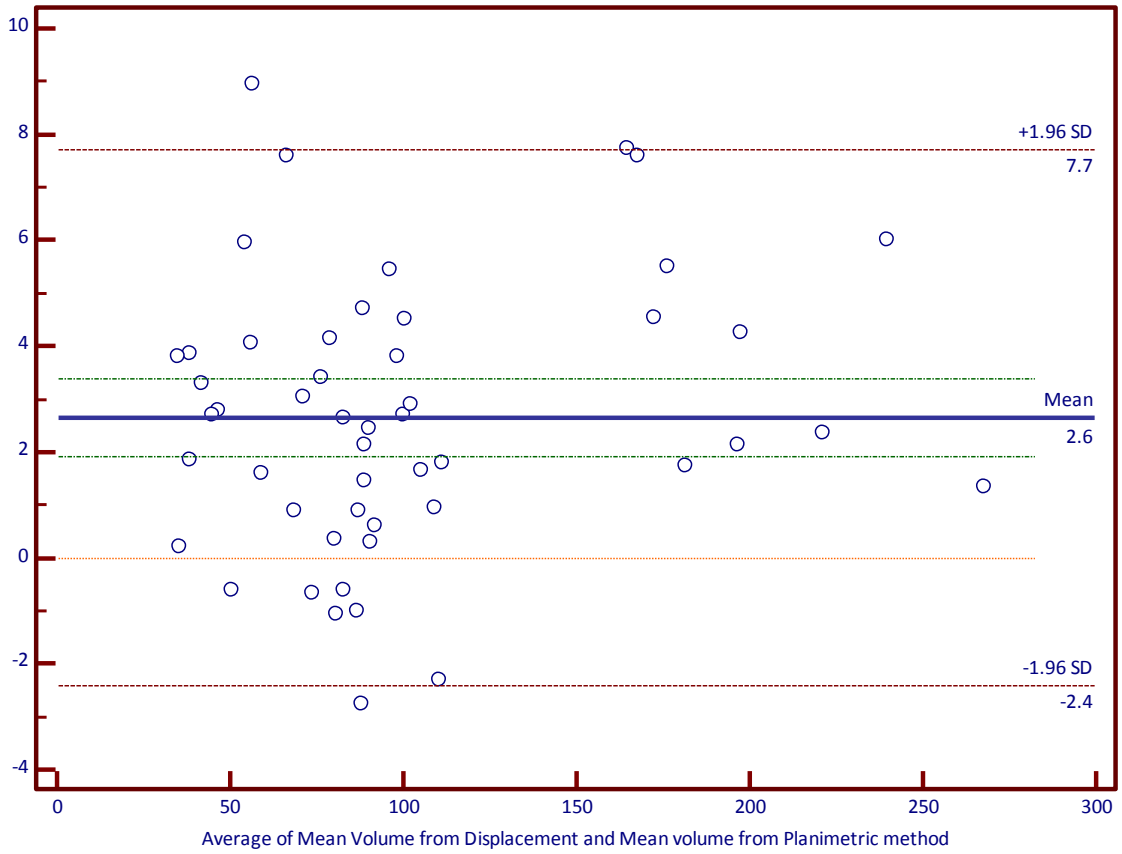
Would you recommend MRgFUS to a friend?

Yes No Not sure

Comments:

Appendix C: Bland Altman Plots for inter-observer correlation

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8
6
4
2
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-2
-4
+1.96 SD
7.7
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-1.96 SD
-2.4
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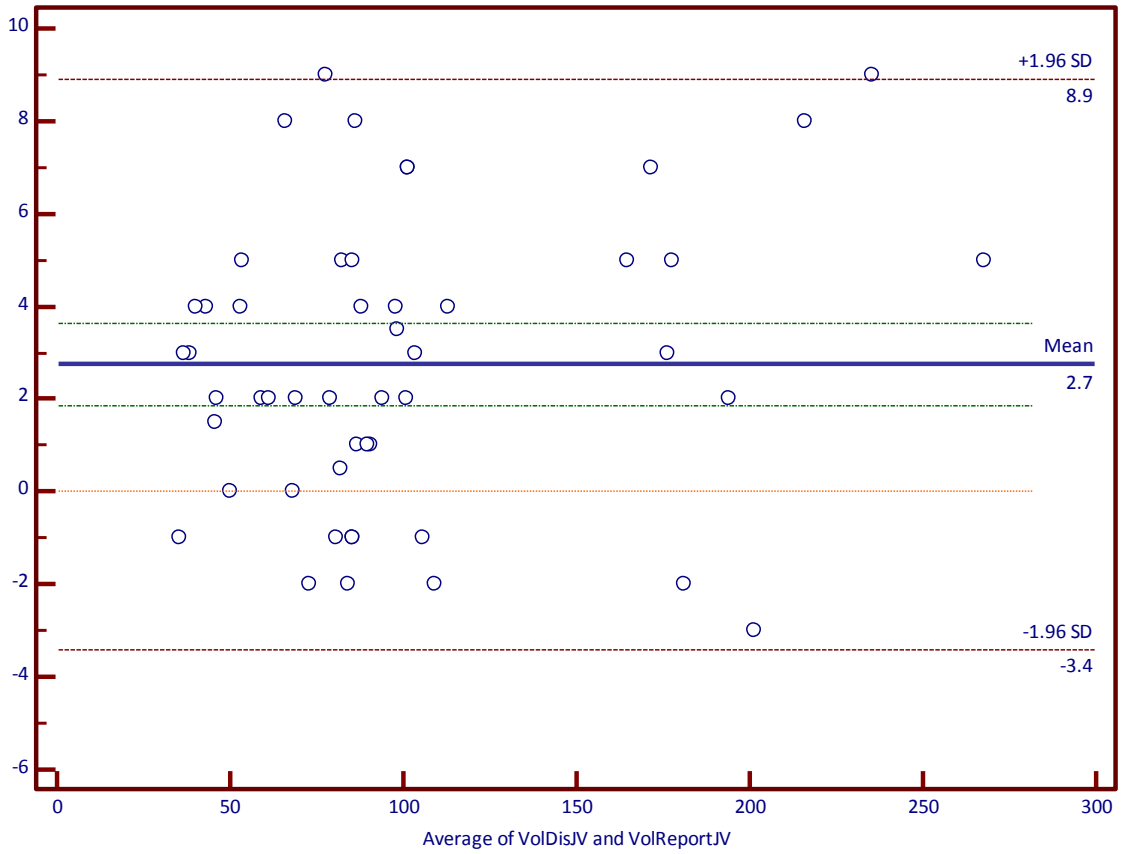
50
100
150
200
250
300
Average of Mean Volume from Displacement and Mean volume from Planimetric method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+1.96 SD
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-3.4
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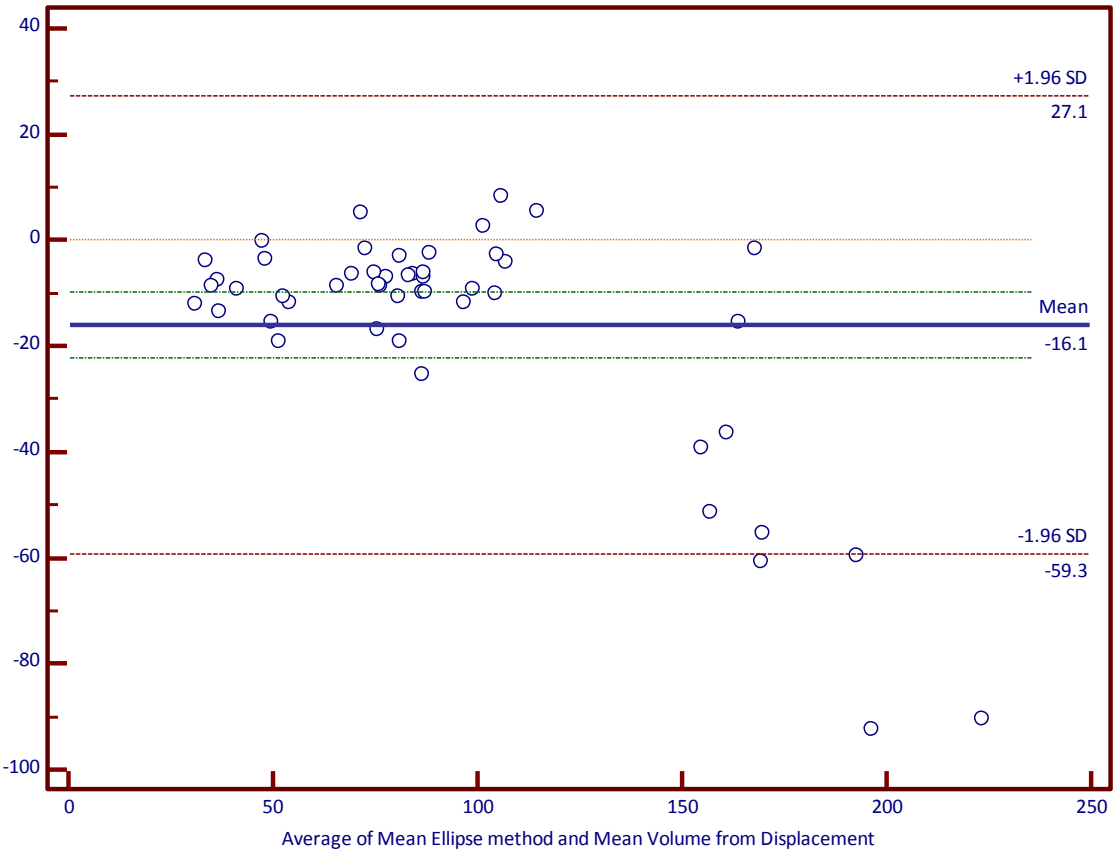
300
Average of VolDisJV and VolReportJV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-60
-80
-100
+1.96 SD
27.1
Mean
-16.1
-1.96 SD
-59.3
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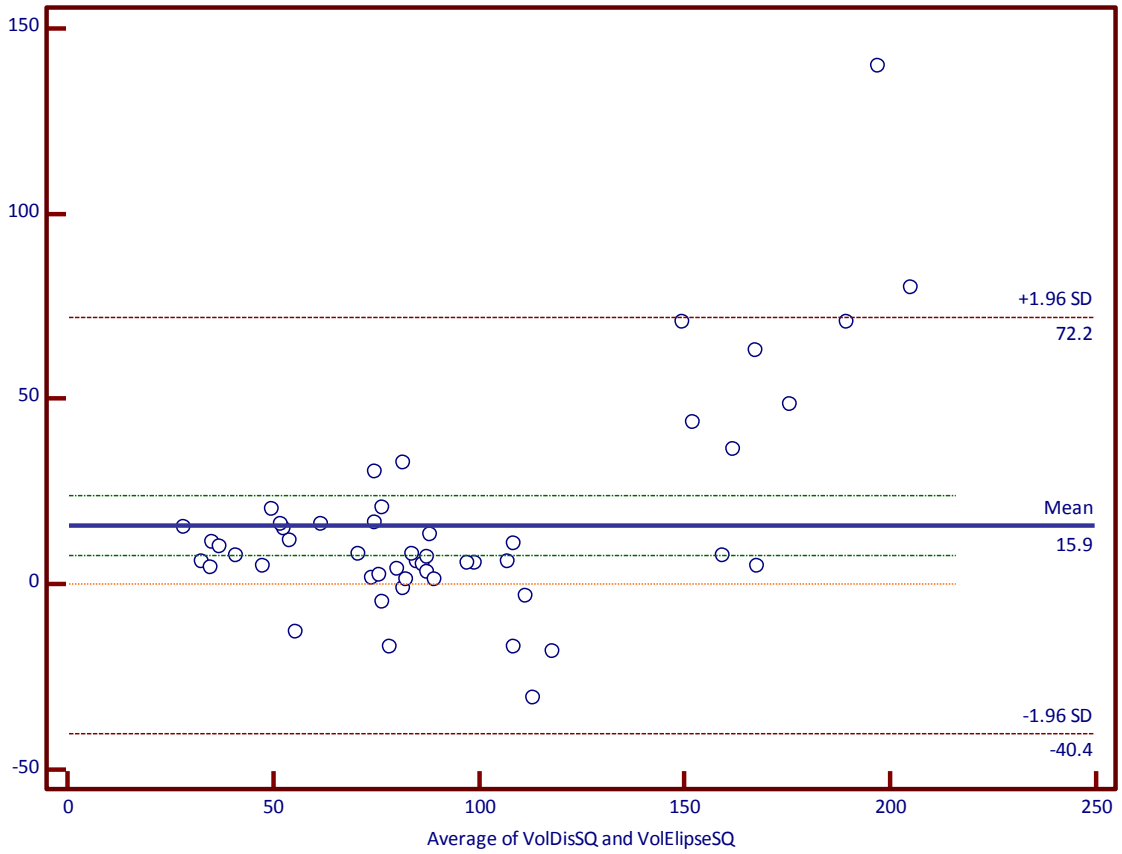
100
150
200
250
Average of Mean Ellipse method and Mean Volume from Displacement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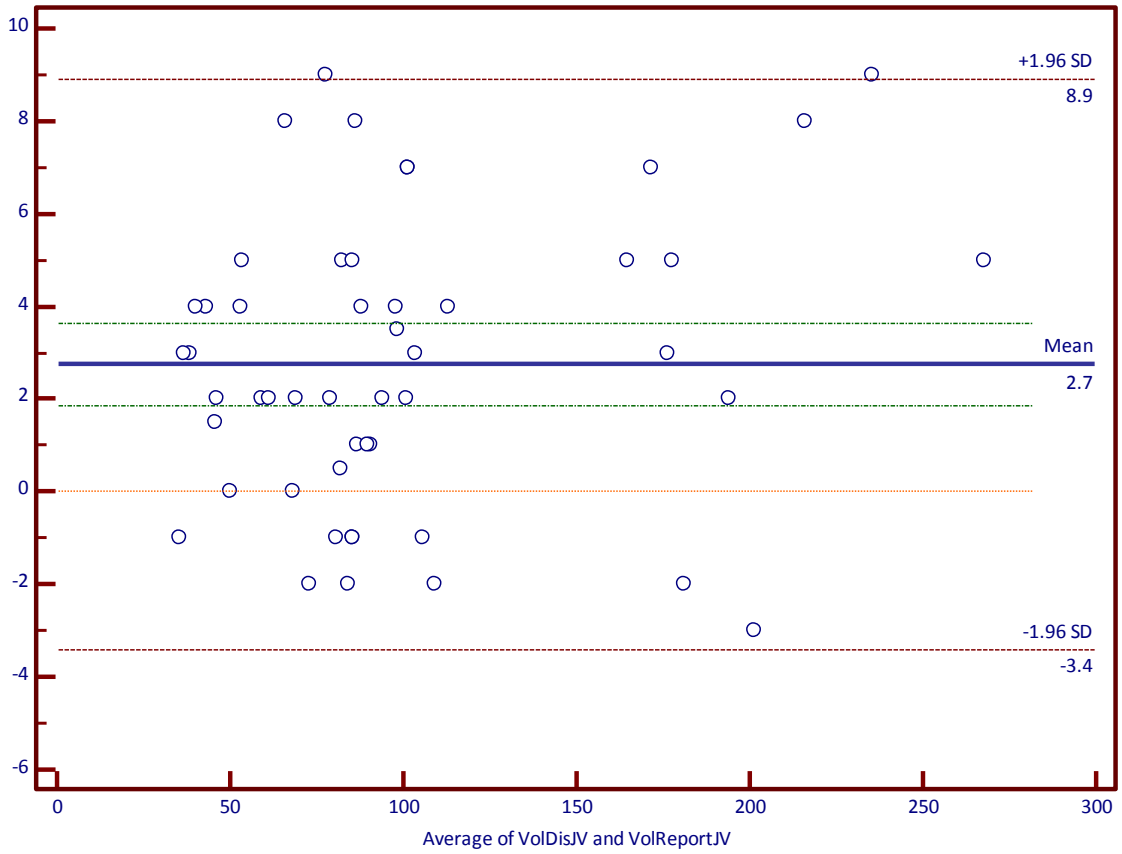
Average of VolDisSQ and VolElipseSQ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-6
+1.96 SD
8.9
Mean
2.7
-1.96 SD
-3.4
0
50
100
150
200
250
300
Average of VolDisJV and VolReportJV

Appendix D: St Mary's Hospital Fibroid Clinic Patient questionnaire

Fibroid Clinic – St Mary's Hospital

Name:…….. Dob:…….Age:… Ethnic Origin……

Occupation…….. Height……. Wgt:…….

Contact Nos:: Home:…….. Work……. Email address……..

Please answer the following questions:

How long ago were your fibroids Diagnosed?.....year(s)…….month(s)

Do your fibroids cause any symptoms? Yes† No† Sometimes †

Section 1

Do you have a regular menstrual cycle? Yes † No†

How many days do you normally bleed for?.....Days

How many days of heavy bleeding do you experience?.....Days

Do you ever pass blood clots? Yes† No† Sometimes†

Do you ever flood through your clothing? Yes† No† Sometimes†

Are your activities limited during your period? Yes †No† Sometimes†

Do you bleed between your periods or after intercourse? Yes†No† Sometimes†

Are you currently, or have you ever been anaemic? Yes† No† Not Sure†

Section 2

How often do you pass urine during the day?.....

How often do you pass urine at night?.....

Do you ever leak urine when you cough or sneeze? Yes ¶ No¶ Sometimes¶

Does your bladder ever empty without warning? Yes ¶ No¶ Sometimes¶

Do you start to pass urine straight away when you want to? Yes¶ No¶

Is your flow of urine the same as before? Yes¶ No¶

Do you feel your bladder is emptying after you have passed urine? Yes¶ No¶

Section 3

Do you experience painful periods? Yes No

Do you experience pain even before your periods start? Yes¶ No¶ Sometimes¶

Do you have pain at any other time of the month? Yes¶ No¶ Sometimes¶

Do you experience pain during sexual intercourse? Yes¶ No¶ Sometimes¶

Do you take painkillers? Yes ¶ No¶ Sometimes¶

If yes, what sort and how many do you take?.....

Section 4

How often do your bowels open?.....

How would you describe your stools/motions? Hard ¶ Soft¶ Loose¶ Watery¶

Do you have to strain to move your bowels? Yes¶ No¶ Sometimes¶

Do you take anything to help your bowels? Yes¶ No¶ Sometimes¶

Do you experience abdominal bloating? Yes¶ No¶ Sometimes¶

Do you have any other bowel problems? Yes¶ No¶

If yes, what?.....

Section 5 – General Gynaecology

When was you last cervical smear test?.....

Was the result normal?	Yes ☐	No ☐	Not sure☐
Have you ever had an abnormal smear?	Yes☐	No☐	Not sure
Are you currently using any contraception?	Yes☐	No ☐	

If yes, what?.....

Have you ever been pregnant?	Yes☐	No☐	
Do you wish to become pregnant in the future?	Yes ☐	No☐	Not Sure☐

Do you have children?	Yes	No
Have you had a pelvic ultrasound scan?	Yes	No
Have you had a pelvic MRI scan?	Yes	No
Have you had any other operations for your fibroids?	Yes	No

If yes what?.....

Section 6 – Medical History

Do you have any other medical problems?.....

Have you had any other surgical operations?.....

…….

……..

Please list any medications you take, including any that you buy without prescription?

1……

2……

3……

4……

Do you drink alcohol? Yes☐ No☐

If yes, how many units per week?.....

Do you smoke cigarettes? Yes☐ No☐

If yes, how many do you smoke?.....

What do you hope to gain from your visit to the Fibroid Clinic?

……

……

……

……

Appendix E: Study Protocol for ExAblate 2100 Safety Study

1. Prior to all treatments women will be given a urine pregnancy test prior to treatment. A positive result will be reason for exclusion from the study. A urinary catheter will be positioned in the bladder to keep it empty during the course of the treatment. An IV line will be positioned for the delivery of medications and will be maintained throughout the procedure. Monitoring of heart rate and pO2 will be maintained throughout the procedure using standard MR-compatible monitoring devices.
2. The patients' abdomen will be cleaned and closely examined for any hair in the treatment path. The abdomen will be shaved from the umbilicus to 1 cm below the crest of the pubic bone.
3. Degassed water and an acoustic gel will be placed on top of the transducers' window to generate acoustic coupling.
4. Patient will be positioned on the ExAblate therapy bed in the prone position. The patient will be positioned in the magnet in the treatment position.
5. An MR scan will be performed with T2 weighted sequences to localize and measure lesion(s) to be treated.
6. The pelvic anatomy will be evaluated to identify any abnormal anatomy that could prevent safe treatment. The intra-abdominal contents will be evaluated to insure that no bowel, bone or other obstruction is present in the pathway of the ultrasound treatment beam. Any prior abdominal scar will be identified on the image and the ultrasound treatment plan will be designed to avoid sonicating through the scar whenever possible.
7. If the fibroid(s) to be treated are identifiable on the MR images, accessible by ExAblate for treatment, and the patient meets all of the inclusion criteria, treatment planning will begin.
8. The physician will draw the planned treatment volume on the MR images using the following guidelines:
    - The investigator should select the fibroids for treatment based on their location in the uterus and presumed clinical relevance to the reported patient symptoms (e.g. mass effect and/or uterine bleeding), and previously treated regions (in the case of second treatments).
    - Pedunculated fibroids either inside the uterine cavity or outside the uterus will not be treated.

- During the planning phase the investigator will draw a region of treatment within the capsule of the fibroid.
- The treatment plan will maintain a 10 mm margin between the planned region of treatment and the serosal surface.
- Low energy density regions (LEDRs) should be drawn around the intestine, pubic bone and far field bone to ensure safe and optimal planning
- Patient positioning should be planned to maximize the incidence angle of the beam with the sacrum.

9. If at this point it is determined that the patient cannot be safely treated, she will be taken out of the scanner, removed from the treatment table, and the catheters will be removed. She will be taken to the recovery area for observation and released. The Procedure Case Report Forms will be completed until that point with the reason for the exclusion.
10. Prior to the delivery of any treatment sonications, the patient may receive analgesia and conscious sedation (example: Fentanyl and Versed, or similar medications) to reduce pain and any unnecessary motion, as well as to help alleviate anxiety and claustrophobia.
11. A central point in the fibroid will be sonicated with a low thermal dose, generating a sub-lethal sonication, to reconfirm targeting accuracy in the patient. Target placement and / or transducer location will be adjusted as necessary.
12. Sonications will be performed at therapeutic power level to confirm thermal image dosimetry. Energy delivered will be adjusted as necessary to achieve a sufficient thermal dose to coagulate the tissue.
13. Sonications will be performed on successive points. Acoustic power will be adjusted throughout the treatment based on MR thermometry, resulting in temperatures between 65°C and 85°C at the focal point. Successive sonications should occur only after adequate cooling which the system calculates automatically. Inadequate cooling time between sonications can lead to thermal build-up that may cause serious damage to normal tissues outside the targeted volume. The thermal map should be monitored constantly throughout the entire duration of treatment to avoid patient injury.
14. Criteria to terminate sonication include inability to visualize the focal treatment spot, patient complaints of unacceptable pain, targeting difficulties due to patient motion or other reasons.

15. After the treatment is complete, a final MR scan will be completed. The scanning will include T1 weighted sequences with / without Gadolinium contrast to evaluate general abdominal anatomy and to assess treatment effect.
16. The patient will be removed from the ExAblate system and taken to a recovery area for a short observation period pending release from the hospital.
17. All MR studies and treatment exports taken during the study will be archived and returned to the Sponsor for later analysis.

The post-treatment images from the first treatment will be used in planning the second treatment to target the remaining perfused volume of the fibroid.

Following treatment women were asked to attend at one week and one month for assessment of any physical adverse events or exacerbated medical conditions.

STUDY VISIT SCHEDULE

Schedule of Events				
Procedures	**Screening**	**Day 0**	**Week 1 (±3 days)**	**Month 1 / Exit (±4 days)**
Written Consent	**X**			
Check Eligibility	**X**			
Demographics, Medical History	**X**			
MRI	**X**	**X**		
Physical Exam	**X**		**X**	**X**
Pregnancy Test	**X**	**X**		
Uterine Fibroid Symptom and Health-Related Quality of Life Questionnaire (UFS-QOL)	**X**			
ExAblate		**X ***		
Adverse Events		**X**	**X**	**X**

* If needed patient may undergo a second ExAblate treatment. Follow-up visits are based on date of last

treatment.

Following each visit data was entered prospectively using an electronic data capture system (EDC). This electronic data capture of the eCRFs is based on the Oracle Software system, and is designed, run and hosted by InSightec (Haifa, Israel).

Appendix F: Participant information leaflet for cytokine study

Imperial College London

St Mary's Hospital, Imperial College London

Participant Information leaflet

CFUS 1.3

Date: 12/05/2011

Cytokine levels and pain following uterine artery embolization and MR-guided focused ultrasound treatment of fibroids.

Dr M. Hamady

Consultant Radiologist

Mr Stephen D. Quinn

Clinical Fellow Gynaecology

We would like you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One our team will go through the information sheet with you and answer any questions you might have. We would suggest this should take about 10 minutes. Talk to others about the study if you wish. Part 1 of this sheet tells you about the purpose of this study and what will happen if you take part. Part 2 gives you more detailed information about the conduct of this study. Please ask us if anything on this sheet is not clear

Part 1

Name of the proposed procedure and trial:

Cytokine levels and pain following uterine artery embolization and MR-guided focused ultrasound treatment of fibroids.

What is the purpose of this study?

Fibroids are benign tumours affecting the uterus (womb) They are thought to affect between 25 and 50% of all women. Traditionally the usual treatment for fibroids causing symptoms was surgical, involving either removal of the fibroids (myomectomy) or hysterectomy (removal of the womb). Over the last 10 years we have developed non-surgical treatments for fibroids which avoid the need for surgery. The pain and discomfort experienced following these treatments varies a great deal. In this study we will look at how these treatments effects certain chemicals in your blood. These chemicals, known as cytokines, are known to effect the sensation of pain experienced, and may be related to how a fibroid responds to these treatments. This study will be the first time that these cytokines have been measured immediately following treatment, and compared with pain experienced. We hope by understanding how these treatments lead to cytokine production we can improve these treatments, and develop new ways of reducing discomfort pain following treatment. This study will form part of the study into uterine fibroids carried out by Dr Stephen Quinn as part of his higher degree from Imperial College London.

Why have I been invited?

As part of your on-going treatment for your uterine fibroids you are due to undergo either uterine artery embolism (UAE)of your fibroids or Magnetic resonance-guided focused ultrasound (MRgFUS) treatment. This makes you eligible for our trial.

Do I have to take part?

No. It is entirely up to you to decide to join the study. We will describe the study in this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at and time, without giving a reason. If you do decide to leave the study, or do not wish to participate at all, this **will not** affect the standard of care you receive at Imperial College NHS Healthcare Trust.

What will happen to me if I take part?

If you decide to take part in the study you will be asked to attend an extra screening visit prior to your treatment (between one and two weeks prior to your treatment).

If you take part in this study, you will be required to undergo blood tests before and after your treatments. You will also be required to attend for three extra visits to the Hospital (St Mary's, Imperial College Healthcare NHS Trust) in order to have these blood tests taken.

Consenting and Screening Appointment

During the first study visit, the following procedures will take place:

- The study will be discussed with you. You will be given the opportunity to ask questions, and if you have had your questions answered to your satisfaction, you will be asked to sign this consent form and will be assigned a patient study number.
- A medical history will be obtained to determine your general health status and current symptoms.
- Your General Practitioner (GP) will be informed of your involvement in the trial and receive a letter from the investigators, including information regarding this study and copy of this information leaflet.

Expenses and payments

You will not be paid for participation in this clinical study.

What will I have to do?

If you are undergoing UAE you have small blood samples (<5ml) taken at the following intervals:

1. Before your treatment
2. Two hours following your treatment
3. Four hours following your treatment
4. Six hours following your treatment
5. Twenty-hour hours after your treatment. This is usually the point at which you may be discharged from hospital.

If you are undergoing MRgFUS you will have small blood samples (<5ml) taken at the following intervals:

1. Before your treatment
2. Immediately after you treatment
3. 2 hours after your treatment. This is usually the point at which you would be discharged from hospital.

In addition to these blood samples you will be asked to complete a visual analogue score describing any pain you may be experiencing at that time, just before blood samples are taken. This simply involves telling what pain you have and grading it out of 10.

What are the alternatives for diagnosis or treatment?

You do not have to be in this study to receive treatment for your condition. If you choose not to participate in this study, your standard medical care will not be affected in any way, and you will still receive the treatment that you normally would have received. You should talk to your doctor about each of your choices before you decide if you will take part in this study.

What are the possible disadvantages and risks of taking part?

Blood samples may be uncomfortable, and may lead to bruising of the arm.

What are the side effects of any treatment received when taking part?

The actual treatment you receive will not be affected by your involvement in this study. The risks of these treatments will be discussed with you before treatment.

What are the possible benefits of taking part?

It is hoped that by better understanding how these treatments for fibroids cause pain, we may be able to improve these treatments in the future, and also improve the pain control required during and following these treatments.

What happens when the research study stops?

Following your treatment you will have a repeat MRI scan at 6 months to assess how your fibroid has responded to the treatment. This is standard practice for any patient undergoing MRI-guided focused

ultrasound or UAE for their fibroids. If you require further treatment, or follow-up this will be arranged by your doctor at the fibroid clinic. Any further treatment will not be affected by your involvement in this trial. Your GP will be informed of your follow-up as per standard practice for NHS patients.

What if there is a problem?

Your participation in this study is voluntary. You do not have to take part in this study, but if you do, you can stop at any time. Your medical care at this institution now or in the future will not be affected whether or not you take part in this study. You do not give up any of your rights by taking part in this study.

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2 of this sheet.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in part2. If the information in the sheet interests you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes we get new information about the treatment being studied. If this happens, your research doctor will tell you and discuss whether you should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. He/she will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, we will tell you and arrange your continuing care.

What will happen if I don't want to carry on with the study?

If you wish to withdraw from the study at any time, you can simply contact the MR-treatment centre. If during the study you do not wish to have any further blood tests, we will stop and you will carry on your

post-treatment care as normal we can arrange an outpatient appointment with you to ensure that you still receive the treatment you require. Withdrawing from this trial will not affect the quality of care you receive at St Mary's Hospital.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you are not satisfied with the action taken or would like assistance in resolving your concerns please speak to our patient advice and liaison service (PALS). The PALS team act independently when dealing with the concerns of patients, carers or visitors and can help negotiate prompt solutions on your behalf. Where your issues are complicated, PALS can listen and advise you about step two of the NHS complaint procedure if appropriate.

If you feel that your concerns have not been resolved informally and you wish us to investigate your complaint, please **write to us**. Send your letter to:

The managing director
Imperial College Healthcare NHS Trust
Trust headquarters
The Bays
South Wharf Road
London W2 1NY
Alternatively you can **email our patient complaint coordinators** at complaints@imperial.nhs.uk.

If you are injured as a result of your participation in this study, you will receive appropriate medical care. Any injury should be discussed with your study doctor who will explain appropriate treatment options. The sponsor will provide compensation for all reasonable and necessary medical services when injuries are determined to be directly related to the use of the study device provided that:

(A) The injury is not the result of the natural course of your disease or some other underlying condition.

(B) Such expenses are not attributable to negligence, malpractice, or misconduct of the doctors or product liability of any third party.

(C) Such expenses are not covered by your medical or hospital insurance coverage.

No financial compensation for lost wages, disability, or discomfort is offered. The sponsor and study doctor will determine whether your injury is related to the study device. You do not give up any of your legal rights as a research subject by signing this consent form.

Will my taking part in this study be kept confidential?

Yes. All information regarding this study will be kept in locked areas, with access only to the study coordinators. Once the trial has completed your details will be filed in your St Mary's NHS notes, subject to routine NHS confidentiality.

Involvement of the General Practitioner/Family doctor (GP)

If you agree to participate in the trial a letter will be sent to your GP, explaining the aims of the trial and the treatment you will receive. Upon completion of the trial, your GP will be contacted with your individual response to the treatment in the same way that would occur if you were having routine MRgFUS. This is important as your GP should have this information in order to correctly manage you fibroid symptoms in the future. If you experience any problems or complication during your treatment your GP will again be informed.

Are there reasons I might leave this study early?

Taking part in this study is your decision. You may decide to stop at any time. You should tell the study doctor if you decide to stop and you will be advised whether any additional tests may need to be done for your safety. In addition, the doctor may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

Will any biological sample(s) be stored and used for research in the future?

Yes, the samples taken will be stored until such time as the analysis can proceed. These samples may be used in a further study to examine metabolic changes experienced following treatment for fibroids. If your samples are used in this study, you will be contacted and your consent will be requested. If you decided that you do not want your samples used in this study, these samples will be destroyed. Following the final analysis, samples will be destroyed.

What will happen to the results of the research study?

The results of this study will be written-up and presented locally and nationally. These results will also be submitted to the medical literature for publication.

Who is organising and funding the research?

This study is organised by Imperial College London clinical trials unit. The funding is provided by the company Celanova Biosciences Ltd.

CeloNova BioSciences of Europe B.V. – London
Pinewood,
Crockford Lane,
Chineham Business Park,
Basingstoke, R
G24 8AL,
United Kingdom
Tel.: +44.1256.698.010

Who has reviewed the study?

This study has been reviewed by a Nationally-appointed research and ethics group, in addition to local research and ethics office at St. Mary's Hospital.

What are my rights if I take part in this research study?
Your participation in this study is voluntary. You do not have to take part in this study, but if you do, you can stop at any time. Your medical care at this institution now or in the future will not be affected whether or not you take part in this study. You do not give up any of your rights by taking part in this study.

Who can answer my questions?

You are encouraged to ask questions about the study or your role as a subject at any time. If you have any questions about your participation in this study, if at any time you feel you have had a research-related injury, or if you have questions, concerns, or complaints about the research, contact:

Mr Stephen D. Quinn
Clinical Research Fellow
Ground Floor Mint Wing
St. Mary's Hospital
South Wharf Road
London
W2 1NY
Tel : 02078862325
E-mail: s.quinn@imperial.ac.uk

Dr Elizabeth Dick
Consultant Radiologist
St. Mary's Hospital,
London,
W2 1NY
Tel : 0207886666
E-mail : e.dick@imperial.ac.uk

Appendix G: GP letter for cytokine study

Department of Women's Health,
St Mary's Hospital,
Imperial College London
South Wharf Road,
London
W21NY
Tel: 02078862325
e-mail: s.quinn@imperial.ac.uk
m.hamady@imperial.ac.uk

Date 12/01/2011

Dear Doctor,

Re: CFUS. Cytokine levels and pain following uterine artery embolization and MR-guided focused ultrasound treatment of fibroids.

This letter is to inform you of your patient _____`s potential involvement in the above trial.

Uterine artery embolization (UAE) and MR-guided focused ultrasound (MRgFUS) treatment of uterine fibroids lead to ischemia and coagulative necrosis of fibroid tissue respectively. At present the mechanisms by which these therapies lead to pain post-procedure is not understood. UAE usually involves in-patient care with patient-controlled –opioid analgesia, whereas MRgFUS is a day-case procedure involving relatively little post-procedure analgesia. We intend to compare the circulating cytokine profiles following these treatments in order to improve the understanding of how circulating cytokine levels are affected by these treatment modalities. This will improve or understanding of the inflammatory mechanisms following these therapies which may allow targeting of these changes in improving patient pain-control in the future.

The inclusion and exclusion criteria are listed below:

Inclusion Criteria

1. Women due to undergo UAE or MRgFUS at St Mary's Hospital.
2. Willingness to have blood samples taken at intervals

Exclusion Criteria:

1. Any clinically significant medical condition or system disease which may contribute to circulating cytokine levels.
    a. Ischaemic heart disease
    b. Acute or chronic infections
    c. Any form of on-going treatment for cancer
    d. Autoimmune disease (e.g. SLE, Rheumatoid arthritis)
    e. Any form of surgery 3 months prior to treatment
    f. Cerebral vascular accident

2. Body mass index > 36;
3. Being pregnant or lactating
4. Participation in another trial of an investigational product or device within the previous 30 days;
5. Unable or unwilling to comply with the requirements of the study, in the opinion of the investigator.

If you have any concerns that your patient may not be suitable for this study could you please contact Mr Stephen D. Quinn at the above address, or Dr M. Hamady. A copy of the patient information leaflet has been enclosed. If you require any additional information regarding this study please contact us at the above address, e-mail or telephone number.

Yours faithfully,

Mr Stephen D. Quinn MB BS MRCOG BSc

Clinical Fellow, Department of Obstetrics and Gynaecology

Dr M Hamady

Consultant Radiologist

Division of Clinical Sciences

Appendix H: Consent for Cytokine study

Imperial College Healthcare NHS Trust

Study Title	**Cytokine levels and pain following uterine artery embolization and MR-guided focused ultrasound treatment of fibroids.**		
Participant identification Number for this trial:		**Site**	St. Mary's Hospital, London
Name of Research Doctor	Dr M. Hamady Consultant Radiologist. St Mary's Hospital		

Please initial box if you agree with the following:

1. I confirm that I have read (or have had read to me) and understand the information sheet dated (Version 1.1 12/5/2011) for the above trial. I have had time to review this information, have had an opportunity to ask questions and had answers in terms I understand. ☐

2. I understand that taking part is completely voluntary and that I am free to stop at any time even without providing any reason and without my normal medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by responsible individuals or representatives of Imperial College Healthcare NHS trust or from regulatory authorities where it is relevant to my taking part in the research. The purpose of this is to check that the research is being carried out correctly. I am willing to allow access to my medical notes but understand that strict confidentiality will be maintained. ☐

4. I am willing for my anonymous research data, and the results arising from the trial, to be used as appropriate. ☐

5. I agree to take part in this research project. ☐

_____	_____	_____
Name of Research Participant	Signature	Date
_____	_____	_____
Name of Research Doctor	Signature	Date
_____	_____	_____
Name of person taking consent if different from Research Doctor	Signature	Date

1 copy for subject, 1 for Investigator Site File, 1 (original copy) for medical records

Appendix I: Cytokine Study trial documentation

Imperial College London

St Mary's Hospital, Imperial College London

CFUS 31/05/2011

Recruitment details

Subject initials and number:	

Subject date of birth:	

Past medical history:

Past surgical history:

Current medications:

Use of hormones in last 12 months:

Menopausal status:

Subject selection

Inclusion criteria

(i) Women due to undergo UAE or MRgFUS at St Mary's Hospital ☐

(ii) Willingness to have blood samples taken at intervals ☐

Exclusion criteria

1. Any clinically significant medical condition or system disease which may contribute to circulating cytokine levels. ☐

   a. Ischaemic heart disease ☐

   b. Acute or chronic infections ☐

c. Any form of on-going treatment for cancer

d. Autoimmune disease (e.g. SLE, Rheumatoid arthritis) ☐

e. Any form of surgery 3 months prior to treatment ☐

f. Cerebral vascular accident ☐

2. Body mass index > 36 ☐

3. Being pregnant or lactating ☐

4. Participation in another trial of an investigational product or device within the previous 30 days; ☐

5. Unable or unwilling to comply with the requirements of the study, in the opinion of the investigator. ☐

Procedures and measurements

Blood samples will be collected by S Quinn (Clinical research fellow) and stored at the laboratory facilities in the Mint Wing until analysis.

Collection of plasma samples before treatment, and at intervals:

UAE patients (n=20):

Pre-treatment Sample number(s):

Pain score:

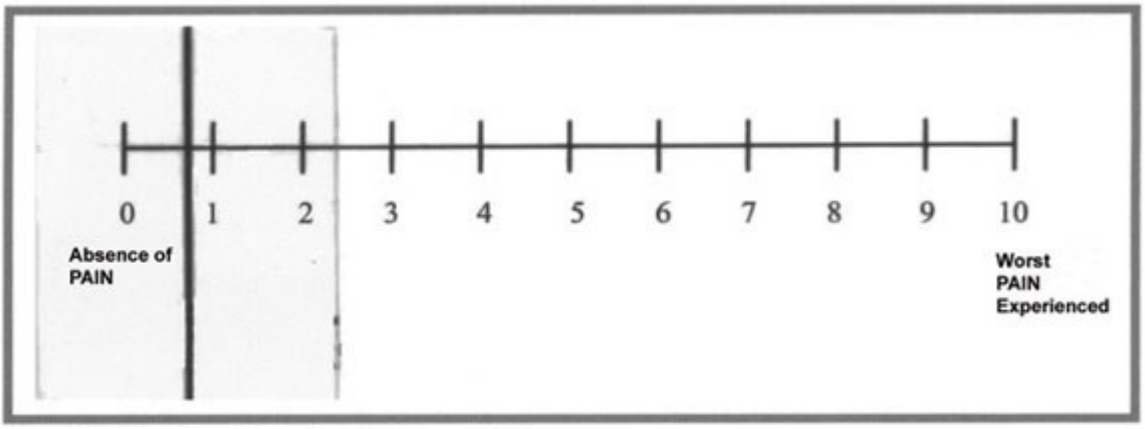

***Figure 2** – Visual analogical scale for pain (VAS).*

2 hours post treatment Sample number(s):

Pain score:

4 hours post treatment Sample number(s):

Pain Score:

6 hours post treatment Sample number(s):

Pain score:

24 hours post treatment Sample number(s):

Pain score:

MRgFUS patients (n=20)

Pre-treatment Sample number(s):

Pain score:

Immediately post treatment Sample number(s):

Pain score:

2 hours post treatment Sample number(s):

Pain score:

Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g, at 2-4°C. Remove serum and assay immediately or aliquot and store samples at -20° C, at the storage facility in the Mint Wing laboratories.

In addition to blood samples, pain-scores will be recorded using a visual analogue scale prior to each blood samples.

Samples will be assayed using Millipore™ Human Cytokine/Chemokine 96-Well Plate assay. Duplicate samples will be used to reduce error. Laboratory facilities will be used at Imperial College London Medical School Labs

Appendix J: Growth factor participant information leaflet

Imperial College London

St Mary's Hospital, Imperial College London

Participant Information leaflet

Protocol Number: GFFUS 1.1

Plasma growth factor production following MR-guided focused ultrasound and uterine artery embolization of uterine fibroids

Dr Mohamad Hamady
Department of Surgery and Cancer

Dr Stephen D. Quinn
Clinical Fellow Gynaecology

We would like you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One our team will go through the information sheet with you and answer any questions you might have. We would suggest this should take about 10 minutes. Talk to others about the study if you wish. Part 1 of this sheet tells you about the purpose of this study and what will happen if you take part. Part 2 gives you more detailed information about the conduct of this study. Please ask us if anything on this sheet is not clear.

Part 1

Name of the proposed procedure and trial:

Plasma growth factor production following MR-guided focused ultrasound and uterine artery embolization of uterine fibroids

What is the purpose of this study?

Fibroids are benign tumours affecting the uterus (womb). They are thought to affect between 25 and 50% of all women. Traditionally the usual treatment for fibroids causing symptoms was surgical, involving either removal of the fibroids (myomectomy) or hysterectomy (removal of the womb). Over the last 10 years we have developed non-surgical treatments for fibroids which avoid the need for surgery. Following these treatments we routinely perform a scan at 6 months to check the size of your fibroid and how your treatment has affected the blood supply. In many fibroids the blood supply continues to be significantly reduced, with an improvement in overall symptoms. Some fibroids however continue to grow despite treatment and their blood supplies may even return to normal. This study aim to measure chemicals in your blood known as growth factor following your treatments and compare their release to how your fibroid responds to the treatments. It is hoped that by understanding how these treatments affect growth factor production is your body, it may be possible to find new ways of improving these treatments in the future. This study will form part of the study into uterine fibroids carried out by Dr Stephen Quinn as part of his higher degree from Imperial College London.

Why have I been invited?

As part of your on-going treatment for your uterine fibroids you are due to undergo either uterine artery embolism (UAE) of your fibroids or Magnetic resonance-guided focused ultrasound (MRgFUS) treatment. This makes you eligible for our trial.

Do I have to take part?

No. It is entirely up to you to decide to join the study. We will describe the study in this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at and time,

without giving a reason. If you do decide to leave the study, or do not wish to participate at all, this **will not** affect the standard of care you receive at Imperial College NHS Healthcare Trust.

What will happen to me if I take part?

If you decide to take part in the study you will be asked to attend an extra screening visit prior to your treatment (between one and two weeks prior to your treatment).

If you take part in this study, you will be required to undergo blood tests before and at one, three and six months after your treatment. You will also be required to attend for three extra visits to the Hospital (St Mary's, Imperial College Healthcare NHS Trust) in order to have these blood tests taken.

Consenting and Screening Appointment

During the first study visit, the following procedures will take place:

- The study will be discussed with you. You will be given the opportunity to ask questions, and if you have had your questions answered to your satisfaction, you will be asked to sign this consent form and will be assigned a patient study number.
- A medical history will be obtained to determine your general health status and current symptoms.
- Your General Practitioner (GP) will be informed of your involvement in the trial and receive a letter from the investigators, including information regarding this study and copy of this information leaflet.

Expenses and payments

You will not be paid for participation in this clinical study.

What will I have to do?

You will be required to have a small blood sample (equivalent to one teaspoon (<5ml)) taken:

4. Before your treatment
5. one month following your treatment
6. three months following your treatment

7. six months following your treatment (at the time of your MRI follow-up scan)

What are the alternatives for diagnosis or treatment?

You do not have to be in this study to receive treatment for your condition. If you choose not to participate in this study, your standard medical care will not be affected in any way, and you will still receive the treatment that you normally would have received. You should talk to your doctor about each of your choices before you decide if you will take part in this study.

What are the possible disadvantages and risks of taking part?

Blood samples may be uncomfortable, and may lead to bruising of the arm.

What are the side effects of any treatment received when taking part?

The actual treatment you receive will not be affected by your involvement in this study. The risks of these treatments will be discussed with you before treatment.

What are the possible benefits of taking part?

It is hoped that by better understanding how these treatments for fibroids cause release of growth factors, we may be able to improve these treatments in the future.

What happens when the research study stops?

Following your treatment you will have a repeat MRI scan at 6 months to assess how your fibroid has responded to the treatment. This is standard practice for any patient undergoing MRI-guided focused ultrasound or UAE for their fibroids. If you require further treatment, or follow-up this will be arranged by your doctor at the fibroid clinic. Any further treatment will not be affected by your involvement in this trial. Your GP will be informed of your follow-up as per standard practice for NHS patients.

What if there is a problem?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal

action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Insert name and contact details). The normal National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.

Will my taking part in this study be kept confidential?

Yes. All information regarding this study will be kept in locked areas, with access only to the study coordinators. Once the trial has completed your details will be filed in your St Mary's NHS notes, subject to routine NHS confidentiality.

Involvement of the General Practitioner/Family doctor (GP)

If you agree to participate in the trial a letter will be sent to your GP, explaining the aims of the trial and the treatment you will receive. Upon completion of the trial, your GP will be contacted with your individual response to the treatment in the same way that would occur if you were having routine MRgFUS. This is important as your GP should have this information in order to correctly manage you fibroid symptoms in the future. If you experience any problems or complication during your treatment your GP will again be informed.

Are there reasons I might leave this study early?

Taking part in this study is your decision. You may decide to stop at any time. You should tell the study doctor if you decide to stop and you will be advised whether any additional tests may need to be done for your safety. In addition, the doctor may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

Will any biological sample(s) be stored and used for research in the future?

Yes, the samples taken will be stored until such time as the analysis can proceed. Following the analysis, samples will be destroyed. These samples may be used in a further study to examine metabolic changes experienced following treatment for fibroids. If your samples are used in this study, you will be contacted

and your consent will be requested. If you decided that you do not want your samples used in this study, these samples will be destroyed. Following the final analysis, samples will be destroyed.

You may be approached to take part in an additional study known as CFUS (Cytokine levels and pain following uterine artery embolization and MR-guided focused ultrasound treatment of fibroids.). This is a sister study, using the same samples but looking at a different type of chemical in your blood known as cytokine. If you are recruited to that study you will be given additional information. Your participation in that study will not affect your participation in this study.

What will happen to the results of the research study?

The results of this study will be written-up and presented locally and nationally. These results will also be submitted to the medical literature for publication.

Who is organising and funding the research?

This study is organised by Imperial College London clinical trials unit. The funding is provided by the company Celanova Biosciences Ltd.

CeloNova BioSciences of Europe B.V. – London
Pinewood,
Crockford Lane,
Chineham Business Park,
Basingstoke, R
G24 8AL,
United Kingdom
Tel.: +44.1256.698.010

Who has reviewed the study?

This study has been reviewed by a Nationally-appointed research and ethics group, in addition to local research and ethics office at St. Mary's Hospital.

What are my rights if I take part in this research study?

Your participation in this study is voluntary. You do not have to take part in this study, but if you do, you can stop at any time. Your medical care at this institution now or in the future will not be affected whether or not you take part in this study. You do not give up any of your rights by taking part in this study.

Who can answer my questions?

You are encouraged to ask questions about the study or your role as a subject at any time. If you have any questions about your participation in this study, if at any time you feel you have had a research-related injury, or if you have questions, concerns, or complaints about the research, contact:

Mr Stephen D. Quinn
Clinical Research Fellow
Ground Floor Mint Wing
St. Mary's Hospital
South Wharf Road
London
W2 1NY
Tel : 02078862325
E-mail : s.quinn@imperial.ac.uk

Dr Elizabeth Dick
Consultant Radiologist
St. Mary's Hospital,
London,
W2 1NY
Tel : 0207886666
E-mail : e.dick@imperial.ac.uk

Appendix K: Consent form for growth factor study

Imperial College Healthcare NHS Trust

Study Title	**Plasma growth factor production following MR-guided focused ultrasound and uterine artery embolization of uterine fibroids**		
Patient Identification Number for this trial:		**Site**	St. Mary's Hospital, London
Name of Research Doctor	Dr M. Hamady. Consultant Radiologist, St Mary's Hospital		

Please initial box if you agree with the following:

1. I confirm that I have read (or have had read to me) and understand the information sheet dated (Version 1.3 12/05/2011) for the above trial. I have had time to review this information, have had an opportunity to ask questions and had answers in terms I understand. ☐

2. I understand that taking part is completely voluntary and that I am free to stop at any time even without providing any reason and without my normal medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by responsible individuals or representatives of Imperial College Healthcare NHS trust or from regulatory authorities where it is relevant to my taking part in the research. The purpose of this is to check that the research is being carried out correctly. I am willing to allow access to my medical notes but understand that strict confidentiality will be maintained. ☐

4. I am willing for my anonymous research data, and the results arising from the trial, to be used as appropriate. ☐

5. I agree to take part in this research project. ☐

_____	_____	_____
Name of Research Participant	Signature	Date
_____	_____	_____
Name of Research Doctor	Signature	Date
_____	_____	_____
Name of person taking consent if different from Research Doctor	Signature	Date

1 copy for subject, 1 for Investigator Site File, 1 (original copy) for medical records

Appendix L: Trial documentation for growth factor study

Imperial College London

St Mary's Hospital, Imperial College London

GFFUS 31/05/2011

Recruitment details

Subject number:

Subject initials:

Subject date of birth:

Consent date:

Patient Information sheet:

Past medical history:

Past surgical history:

Current medications:

Use of hormones in last 12 months:

Menopausal status:

Subject selection

Inclusion criteria

1. Women due to undergo UAE or MRgFUS at St Mary's Hospital ☐
2. Willingness to have blood samples taken at intervals ☐

Exclusion criteria

1. Any clinically significant medical condition or system disease which may contribute to circulating cytokine levels. ☐
    a. Ischaemic heart disease ☐
    b. Acute or chronic infections
    c. Any form of on-going treatment for cancer ☐

d. Autoimmune disease (e.g. SLE, Rheumatoid arthritis) ☐

e. Any form of surgery 3 months prior to treatment ☐

f. Cerebral vascular accident ☐

2. Body mass index > 36; ☐

3. Being pregnant or lactating ☐

4. Participation in another trial of an investigational product or device within the previous 30 days; ☐

5. Unable or unwilling to comply with the requirements of the study, in the opinion of the investigator. ☐

Procedures and measurements

Blood samples will be collected by S Quinn (Clinical research fellow) and stored at the laboratory facilities in the Mint Wing until analysis.

Collection of plasma samples before treatment, and at intervals:

UAE patients (n=20):

Pre-treatment	Sample number(s):	
	SSS	
2 hours post treatment	Sample number(s):	
1 week post treatment	Sample number(s):	
	SSS:	
3 months post treatment	Sample number(s):	
	SSS	
6 months post treatment	Sample number(s):	
	SSS	

MRgFUS patients (n=20)

Pre-treatment	Sample number(s):	
	SSS:	
2 hours post treatment	Sample number(s):	
	SSS:	
1 week post treatment	Sample number(s):	
	SSS:	
1 month post treatment	Sample number(s):	
	SSS:	
3 months post treatment	Sample number(s):	
	SSS:	

6 months post treatment Sample number(s):

SSS:

Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g, at 2-4°C. Remove serum and assay immediately or aliquot and store samples at -20° C, at the storage facility in the Mint Wing laboratories. Duplicate samples will be used to reduce error. Laboratory facilities will be used at Imperial College London Medical School Labs.

Appendix M: MRgFUS subjects and VEGF levels

Subject number	Age	Uterine volume	%NPV	Signal Intensity	Baseline VEGF	VEGF Immediately	VEGF 2 hours	VEGF at 1 week	VEGF at 1 month	VEGF at 3 months
1	35	468.70	49.29	Hypo	241.39	275.48	-	-	63.84	53.00
2	44	547.9	60.25	Hypo	135.27	74.56	112.34	-	74.56	-
3	51	558.2	38.10	Hypo	131.32	-	244.64	-	127.34	75.62
4	43	669.4	45.03	Hyper	67.88	86.53	39.56	-	-	-
5	40	932.80	64.58	Hypo	263.49	298.24	-	-	150.55	69.12
7	28	348.50	-	Hypo	70.64	88.44	119.73	-		
9	34	887.60	64.64	Hyper	52.56	84.60	57.05	-	91.11	86.92
10	28	370.10	35.82	Hyper	117.15	179.80	130.10	156.12	89.19	160.27
11	36	531.50	78.63	Hypo	96.03	193.13	-	170.89	99.03	-
12	28	247.80	38.46	Hypo	299.48	180.86	84.15	-	-	-
13	36	591.10	19.45	Hyper	197.18	176.70	114.60	192.38	158.99	161.51
15	29	1557.00	43.56	Hypo	123.53	146.61	43.80	-	-	-
16	40	664.70	65.23	Hyper	323.16	192.89	311.23	-	-	-
19	42	766.60	81.79	Hypo	173.02	74.49	223.01	-	76.26	147.69
20	39	885.10	43.13	Hypo	51.23	27.35	69.55	75.55	-	41.56
21	45	649.10	99.58	Hypo	125.98	145.06	85.39	-	-	-
22	45	1025.60	67.56	Hypo	180.35	75.99	151.07	111.81	200.45	191.17
25	40	492.80	-	Hypo	124.35	67.72	38.90	52.04	-	-
26	43	680.50	24.19	Hypo	83.50	66.57	100.51	87.68	54.62	26.38
30	29	429.90	20.59	Hypo	208.75	122.34	-	-	-	-
38	47	715.80	47.53	Hypo	305.94	267.04	278.99	188.00	-	-

Subjects undergoing UAE and their subsequent VEGF levels

Subject number	Age	Uterine Volume	Signal intensity	Baseline VEGF	VEGF immediately	VEGF 2 hours	VEGF 24 hours	VEGF 1 week	VEGF 1 month	VEGF 3 months
6	40	817.70	Hypo	61.90	54.61	86.16	-	-	-	-
8	46	1374.80	Hypo	145.15	122.31	115.81	-	-	-	-
14	51	682.60	Hyper	93.03	108.91	88.64	73.73	-	-	-
17	42	985.90	Hypo	221.55	174.45	155.75	124.1	-	-	-
18	41	755.90	Hypo	49.91	35.69	-	-	132.89	85.66	126.96
23	38	741.60	Hypo	139.73	183.90	-	264.42	259.82	127.81	52.29
24	35	576.90	Hypo	81.92	66.00	-	-	-	-	89.71
27	38	2909.70	Hyper	244.80	322.95	-	245.98	-	-	-
28	42	506.00	Hyper	31.57	64.94	-	88.99	76.37	59.92	-
29	43	462.90	Hypo	70.31	82.73	45.94	40.29	62.25	27.11	-
31	45	808.00	Hyper	100.06	-	155.38	-	203.43	58.98	113.77
32	46	962.30	Hypo	49.18	-	-	40.09	51.10	-	204.21
33	39	596.50	Hyper	67.64	-	51.58	56.39	134.20	-	-
34	40	601.00	Hypo	71.89	33.30	-	15.20	146.66	-	-
35	41	1066.40	Hypo	68.63	-	-	79.56	160.04	-	-
36	45	809.30	Hyper	104.79	-	-	150.20	146.66	-	-
37	43	339.10	Hypo	94.24	-	-	181.76	129.83	-	-

Appendix N: Peer-Reviewed Publications from this Thesis

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Measurement of uterine fibroid volume: a comparative accuracy and validation of methods study

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ABSTRACT

Objective: A range of measurement techniques have been described which may be used to calculate uterine fibroid volume. A commonly-reported method involves application of a formula for the volume of an ellipsoid sphere to three orthogonal axes of a fibroid as measured on cross-sectional images. We aimed to compare this method and a second method, that of software-computed parallel planimetric uterine fibroid computation on MRI images, to a gold standard: the volume of objects measured by water displacement. We also compared these methods in volume estimation of patient fibroids using MRI data.
Study design: Mixed observational study and blinded cross-sectional analysis of imaging data.
Results: Large inter-observer variability was noted when using the ellipsoid formula method, which was also inaccurate when compared to the gold standard. Conversely, the parallel planimetric method showed excellent interobserver correlation and a high degree of correlation with gold standard volume measurements.
Conclusion: We conclude that the parallel planimetric method, although a more complex and time consuming technique, is the more accurate and therefore preferred method for measuring uterine fibroid volume.

1. Introduction

The size, shape and MRI signal intensity of uterine fibroids, the most common tumour affecting women, varies considerably. This makes measuring the volumes of these tumours particularly problematic. The size of fibroids and uteri has often been described by either their maximum diameter, or volume calculated by the ellipsoid formula or the parallel planimetric area method (Cavalieri method) [1–6]. The ellipsoid formula estimates the volume of an object by assuming an ellipsoid shape, whereas the parallel planimetric area method calculates a volume based on the sum of the multiple areas recorded on either MRI or computerized tomography (CT) images, multiplied by the thickness of each individual slice. An interpolation formula is then applied by software to smooth the boundaries of the object between slices, and at the peripheral margin of the object, a feature which more accurately simulates the true shape of the object. It is common for studies investigating treatment of fibroids to measure volume before and after treatment of fibroids, but if different measurement techniques are in use, it is important for radiologists and gynaecologists to understand how accurate and comparable these techniques are.

This study was therefore conducted in two parts. Firstly, we aimed to compare (a) the parallel planimetric area and (b) ellipsoid formula to (c) a gold standard (that of volume of water displaced by an object) to assess the relative accuracy of each method, using a variety of organic objects simulating potential tumour or uterine shape. We also concurrently assessed the inter-observer variability of these measurements as part of this evaluation. Secondly, we compared the inter-observer correlation of the parallel planimetric area method in estimating the volumes of a variety of randomly chosen fibroid uteri and non-perfused volumes after MR-guided focused ultrasound treatment.

2. Materials and methods

The volumes of 50 different, irregularly shaped objects were calculated using the water-displacement method. To reproduce

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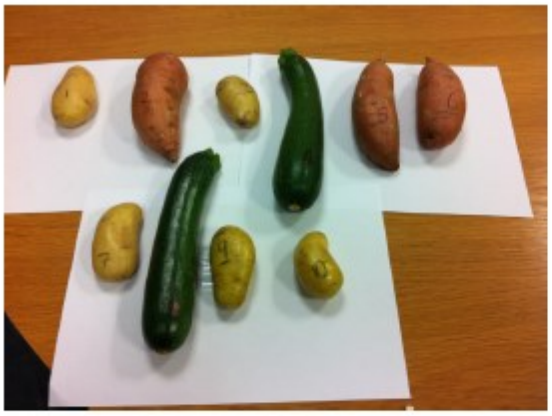

Fig. 1. Organic objects measured by volume displacement.

the organically irregular shape of fibroids, a variety of differently shaped fruit and vegetables were measured (potatoes, courgettes, plums, apples, and avocados) (Fig. 1). The volume of normal saline displaced by the objects was calculated using a 1000 ml plastic measuring cylinder with 5 ml gradations. Following this, all objects were scanned using a GE 1.5T MRI (GE Healthcare, Milwaukee, USA) and tri-planar T2 weighted images obtained. MRI was performed within 4 h of the volume displacement measurements.

The volume of an ellipsoid object (v) was calculated as: $v = 4/3\pi abc$ where a, b and c are the maximum diameters in three orthogonal planes. To calculate the volume using the ellipsoid method, the maximum diameter of each object in three separate planes was recorded for each object using T2 weighted images and the volume calculated using the formula above.

The MRI images were then uploaded into a software package for planimetric volume assessment, GE Reportcard©. Using this software the area of each slice was calculated by assigning multiple points on each sagittal slice around the perimeter of the object and a total object volume calculated automatically by the programme using the software's interpolation formulae (Fig. 2).

Following this initial assessment, the T2-weighted tri-planar MR images of 50 women with uterine fibroids, obtained at 1.5 T

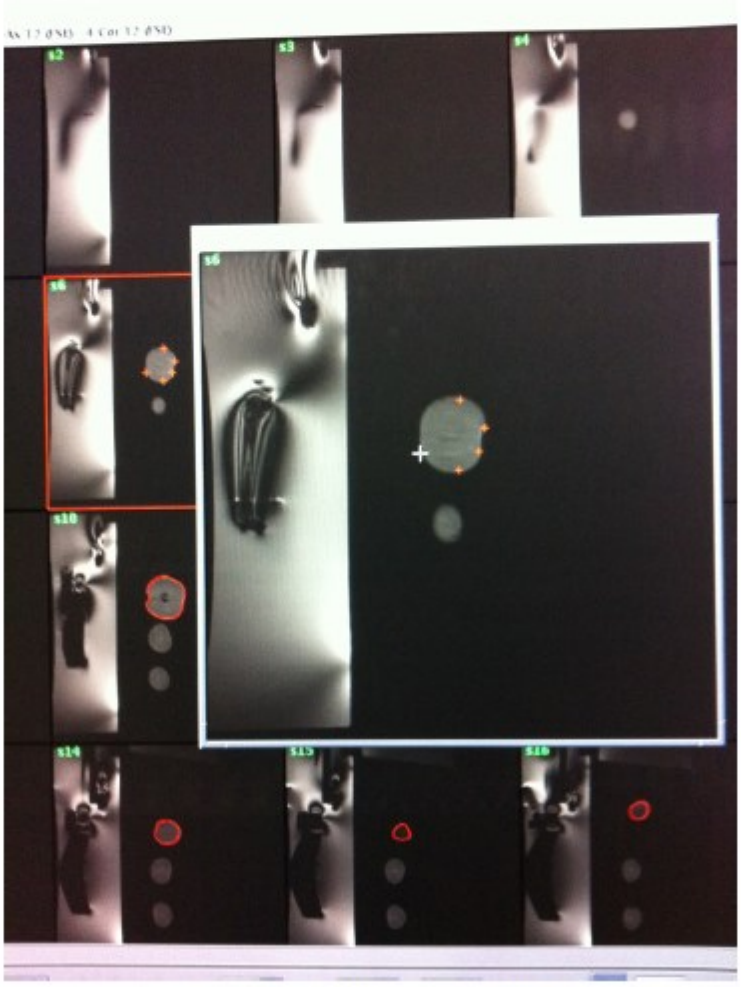

Fig. 2. Planimetric software trace outline of organic object.

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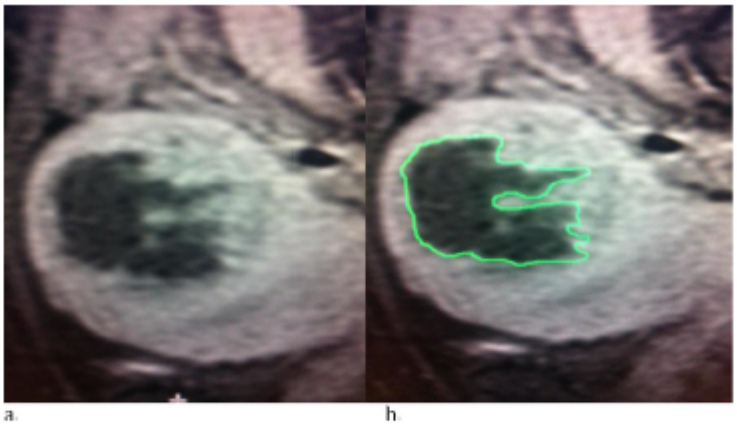

a. b.

Fig. 3. (a) Sagittal MRI image of uterine fibroid post focused ultrasound ablation treatment. (b) Outline of non perfused volume of fibroid (traced in green) using parallel planimetric method to determine non-perfused volume.

and undergoing treatment with magnetic resonance guided focused ultrasound ablation, were then examined by two independent investigators (SQ and JV) at different times. Each investigator was blinded to the other's results. Each observer used the parallel planimetric method to calculate the total uterine volume (TUV), volume of the largest fibroid (VolFib), and final non-perfused volume following administration of the gadolinium contrast agent post-procedure. The area of uterus, fibroid and NPV was marked on each sequential sagittal slice. The total volume was automatically calculated by the Reportcard© software, summation of the adjacent volumes and application of interpolation formulae between slices (Fig. 3).

2.1. Statistical analysis

The inter- and intra-observer agreement was calculated using the coefficient of variation. Statistical graphs and calculation were produced using MedCalc Software Version 12.1.0 – © 1993–2011. The degree of agreement between different methods of volume calculation was assessed according to the method of Bland and Altman [7,8], plotting the difference between each result vs. the mean of the two results. Pearson correlation coefficients were also used to compare measurements made by the two readers, and linear regression was used to obtain the individual slope and 95% confidence intervals (CI). A p-value of less than 0.05 was considered statistically significant. Data analysis was performed using commercially available software (SPSS 19, Chicago, IL, USA and GraphPad Prism 5.0, CA, USA).

3. Results

3.1. Comparison of volume of water displacement with the parallel planimetric method and ellipsoid method: organic objects

The range of volumes measured in the 50 objects (fruit and vegetable) group was 35–270 ml as measured by the volume displacement method. The correlation between observers recording the volume of water displaced by the objects was very favourable. The difference between the volumes calculated by two observers, a radiologist (JV) and a gynaecologist (SQ), was plotted against the mean of their two results. The volume calculated using the displacement method by JV (VolDisJV) and the volume calculated using the displacement method by SQ (VolDisSQ) is shown in Table 1. In all cases, the difference between the observed volumes fell between 1.96 standard deviations of the mean (0.0 ml). From this we concluded that there was no significant disagreement between investigators when using the volume displacement method.

The mean difference was 0.01 ml, with 95% confidence intervals (CI) of −0.6101 to 0.6301 and a standard deviation (SD) of 2.1818. The mean volume calculated by SQ and JV for each object was used as the gold standard by which we assessed the other methods of volume calculation.

We then compared this mean volume obtained using the water displacement method to the parallel planimetric method, and constructed a Bland–Altman plot. The mean difference between these groups was 2.63 ml, with 95% confidence intervals (CI) of 1.92 to 3.34 and a standard deviation (SD) of 2.58. Conversely, in comparing the mean volume of the water displacement method with the mean volume of the ellipsoid method a mean difference of −16.08 ml, with 95% CI of 9.97 to 22.19 and an SD of 22.05 was observed.

The range and mean of each method is reported in Table 2. In short, the planimetric method was, by a considerable margin, the

Table 1
Bland Altman plot of two observers (SQ and JV) measuring volume of displacement of a variety of organic objects. *X* axis: volume measured; *Y* axis: average difference of means.

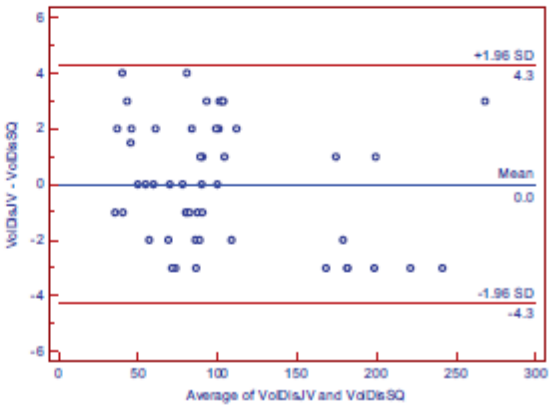

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Table 2
Measurements by SQ and JV by alternative methods.

	Uterine volume SQ	Uterine volume JV	Fibroid volume SQ	Fibroid volume JV	NPV SQ	NPV JV
Mean (ml)	726.60	725.67	314.76	319.76	138.87	140.98
Standard deviation	383.31	386.02	266.19	275.76	96.83	99.03
Range (ml)	216.20–1905.30	220.30–1894.00	25.20–1169.40	26.00–1201.00	7.40–529.70	10.00–524.30

method which showed, by both range and mean, the closest approximation to the 'gold standard' volume of water displaced.

3.2. Inter and intra observer variability assessment

We performed seven pairwise comparisons of method, firstly comparing the results between investigators for each method, and then for each observer between their volume calculations by the displacement, parallel planimetric, and ellipsoid methods respectively (Table 3).

There was very little variability between observers when measuring the volume of each object by the water displacement method as shown by Pearson correlation reported in Table 3. There was also very good correlation within each individual observer between the volume measured by water displacement and the volume as measured by the planimetric method. Very good inter-observer correlation using the planimetric method was observed. Conversely, the ellipsoid measurements resulted in markedly different volume calculations between observers. Further, those volumes obtained using the ellipsoid method were not at all well correlated with the 'true' volume as measured by water displacement.

3.3. Utility of parallel planimetric method in fibroid measurement

As we had found excellent correlation between observers in using the parallel planimetric method measuring organic objects, and excellent correlation of this method with the gold standard as calculated by volume of water displaced, we proceeded to utilize this method to calculate fibroid volume in 50 patients selected at random. The results of this second part of our study are given in Table 4. Pearson correlation was excellent between observers for uterine volume, fibroid volume and non-perfused volume. As expected, the standard deviation of bias was greatest in the uterine volume calculations where the range of values was the largest.

4. Comments

Whilst clinical symptomatology is the primary concern after fibroid therapy, accurate measurement of uterine fibroid volume is desirable for a variety of reasons. Accurate volume measurement is of importance in the assessment of fibroids being considered for uterine-sparing treatments such as focused ultrasound therapy or laparoscopic myomectomy and in the comparison of studies evaluating treatment options. Large fibroids may be considered a relative contra-indication for focused ultrasound therapy or laparoscopic myomectomy. Excessively rapid growth of a lesion may raise suspicion of more sinister non-leiomyoma pathology, or indicate earlier follow-up or intervention. The irregular margins of a fibroid following focused ultrasound therapy may make volume measurement based on a simple three-axis calculation not a true reflection of the complexity of the structure. Monitoring the efficacy of medical hormonal therapy also relies on accurate serial determination of fibroid and uterine volume.

Our results suggest that, although it is the quickest and most simple method available, there is wide inter-observer variability when using the ellipsoid method of volume calculation, and that, further, it often does not reflect the true volume of an irregular ovoid structure. This significant degree of inter-observer variability could lead to inaccurate assessment of fibroid volume, growth or

Table 3
Correlation between volume of displacement, parallel planimetric and ellipsoid methods between two observers (JV and SQ).

Comparison	Mean difference[a]	Pearson correlation	P value (two tailed)	Bland Altman analysis		
				Bias	SD of bias	95% limits of agreement
Volume displacement method JV vs. SQ	−0.05 ± 4.59	0.997	0.000	0.05	4.58	−8.94 to 9.04
Reportcard method JV vs. SQ	−0.28 ± 6.59	0.993	0.000	0.28	6.593	−13.20 to 12.64
Ellipsoid method JV vs. SQ	−0.4 ± 20.3	0.882	0.000	0.40	20.3	−40.19 to 39.39
Volume displacement vs. Reportcard						
SQ	2.52 ± 4.14	0.997	0.000	2.52	4.138	−5.59 to 10.63
JV	2.75 ± 3.14	0.998	0.000	2.75	3.145	−3.414 to 8.39
Volume displacement vs. ellipsoid method						
SQ	15.90 ± 28.75	0.879	0.000	15.91	28.73	−40.41 to 72.22
JV	16.25 ± 19.06	0.957	0.000	16.26	19.06	−21.10 to 53.61

[a] Data are means ± SD.

Table 4
Range, mean difference and correlation of uterine and fibroid volume measurement between two observers (SQ and JV) using parallel planimetric method.

Comparison	Range (ml) n = 50	Mean difference[a]	Pearson correlation	P value (two tailed)	Bland Altman analysis		
					Bias	SD of bias	95% limits of agreement
Uterine volume calculation JV vs. SQ	220.3–1905.3	0.93 ± 19.85	0.99	0.000	−0.92	19.85	−39.82 to 37.97
Fibroid volume calculation JV vs. SQ	25.2–1201.0	5.24 ± 16.45	0.998	0.000	5.24	16.46	−27.01 to 37.50
NPV JV vs. SQ	7.4–534.3	2.11 ± 9.01	0.996	0.000	2.11	9.01	15.56 to 19.77

[a] Data are means ± SD.

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regression, before or after therapy. This finding may be of particular relevance to fibroid volume calculations obtained using ultrasound images, which are generally performed using the ellipsoid method. On the basis of our results, we contend that the optimum method of pre- and post-treatment fibroid volume assessment is the planimetric method using T2-weighted MRI images.

The planimetric method has been validated in volume measurement of other structures and organs in the body, including measurement of the chambers of the heart and the prostate [9,10], and has been used to measure fetal, placental, prostate and uterine volume [1,11,12]. Validation studies have been performed using both ultrasound and MRI modalities.

A potential disadvantage of the planimetric technique is the amount of time required to calculate the volume of an object using this method. On each slice, multiple points outlining the perimeter of the object must be marked with callipers manually, until the entire perimeter has been circumnavigated and defined. This represented the most time-consuming part of the process; in our experience the planimetric method may take many times longer than the simple application of a prolate formula to three diameters in the *x*, *y* and *z* axes. In the future, software advances which automatically define the perimeter of an object may be of advantage in streamlining this process, and allow the radiologist or gynaecologist to perform the volumetric calculation in a fraction of the time currently required.

Our study was limited by several technical constraints which could have potentially introduced small, and we believe insignificant, sources of error. The interpolation algorithm of the software defines the outline of an object between two points which have been marked manually by the observer on the perimeter of the object. Depending on where these points are defined, and how many, we noticed that occasionally the automatically calculated trace of the outline of the object could be drawn deep to its surface, leading to a potential underestimation of its volume. Irregular edges or margins of certain objects would exacerbate this effect. We endeavoured to redraw any instances where this occurred. When measuring the volume of water displacement, we were also unable to confidently perform measurements to the nearest millilitre using the measuring flask, because the gradations on the side of the flask were at 5 ml intervals. Our measurements represented our best interpretation, taken at the horizontal limit of the meniscus of fluid, to the nearest millilitre. Such potential sources of error, we believe, are very minor when compared to the differences between two observers using the formula for an ellipsoid volume. Defining exactly which measurement points constitute a fibroid's largest dimensions, at which angles such measurement lines should be drawn and on which slices and planes, are all factors open to wide inter- and intra-observer variation and, we suggest, account for the large degree of variation between the two observers noted in our study.

5. Conclusion

Although relatively time-consuming and requiring dedicated software, we contend that the parallel planimetric method is likely to be more accurate in estimating uterine fibroid volume, and closely reflects the true volume of an object. Fibroid size following ultrasound ablation therapy or embolization is an often-quoted measure of a fibroid's response to treatment; accurate determination of size is therefore important in comparing studies quoting volumetric parameters. Following focused ultrasound ablation or uterine fibroid embolization, an important measurement is the non-perfused volume (NPV) of the fibroid tissue, which is often of an irregular shape. The NPV, in particular, is best suited to the parallel planimetric area method. A variety of measurement techniques have been reported in measuring fibroid growth and response to therapy; our results indicate that calculating the volume of uterine fibroids using the formula for an ellipsoid sphere is considerably less accurate and could introduce error into results. We therefore recommend the planimetric method using T2-weighted MRI images as the most accurate and consistent measurement technique for future studies investigating fibroid size.

Conflict of interest statement

No conflict of interest is declared.

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Quinn et al. Journal of Therapeutic Ultrasound 2013, 1:20
http://www.jtultrasound.com/content/1/1/20

JOURNAL OF THERAPEUTIC ULTRASOUND

RESEARCH ARTICLE Open Access

Safety and treatment volumes achieved following new developments of the magnetic resonance-guided focused ultrasound system in the treatment of uterine fibroids: a cohort study

Stephen Derek Quinn[1*], John Vedelago[2], Lesley Regan[1] and Wladyslaw M Gedroyc[2]

Abstract

Background: This research investigates whether modifications to the magnetic resonance-guided focused ultrasound ablation of uterine fibroid (MRgFUS) system used resulted in improved treatment volumes of uterine fibroids, while maintaining safety.

Methods: This study is a prospective cohort analysis of 34 women undergoing the ExAblate 2100 MRgFUS treatment for their uterine fibroids.

Results: The percentage of non-perfused volume (NPV) achieved with the ExAblate 2100 system was 54.92% compared with 50.49 % with the ExAblate 2000 system over the preceding year ($p = 0.543$). The ExAblate 2100 system resulted in a greater NPV in hyper-intense fibroids compared with the ExAblate 200 system (43.20% versus 36.33%, $p = 0.005$). There have been no recorded hospital admissions, no skins burns, and no reported major adverse events since the introduction of this new system.

Conclusion: Overall, the new system has thus far shown an encouraging safety record and an improvement in non-perfused volumes achieved, especially in hyper-intense fibroids.

Keywords: Uterine fibroids, Magnetic resonance-guided focused ultrasound, Leiomyoma

Background

Magnetic resonance-guided focused ultrasound (MRgFUS) treatment of uterine fibroids uses MR guidance to direct high-intensity focused ultrasound into a fibroid, resulting in coagulative necrosis within the tissue. This treatment was first performed in 2002 [1] and received approval from the US Food and Drug Administration in 2004. It is known that the signal intensity of the uterine fibroids (UF) at baseline T2-weighted MR imaging and the non-perfused volume (NPV) of the fibroid post-treatment are important determinants of outcome following MRgFUS [2]. Compared to other treatment options for uterine fibroids, focused ultrasound has been shown to carry a very low risk of complications and side effects [1]. At our institution, uterine fibroids have been treated using focused ultrasound since 2003. In January 2011, a new transducer and software system, the ExAblate 2100 (InSightec, Haifa, Israel), replaced the previous system in use, the ExAblate 2000 (InSightec).

The ExAblate 2100 system incorporates several software and hardware upgrades which were not components of the ExAblate 2000 system. This includes a new 3D axis ultrasound transducer, capable of rotation, tilt, and movement in the vertical plane to allow the transducer to be placed much closer to the patient's skin surface than formerly. The system also allows transducers to be shut off in banks which allows for sculpting and steering of the ultrasound beam. This affords the operator enhanced control in avoiding non-target structures and defining the location of energy dissipation posterior to the fibroid. As with the earlier system, focal spot size, location, and angle, in addition to the exact amount of energy deposited, can all be manually controlled using

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the 2100 system. In real time, MR thermal maps depict the precise location of treated tissue, providing the operator with a high degree of control throughout the procedure. In addition, the introduction of 'scar tape', an adhesive barrier that prevents build-up of heat within the scar tissue, reduces the risk of cutaneous thermal injury for treating patients who have an anterior abdominal wall scar [3]. This tape enables women with low transverse abdominal scars to be treated safely while avoiding the build-up of excess heat within the scar and subsequent burns. This tape was not available to us when we were treating patients with the ExAblate 2000 system; however, this could be used by other units using the earlier system.

The overall effectiveness of MRgFUS has been linked to the overall volume of fibroid tissue treated, as measured by the NPV [4]. The NPV refers to the volume of fibroid tissue identified as non-enhancing following administration of radio-opaque contrast agent.

We aimed to assess the effectiveness of this new technology by comparing the non-perfused volumes achieved with the newer technology to those obtained using the ExAblate 2000 system. We also aimed to compare the safety profile of the two systems.

Methods

This was a prospective cohort study of women undergoing treatment for uterine fibroids with the ExAblate 2100 MRgFUS system ($n = 34$). The results were compared with a data collected retrospectively from a cohort of women undergoing treatment using the ExAblate 2000 system prior to January 2011 ($n = 238$).

All patients underwent standard pelvic MRI including T1 and T2 images prior to treatment. All MRI images were reviewed by two independent investigators (SQ and JV). The fibroids were graded as hypo-intense or hyper-intense relative to myometrium and rectus abdominus skeletal muscle. The non-perfused volume following both systems was calculated using the same software package utilizing the planimetric method (Report Card Software, GE Healthcare, Milwaukee, USA) using the images from the sagittal T2 images (5-mm slices). Volume calculation between investigators was tested for agreement using the Bland-Altman method [5] and interclass correlation coefficient.

A range of patient demographic data was recorded, including body mass index, symptom severity score, parity, ethnicity, primary symptoms, and previous treatment. Prospective data was collected for those women undergoing the newer ExAblate 2100 system for 1 year post-treatment. All data regarding complications were recorded via review of patients' notes, interview with patients at follow up, telephone and written questionnaires, and direct observations of patients' post-procedures.

The statistical package SPSS version 20.0 (IBM, Armonk, NY, USA) was used to analyze differences between variables in both groups. Continuous data were tested for normal distribution by Shapiro-Wilk test. Normally distributed data were tested using Student's t test; where variables were not normally distributed, Mann–Whitney test was performed to investigate differences between these groups. For the categorical data of hyper-intensity, the differences between those undergoing the ExAblate 2100 and 2000 systems were tested using chi-square testing between these two groups.

The NPV achieved between the groups was investigated for the possible effect of signal intensity differences between groups using Mann–Whitney non-parametric testing. Analysis of covariance was used to adjust for confounding effect of different signal intensities of the fibroids treated. Differences in fibroid numbers and signal intensity between those undergoing the ExAblate 2100 and 2000 systems were examined using chi-square test. A p value less than 0.05 was taken to indicate a significant difference between groups.

Results

The MR images and treatment details from 238 women who had undergone treatment using the ExAblate 2000 system and 34 women undergoing treatment using the ExAblate 2100 system were examined. Mean follow-up from ExAblate 2000 patients was 3.81 years (SD 1.98). ExAblate 2100 patients were followed up over the first year post-treatment. The test for agreement of volume calculation between investigators using the Bland-Altman method [5] found a bias of 5.24 ml (SD 16.46) and interclass correlation coefficient of 0.998. This degree of agreement between the two investigators was judged to be acceptable.

The demographics for the patient cohort are described in Table 1, and pre-treatment clinical features for the two groups are compared in Table 2.

The percentage NPV achieved with the ExAblate 2100 system was 54.92% (SD 19.28) compared with 43.72% (SD 22.26) with the ExAblate 2000 system over the whole 9 years, a significant difference ($p = 0.007$). When signal intensity of the fibroids was corrected for differences, the %NPV between the two treatments remained statistically significant ($p = 0.012$). Of the women treated by the ExAblate 2100 system, 17.6% had fibroids found to be hyper-intense on T2-weighted MRI images, compared with 28.2% of those undergoing treatment with the ExAblate 2000 system ($p = 0.137$). The mean NPV achieved in women with hyper-intense fibroids using the ExAblate 2100 was 43.20% compared with 57.43% in those women with hypo-intense UF. This compares favorably with the 36.33% NPV achieved in hyper-intense UF treated with the ExAblate 2000 system.

Table 1 Demographics of subjects undergoing treatment with the ExAblate 2000 and 2100 systems

		ExAblate 2000 ($n = 238$)	ExAblate 2100 ($n = 34$)	p Value
Age	Mean years (range)	42.2 (25–84)	39.47 (25–52)	0.26[a]
BMI	Mean (SD)	25.1 (4.6)	23.41 (3.79)	0.038[a]
Race	White, *N* (%)	135 (48.0)	14 (41.18)	0.01[b]
	Black, *N* (%)	76 (27.0)	12 (35.30)	0.01[b]
	Asian, *N* (%)	18 (6.4)	8 (23.52)	0.01[b]
	Arabic, *N* (%)	7 (2.5)	0	0.01[b]
	Mixed, *N* (%)	4 (1.4)	0	0.01[b]
Parity	Mean (SD)	0.51 (0.89)	0.38 (0.82)	0.328[c]
Symptom severity score	Mean (SD)	62.64 (17.80)	61.71 (17.86)	0.841[c]
Hyper-intense fibroids	*N* (%)	67 (28.2)	6 (17.6)	0.196[b]
Previous treatment	*N* (%)	43 (15.4)	5 (13.9)	0.88[b]
Number of fibroids	Mean (SD)	6.43 (6.54)	4.18 (3.85)	0.044[c]
Mean uterine volume	ml (SD)	788.12 (408.01)	659.62 (259.91)	0.093[c]

BMI body mass index.
[a]Two-tailed t test.
[b]Chi-square test.
[c]Mann-Whitney test.

Three of the women treated with the ExAblate 2100 required a second treatment for their uterine fibroids in order to complete the treatment. These second treatments were done at 1, 2, and 7 months following the initial treatment and were deliberately planned as the volume of fibroid tissue to be treated could not be achieved in a single session.

The details of the ExAblate 2000 treatments by year since 2003 are detailed in Table 3. When compared to the NPVs initially achieved with the ExAblate 2000 system, the NPV increased and reached a statistically significant improvement by 2011 with the introduction of the ExAblate 2100 system ($p = 0.007$). When compared to the NPV achieved in recent years (2009 and 2010), the NPVs are greater with the ExAblate 2100 system; however, this did not reach statistical significance ($p = 0.543$).

Following the treatment of 34 women using the new ExAblate 2100 system, there have been no recorded hospital admissions, no skins burns, and no reported major adverse events. All women were discharged from the MRgFUS center within 3 h of their treatment, and only one woman required analgesia to take home (diclofenac orally for 3 days). There were no reported urinary tract infections and no persistent neurological problems or other complications. For patients treated with the ExAblate 2000 system, minor complications were experienced by 4% of patients and included urinary tract infection, urinary retention, PV bleeding, and transient buttock pain. One woman experienced PV fibroid expulsion, there was one skin burn requiring a small surgical intervention, and one case of a persistent neuropathy. No emergency hysterectomies were required following MRgFUS therapy in either group.

Discussion

Magnetic resonance-guided focused ultrasound ablation, through controlled deposition of high acoustic energy, causes thermally induced coagulative necrosis of leiomyoma cells. The procedure is performed using real-time MRI guidance, allowing the operator to precisely control the location, intensity, and size of the focus of energy deposited. Control with MRI also allows the operator to monitor the temperature of the tissue treated. This technique has been in use at our institution

Table 2 Comparison between ExAblate 2000 and 2100 treatments

	ExAblate 2000 system ($n = 238$)	ExAblate 2100 system ($n = 34$)	p Value
Mean volume of fibroids treated, ml (SD)	337.78 (252.75)	305.12 (206.28)	0.04[a]
Mean NPV, ml (SD)	165.41 (136.39)	162.39 (19.28)	0.65[a]
Mean percentage NPV (%)	43.72	54.92	0.007[a]
% NPV in hypo-intense fibroids	46.79	57.43	0.005
% NPV in hyper-intense fibroids	36.33	43.20	0.005[a]

NPV non-perfused volume.
[a]Mann-Whitney test.

Table 3 ExAblate treatments in 2003–2011

Year	Mean age, years (SD)	Mean BMI (SD)	Mean SSS (SD)	Mean uterine volume, ml (SD)	Mean total fibroid volume, ml (SD)	Mean %NPV (SD)	% HI dominant fibroid
2003	41.97 (5.44)	24.95 (4.05)	64.79 (17.47)	809.73 (445.48)	333.8 (209.75)	41.22 (25.66)	29.2
2004	43.87 (5.09)	24.11 (3.17)	62.1 (14.05)	734.20 (323.86)	387.0 (198.31)	47.33 (23.21)	29.0
2005	43.67 (8.93)	27.23 (4.14)	78.12 (16.24)	949.51 (383.33)	544.23 (276.52)	33.4 (20.34)	30.8
2006	42.74 (10.20)	25.00 (4.39)	64.1 (19.13)	958.1 (469.46)	495.1 (291.49)	39.4 (24.31)	26.9
2007	42.25 (5.93)	26.10 (5.55)	59.4 (16.93)	828.65 (446.98)	431.7 (289.64)	40.7 (22.04)	32.0
2008	43.15 (6.97)	23.75 (3.08)	64.1 (19.26)	861.90 (469.25)	445.1 (314.95)	44.54 (21.10)	36.4
2009	41.1 (6.00)	25.37 (5.97)	57.9 (19.04)	611.01 (249.98)***	314.3 (195.75)	51.53 (19.63)	25.92
2010	40.4 (7.67)	25.5 (4.00)	65.46 (19.18)	658.38 (296.37)***	319.2 (241.88)	50.49 (18.23)	13.78****[b]
2011 (ExAblate 2100)	39.47 (6.53)	23.41 (4.25)	61.02 (17.13)	655.06 (257.49)***	305.12 (203.43)	54.78 (19.01)****	17.1

BMI body mass index, *SSS* symptom severity score, *NPV* non-perfused volume, *HI* hyper-intensity of treated fibroid.
*$p = 0.004$.
**$p = 0.015$.
***$p = 0.005$.
****$p = 0.007$.
*****$p = 0.049$.
[a]Mann-Whitney test.
[b]Chi-square test.

since 2003 in the treatment of uterine fibroids; these data describe and reflect the evolution which has occurred in patient selection and non-perfused volumes since the introduction of the technology.

We have found an overall improvement in the NPV achieved since the introduction of MRgFUS, and the mean NPV achieved so far with the ExAblate 2100 system (54.92%) is the highest in our unit. However, when compared with the mean NPV achieved with the previous system the year before (50.49%), this was not found to be a significant overall improvement. It is possible that the year-on-year difference in NPV from 2003 to 2010 is a reflection of the increasing experience with the MRgFUS procedure and treatment apparatus, and that a similar curve of improvement aided by familiarity with the technology and better patient selection will continue to evolve. This increase in experience will inform future research directions in this area. Recent publications have reported a 54% NPV following prolonged experience with the ExAblate 2000 system [4]. A survey of MRgFUS operators found a target percentage NPV of 76% and a reported achieved NPV of 58% [6].

The year-on-year results do suggest that patient selection for MRgFUS has favored women with smaller uteri, smaller overall fibroid bulk, and a tendency to treat women with fewer hyper-intense fibroids. There is a known reduced efficacy of this treatment in patients with hyper-intense fibroids on T2-weighted MR imaging [2]. As such, a tendency towards treating single hypo-intense fibroids, rather than multiple fibroids or single hyper-intense fibroids, has become evident since the introduction of the technique in 2003, while patient demographic data remain largely unchanged between the two groups. The higher non-perfused volumes achieved with the earlier ExAblate 2000 technology in hypo-intense fibroids in recent years would seemingly support the rationale for this shift in patient selection. The initial experience with the ExAblate 2100 system suggests that we are able to achieve greater treatment volumes even in those women with hyper-intense fibroids.

The safety of the newer ExAblate 2100 system has proven to be encouraging, with no adverse events recorded at present. There were no complications experienced using the new system. In particular, there were no skin burns or evidence of neurologic injury, which were the two most significant complications seen (albeit very rarely) using the ExAblate 2000 system. It is perhaps worth noting that the complications experienced using either the ExAblate 2000 or ExAblate 2100 were, when compared to the reported complication profile of hysterectomy or myomectomy, relatively modest [7].

As with the introduction of any new technology, a learning curve aided by experience and familiarity with the system is to be reasonably expected. We would hope that once operators using the new ExAblate 2100 system gain more experience with this system, the trajectory of NPV improvement should show further positive improvement. A more evidence-based approach to patient selection, favoring patients with smaller, less numerous, hypo-intense fibroids may augment this anticipated process of improvement. Longer follow-up of these patients is required to determine if increases in non-perfused volume in these patients translate to higher patient satisfaction and a reduced re-intervention rate.

Conclusions

Overall, the new ExAblate 2100 system has thus far shown an encouraging safety record and an improvement in non-perfused volumes achieved, especially in hyper-intense fibroids.

Consent

All participants consented to be part of this study. Approval from the research ethics committee of St. Mary's Hospital was obtained for the women undergoing treatment and follow-up by the ExAblate 2000 system (00/EA/76E) and for the women undergoing treatment by the ExAblate 2100 system (10/H0724/29).

Abbreviations

MR: Magnetic resonance; MRgFUS: Magnetic resonance-guided focused ultrasound; MRI: Magnetic resonance image; NPV: Non-perfused volume; SD: Standard deviation.

Competing interests

This work was carried out while Stephen Quinn was working as the clinical research fellow at Imperial College London, and the funding for this post was provided by the company InSightec.

Authors' contributions

This paper was written by SQ, with collaboration with collection of data and statistics with JV under the direct supervision of WG and LR. All authors read and approved the final manuscript.

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