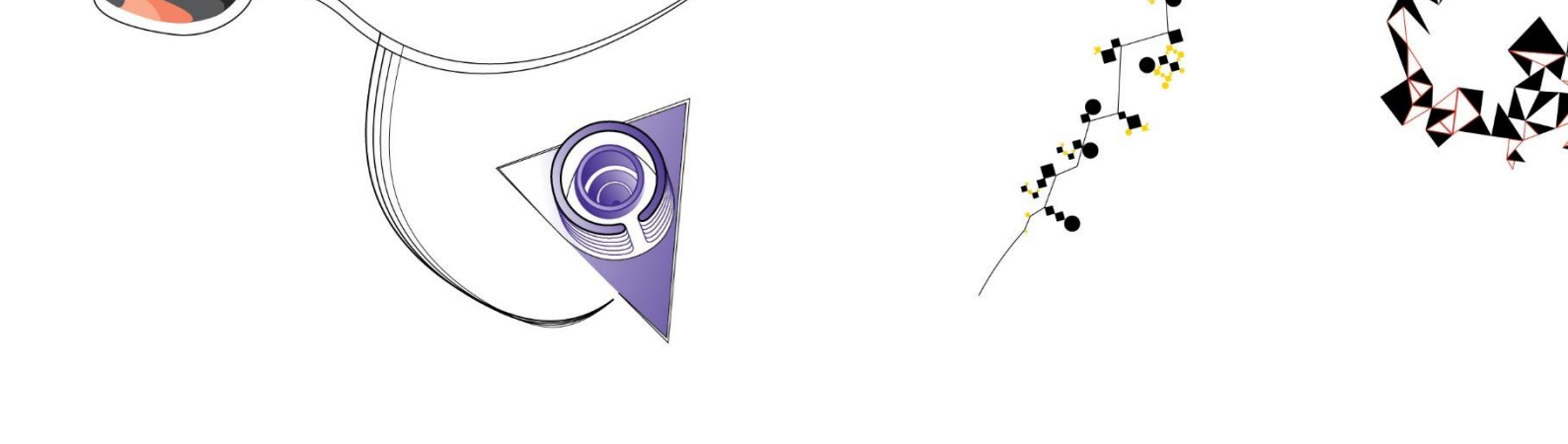
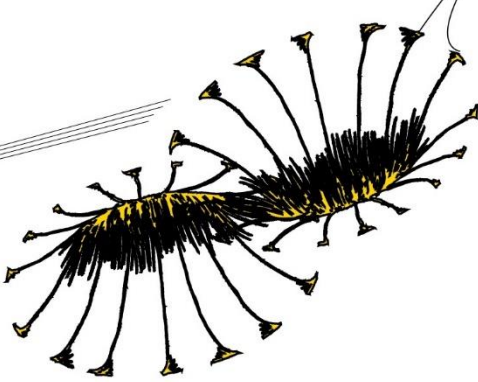




IN DEPTH ANALYSIS OF BIOLOGICAL TISSUE CHARACTERISTICS OF UTERINE FIBROIDS USING NEW MRI TECHNIQUES

Master Thesis
Technical Medicine
Medical Imaging & Interventions



Inge Slouwerhof
February 2016
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Aim and outline of the thesis

Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU) is a promising minimal-invasive therapy for the treatment of uterine fibroids, a frequent appearing benign gynaecological tumor.

The first aim of this thesis is design, clinically implement and study new multi-parametric MRI techniques for characterization of uterine fibroids and compare them with the traditional MRI screening parameters in an exploratory study. Knowledge about MRI-based characterization parameters contributes to an optimization of patient selection, treatment planning and treatment outcomes of the MR-HIFU treatment for uterine fibroids. Also, identification and characterization of the patient population in the Isala suffering from uterine fibroid-related symptoms is performed. This outline of the patient population in the MASS I study (Myoma Screening Study) will contribute to the preparation and implementation of the HIFU treatment in the clinical gynaecological treatment spectrum. Finally, clinical feasibility can be grounded by means of this study.

Second part of this thesis is the design of a subsequent study treating patients with MR-HIFU. This study (MASS II) is a continuation of the MASS I study and will study the prognostic value of the new MRI parameters developed in the MASS I study in treatment effectiveness, planning and treatment outcome.

In **chapter 3** of this thesis a general introduction is given about the background and rationale of the conducted study.

Chapter 4 presents the design and results of the MASS I study.

The design of the subsequent MASS II study is elaborated in **chapter 5**.

In **chapter 6** the overall conclusions are given with future perspectives.

The thesis will end with the references and some Appendices.

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1 English Summary

Uterine fibroids are the most common gynaecological tumors affecting a high percentage of women. Symptoms occurring from the fibroids are abnormal bleeding, pelvic discomfort and reproductive dysfunction. The treatment possibilities vary from drug therapy to total surgical removal of the uterus. Recently, the minimal-invasive, Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU) is added as a treatment option. This technique combines the anatomical and functional imaging of MRI with the thermal ablation possibilities of high intensity focused ultrasound. Patient selection, based on screening MRIs, before MR-HIFU treatment is an essential step towards obtaining good treatment results.

Currently, the Funaki classification is used as the primary MRI classification parameter for determining patient suitability by dividing the patient population in three groups. From literature we have seen treatment results vary among patients. We hypothesize that visualizing fibroid characteristics with new MRI sequences and image biomarkers contributes to a better patient selection and therefore better treatment results. The goal of this study is to implement a novel and extensive MRI protocol in the diagnosis of uterine fibroids in the current practice of the Isala Clinics.

The implemented MRI protocol consists of the conventional T_2 and T_1 weighted sequences. Also a contrast enhanced T_1 image is acquired. Besides these conventional sequences, we implemented T_2 relaxometry for the measurement of the T_2 relaxation time, a diffusion weighted imaging (DWI) sequence and a DWI sequence with a long echo time. Also, a clinical questionnaire is obtained in these patients.

We performed the MRI scans on 10 patients. For all patients, the whole MRI protocol could be executed. Funaki classifications are determined: two 2 patients fell in Funaki classification 1, seven patients fell in classification 2 and one patient fell in classification 3. All 10 patients completed the symptom severity questionnaire. The mean symptom severity score was 37.1 ± 20.3 . We found that measurements of the T_2 relaxometry maps and ADC maps can be performed in all patients by means of a Matlab developed software. The different functional imaging parameters show a difference between normal myometrium tissue and uterine fibroid tissue. Because a variety is seen in fibroid volume, symptom severity score and the values of the different imaging parameters among patients underline our hypothesis: we find a wide variety and heterogeneity among patients suffering from uterine fibroids.

The initiation and first results of this study show a promising start to the implementation of the HIFU treatment in a non-academic high-volume hospital. We developed an extensive MRI protocol, including functional imaging sequences producing non-invasive biomarkers for the characterization of uterine fibroid tissue. We also developed a Matlab code for the analysis of the MRI parameters. The study will be continued until 80 patients are included allowing an enlargement of the analysis.

We established the next step in the line of research in a new research protocol (MASS 2 study). By continuing to apply the extensive MRI protocol before and after MR-HIFU treatments, we are looking for imaging-based predictors of treatment success.

2 Nederlandse Samenvatting

Uterus myomen zijn de meest voorkomende goedaardige gynaecologische tumoren, voorkomend bij een hoog percentage vrouwen. Symptomen die optreden zijn abnormale bloedingen, ongemak in de onderbuik en voortplantingsdisfunctie. The behandel mogelijkheden variëren van medicamenteuze therapie tot een totale operatieve baarmoeder verwijdering. Recent, de minimaal-invasieve behandeling Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU) is toegevoegd aan het behandel spectrum. Deze techniek combineert de anatomische en functionele beeldvorming van MRI met de thermale ablatie mogelijkheid van de gefocusseerde ultrageluid met hoge intensiteit. Patiënt selectie, gebaseerd op de screening MRI, voorafgaand aan een MR-HIFU behandeling is een essentiële stap voorwaarts in het verkrijgen van klinisch goede behandelresultaten.

In de huidige praktijk, de Funaki classificatie wordt gebruikt als de primaire MRI classificatie parameter voor het bepalen van patiënt geschiktheid, waarbij patiënten in 3 groepen worden ingedeeld. Er wordt gezien dat de behandelresultaten verschillend tussen patiënten. Wij veronderstellen dat het in beeld brengen van myoomkarakteristieken met MRI, bijdraagt aan de patiënt selectie en behandelresultaten. Het doel van deze studie is het implementeren van een uitgebreid MRI protocol in de diagnose van uterus myomen in de huidige klinische praktijk van de Isala. Mogelijkheden en onmogelijkheden worden bestudeerd.

Het geïmplementeerde MRI protocol bestaat uit de conventionele T_2 en T_1 gewogen sequenties. Ook worden contrast gewogen T_1 sequenties verkregen. Naast deze sequenties, implementeren we T_2 relaxometrie en verschillende diffusie gewogen sequenties. Daarbij wordt een klinische vragenlijst afgenomen.

Een MRI volgens het nieuwe protocol is gemaakt in 10 patiëntes. Bij alle patiëntes kon het gehele protocol worden uitgevoerd. De Funaki classificaties zijn als volgt: twee patiëntes vielen in Funaki classificatie 1, zeven patiëntes in classificatie 2 en één patiënte viel in Funaki classificatie 3. Alle 10 patiëntes hebben de symptoom vragenlijst ingevuld. De gemiddelde 'symptom severity score' was 37.1 ± 20.3 . Metingen in the T_2 relaxometrie maps en de ADC maps konden worden uitgevoerd in alle patiënten door middel van Matlab software. De verschillende functionele beeldvormende parameters laten een verschil zien tussen normaal myometrium weefsel en myoom weefsel. Een variëteit wordt gezien in myoom volume, symptoom hevigheid en de waardes van verschillende MRI parameters tussen patiëntes. Dit onderstreept onze hypothese: we vinden een hoge variëteit en heterogeniteit in patiëntes met gediagnosticeerde uterus myomen.

De initiatie en eerste resultaten van deze studie laten een veelbelovende start zien voor de implementatie van de MR-HIFU behandeling in een niet-academisch hoog-volume centrum. We hebben een uitgebreid MRI ontwikkeld, met daarin functionele sequenties voor non-invasieve biomarkers te gebruiken bij de karakterisatie. Ook hebben we een Matlab code ontwikkeld voor de analyse van de MRI parameters. Deze studie zal worden voortgezet tot er 80 patiëntes zijn geïnccludeerd, wat zal leiden tot een vergroting van de analyse.

We maakten de volgende stap in deze onderzoekslijn door het opstellen van een nieuw onderzoeksprotocol (MASS 2 studie). Door het blijven toepassen van het uitgebreide MRI protocol voor en na MR-HIFU behandelingen, gaan we op zoek naar voorspellers van goede behandelresultaten met behulp van MRI.

3 General Introduction

3.1 Uterine Fibroids

Leiomyomata, also called uterine fibroids, are the most common benign gynaecological tumors affecting a high percentage of women. The life-time prevalence of fibroids lies between 70 to 80%. Uterine fibroids are benign hormone-sensitive tumors arising from smooth-muscle cells of the uterus. The fibroids may occur in different locations in the uterus. The classification of this location is of important interest when choosing therapy. Intramural fibroids are situated within the uterine wall, while subserosal fibroids are developed on the outer uterine wall. Submucosal uterine fibroids often intrude the cavum uteri. The international federation of gynaecology and obstetrics (FIGO) uses the following sub classification system: submucosal fibroids (FIGO type 0, 1, 2), intramural fibroids (FIGO type 3, 4), subserosal fibroids (FIGO types 5, 6, 7) and other (FIGO type 8).

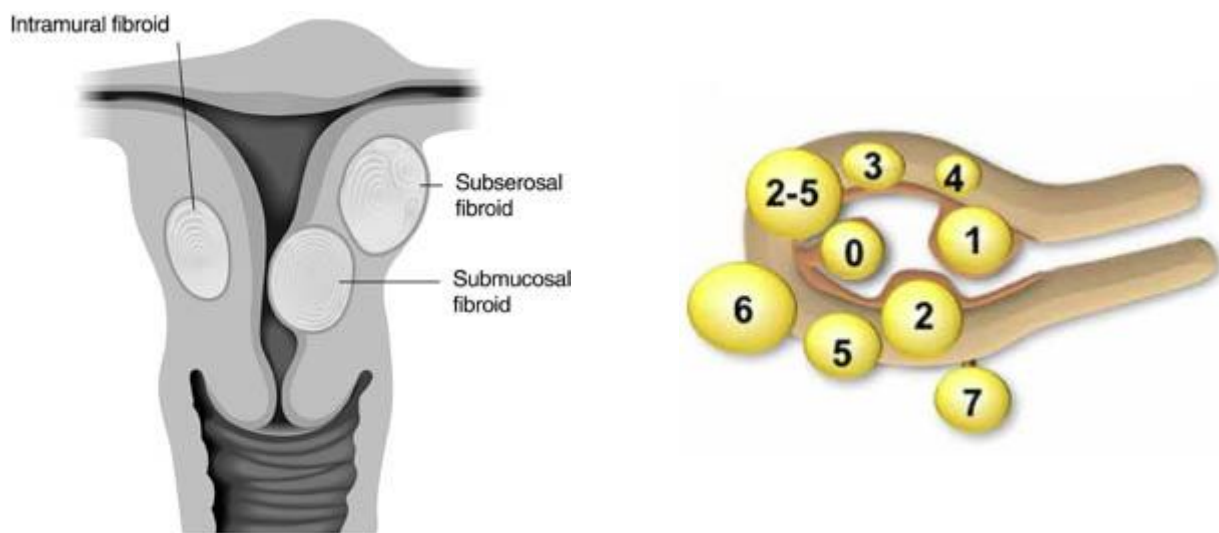


Figure 1: Different types of uterine fibroids and Leiomyoma Sub classification System

The majority of women with uterine fibroids is asymptomatic. However, 20-50% of the patients show symptoms¹, such as abnormal uterine bleeding, pelvic discomfort due to the existing mass and reproductive dysfunction. All these symptoms are leading to a significant negative effect on the quality of life. The above described locations of uterine fibroids are an important determining factor in the symptom occurrence. Submucosal fibroids often show uterine bleeding and reproductive dysfunction, while subserosal fibroids tend to lead to pelvic discomfort. Treatment possibilities include drug therapy, hysterectomy, myomectomy, uterine artery embolization and magnetic resonance guided “High Intensity Focused Ultrasound” (MR-HIFU). The choice of treatment and rate of success is dependent on the morphology and tissue characterization of the uterine fibroids². Hysterectomy generally was the choice of treatment in patients with severe symptoms. However, this treatment has a great impact on the patient’s life. The hormonal status is changed, childbearing is eliminated and treatment complications risks are significant. Therefore, uterus preserving strategies are becoming more and more popular nowadays. In particular minimal invasive treatments such as uterine artery embolization and MR-HIFU.

3.1.1 Histology of Uterine Fibroids

Uterine fibroids are characterized by the proliferation of smooth muscle cells in the myometrium and an exaggerated deposition of collagenous extracellular matrix^{3,4}. From literature it is known that the histopathology of uterine fibroids varies among patients⁴. Based on the histopathology, multiple subtypes of uterine fibroids are recognized. All uterine fibroids are composed of smooth muscle fiber bundles, surrounded by collagenous fibrous connective tissue within which are blood vessels. Also variable numbers of mast cells are present. The density of each of these cell types is different for the various types of uterine fibroids⁴. Large leiomyomata can degenerate due to an outgrowth on their blood supply. Degeneration can manifest in hyaline, myxoid, cystic and red degeneration. After necrosis due to degeneration of the tissue, calcifications tend to develop¹.

Histological and biological characterization of fibroid tissue is useful knowledge in the choice and suitability of treatment options^{2,4}. For example, the treatment effects of drug therapy are lower in patients with a high proportion of collagenous tissue because of its unresponsiveness⁴. Also in MR-HIFU treatment, tissue characteristics are an important predictor of success and therefore are included in the regular inclusion guidelines.

3.2 MR guided High Intensity Focused Ultrasound treatment

A recently introduced minimal invasive treatment for uterine fibroids is MR guided High Intensity Focused Ultrasound (MR-HIFU). This form of therapy makes use of the imaging capabilities of MRI combined with high intensity focused ultrasound to treat the fibrous tissue.

3.2.1 Basic principles

MR guided High Intensity Focused Ultrasound (MR-HIFU) uses ultrasound as a therapeutic application to thermally ablate tissues. An array of piezoelectric ultrasound transducers, integrated in the MRI table, generates a converging beam of ultrasound. Ultrasound is a mechanical pressure wave with a frequency higher than the audible limit. Focusing this ultrasound beam will lead to high levels of acoustic energy in a focal spot. The acoustic energy is converted to heat in the tissue. Focusing of the ultrasound bundle makes it possible to deposit the heat in a controllable and precise manner in the focal spot, while the surrounding tissue is minimally heated. Heating caused by high intensity ultrasound causes a thermal effect, coagulative necrosis through denaturation of cellular proteins, in the tissue at the focal spot.

The use of ultrasound together with MRI eliminates the use of any ionizing radiation, and therefore can be repeated as needed. The use of ultrasound also entails some disadvantages. Ultrasound propagates with difficulty through gas. Also, it attenuates very quickly in bone. These restrictions limit the biological targets possible to treat. In figure 2 a schematic illustration of a MR-HIFU setup is shown. Patients are positioned in a prone position on the MRI table. Because the ultrasound beam cannot propagate properly through air, a coupling gel pad is used between the skin of the patient and the transducer to provide adequate direct contact.

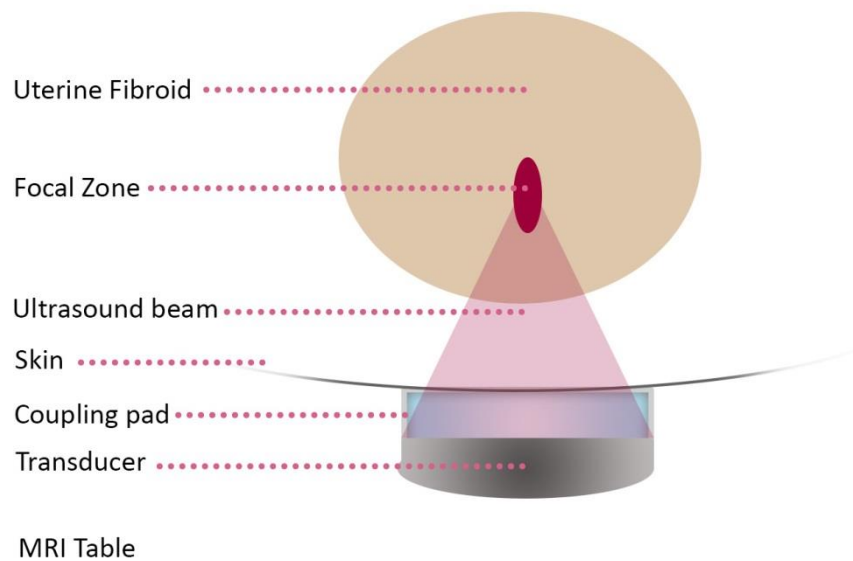


Figure 2: Schematic illustration of a MR-HIFU system

MRI is of great importance in MR-HIFU treatments. Not only is MRI used in patient selection and treatment planning, also real-time temperature mapping and post-treatment success conformation are important MRI features during and after the procedure.

3.2.2 Patient selection

Several clinical studies show that MR-HIFU is an effective and safe treatment for symptomatic uterine fibroids⁵. However, not all patients are suitable candidates for the HIFU procedure and distinguishing between suitable and unsuitable patients for HIFU treatment is an ongoing challenge.

The first screening round for potential candidates takes place at the gynaecologists office. By means of anamnesis and vaginal ultrasonography, an initial screening is performed. Potential candidates for HIFU treatment are then screened with a pelvic MRI protocol. The most important factors which are examined are the location of the uterine fibroid, the interposition of bowel or ovaries in the ultrasound pathway and the position of the fibroid in relation to nearby anatomical structures⁶. Extensive research about the correlation between T_2 signal intensity and treatment results show that hypervascular fibroids with a high T_2 signal intensity are difficult to treat and therefore are generally excluded for MR-HIFU treatment⁷.

3.2.3 Treatment effects

Treatment effects of a MR-HIFU treatment for uterine fibroids can be measured in different manners and there is no uniform consent about treatment effect parameters. The non-perfused volume (NPV) ratio and the Uterine Fibroid Symptom and Health related Quality of Life questionnaire (UFS-QoL) score improvement are the most frequently used parameters. From literature it is known that the area of the NPV within the treated uterine fibroid corresponds to the volume of necrotic tissue⁸. The NPV ratio (ratio between the baseline fibroid volume and the NPV post-treatment) is a predictor for fibroid volume reduction as well as symptom relief after a short follow-up^{9,10}. Because symptoms are the most important factor to treat uterine fibroids, symptom reduction is the second important treatment effect parameter. The symptom score is measured with the standardized, disease specific UFS-QoL questionnaire. The symptom scores are measured in 6 fields (concern, activities,

energy/mood, control, self-consciousness and sexual function) and transformed into a 0 to 100-point scale¹¹. A reduction of 10 points after follow-up is normally seen as a significant symptom improvement.

Despite extensive patient selection as described in section 4.2.2, treatment results are very diverse as shown in the systematic review by Gizzo et al.⁵. The NPV ratio varies between 16.3% and 98%. A NPV ratio of >50% was achieved in 60% of the studies. UFS-QoL improvement varies between 15 and 66 points (mean = 31). There already has been some research on predictors of success of MR-HIFU treatment. As earlier described, the signal intensity of the fibroid on T₂ weighted images is the main parameter of characterization. However, this parameter may be too limited as a predictor of treatment outcome. Yoon et al. show a case report of a successfully treated hyper-intense type 3 uterine fibroid⁶. This suggests that additional screening parameters are required to make better defined subpopulations of patients potentially suitable for MR-HIFU treatment. Knowledge of MRI-based predictors of success prior to treatment may contribute to an optimization of patient selection and therefore treatment outcomes.

3.3 Diagnostic imaging of uterine fibroids in current practice

In current practice, uterine fibroids are diagnosed based on anamnesis, physical examination and trans-vaginal ultrasonography at the gynecologist's office. Women complaining about abnormal bleeding and pelvic discomfort will always be examined for uterine fibroids. In addition, uterine fibroids can be found incidentally on computed tomography (CT) scans performed for other reasons.

The trans-vaginal ultrasound (TVA) gives rudimentary information about the size and the location of the uterine fibroid(s). Also calcifications inside of the fibroid can be identified. However, it does not give information about any underlying disease or all the existing fibroids. Generally, uterine fibroids appear on the TVA as solid masses with a similar echogenicity as the surrounding myometrium, although they can be hypo-echoic. The fibroids can cause acoustic shadowing, even if they are not calcified. Degeneration on TVA can be identified as areas with cystic change or calcifications.

In current practice, when the origin of the pelvic mass is doubted, an additional examination using Magnetic Resonance Imaging (MRI) should be performed.

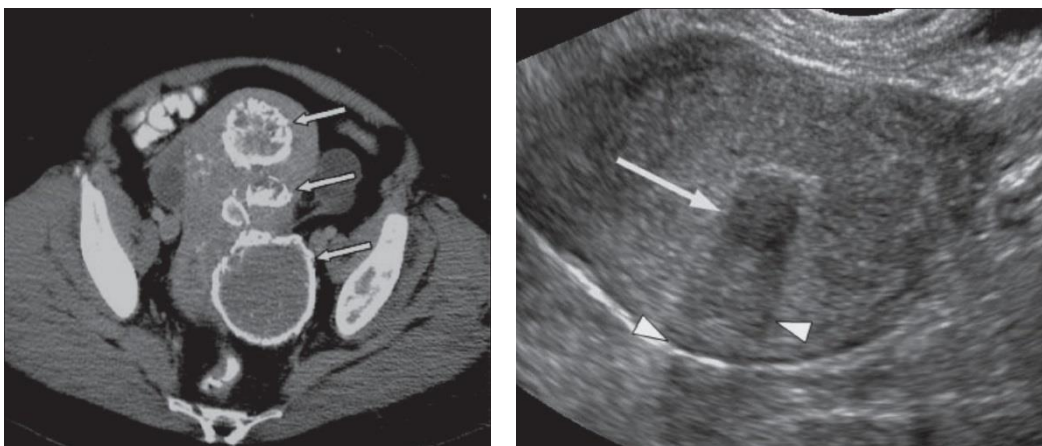


Figure 3: Left: example of an incidentally found uterine fibroid with calcifications on CT. Right: example of an TVA showing an 1.1 cm submucosal uterine fibroid.

3.4 Magnetic Resonance Imaging for Uterine Fibroids

Modern minimal-invasive treatments of uterine fibroids make an exact evaluation of the uterine fibroid size, number, location and tissue characteristics a relevant issue. In current practice, with only a transvaginal ultrasonography (TVS) as an imaging method, it is not possible to accurately characterize these characteristics before uterine fibroid treatment. Magnetic Resonance Imaging (MRI) is more sensitive in identifying uterine fibroids than ultrasonography¹² and has a high diagnostic performance in leiomyomata imaging. Therefore, is not only used when there is doubt about the origin of the pelvic mass, but also as an additional uterine fibroid characterization tool.

3.4.1 Current MRI classification: Funaki Classification

Current imaging-based biological characterizations of uterine fibroids are limited. The signal intensity on T₂-weighted MRI images is the main parameter of characterization. This parameter is described by the Funaki classification, defined by Funaki et al. in 2007⁷. In the Funaki classification, patients are stratified in 3 groups based on T₂ signal intensity in selection of MR-HIFU treatment. The mean T₂ signal intensity of the fibroid is compared to the signal intensity of skeletal muscle and abdominal fat. Details of the Funaki classification are listed in appendix I. In figure 4, an example of the Funaki classifications is shown. This subdivision of uterine fibroids is correlated with pathohistologic structures, demonstrated by Zhao et al. in 2014¹³. The Funaki classification is proven to be a predictor of treatment outcome.

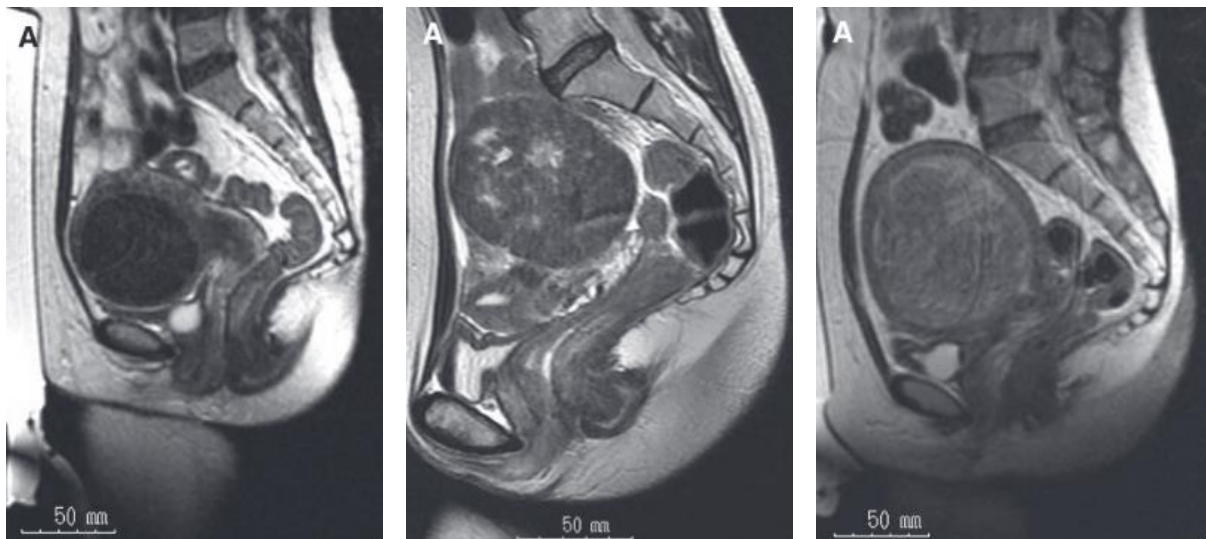


Figure 4: Left: Funaki classification 1; Middle: Funaki classification 2; Right: Funaki classification 3. T₂ signal intensity compared to skeletal muscle and subcutaneous fat is low in Funaki classification 1 and high in Funaki classification 2.

However, we think the Funaki classification alone is a limited parameter. The whole patient population is divided in only three groups. Fibroids classified as Funaki classification 1 are good candidates for HIFU treatment, as the vascularity is relatively low. A high signal intensity comparing to skeletal muscle is seen in Funaki classification 3. This could represent a high vascularity, which is a disadvantage for HIFU treatment, because the deposited heat is rapidly distributed and drained, resulting in poor treatment outcomes^{7,1414}. Most fibroids are characterized as Funaki classification 2. However, treated fibroids in this classification demonstrate varying treatment results.

3.4.2 MR characteristics of uterine fibroids

Because we think the Funaki classification is too limited for the characterization of uterine fibroids, other MRI parameters are discovered. Recently, new multi-parametric MRI parameters are developed by Philips Healthcare (Eindhoven) that can be used as non-invasive biomarkers to

characterize fibroid tissue. This set of new MRI parameters can be used to visualize and gain knowledge about the biological variation and characterization of uterine fibroids. This could be important in the choice of the most appropriate minimal invasive therapy. The MRI parameters potentially able to describe useful tissue characteristics are Apparent Diffusion Coefficient maps (ADC maps) by means of diffusion weighted imaging and T2 maps. Below, the choice of the different parameters is explained further.

3.4.2.1 Diffusion Weighted Imaging

Diffusion weighted imaging (DWI) provides information about the movement of water within a tissue. The image contrast is resolved by micro-diffusion of water protons in intracellular and extracellular environments. Changes in diffusion of water result in an altered signal intensity.

MR-DWI produces a quantitative MRI parameter; the apparent diffusion coefficient (ADC, which is a quantitative measure of the water movement (diffusion) within a tissue. The ADC value gives an average value of the flow and the distance a water molecule is moved. ADC maps are created by acquiring diffusion weighted images with different diffusion weightings (i.e. different b-values). To calculate the b-value (s/mm²), the following equation is used:

$$b = \gamma^2 \times G^2 \times \delta^2 \times (\Delta - \delta/3)$$

In which γ is the gyromagnetic ratio, G the magnitude of the gradient, δ the duration of the gradient and Δ the interval between the gradients. In figure 5, an example of a uterine fibroid imaged with different diffusion weightings (i.e. b-values) is shown. The ADC is the natural logarithm of the ratio of the signal intensities of two or more images acquired with different b-values, expressed in mm²/s. The equation is:

$$S_b = S_0 \times e^{-b \times ADC}$$

$$ADC = \frac{\ln(S_b/S_0)}{b_0 - b_b}$$

In which S_0 is the signal intensity at b-value b_0 ($b_0 = 0$ s/mm²), S_b the signal intensity at b-value b_b . The higher the ADC value, the more signal loss and the higher the diffusion. Because ADC maps are a quantitative measure, they can be used to compare uterine fibroids in different patients. The goal is to find different patient groups, established from ADC map measures, which potentially can lead to a more specific patient selection for MR-HIFU. In Appendix II, a more extensive theoretical background of diffusion-weighted images, b-values and ADC maps is given.

As stated in section 3.2.1, MR HIFU treatment uses thermal coagulation to treat the uterine fibroid. Due to this thermal coagulation tissue changes occur, including protein denaturation, rupture of the cell membrane, vasoconstriction and cauterization of the blood vessels. These tissue changes will lead to a change in the ADC value compared to the uterine fibroid tissue before treatment. Therefore it can potentially be used as a measure to visualize treatment effects without the use of a contrast agent. As shown in the article of Ikink et al., ADC maps contain information about the diffusion as well as the perfusion in the uterine fibroid⁸. By varying the b-values, Ikink et al. showed a strong post-treatment correlation between the ADC map with low b-values and the non-perfused volume (NPV). This finding implies that low b-values are associated with the perfusion, i.e. the micro-circulation of the fibroid.

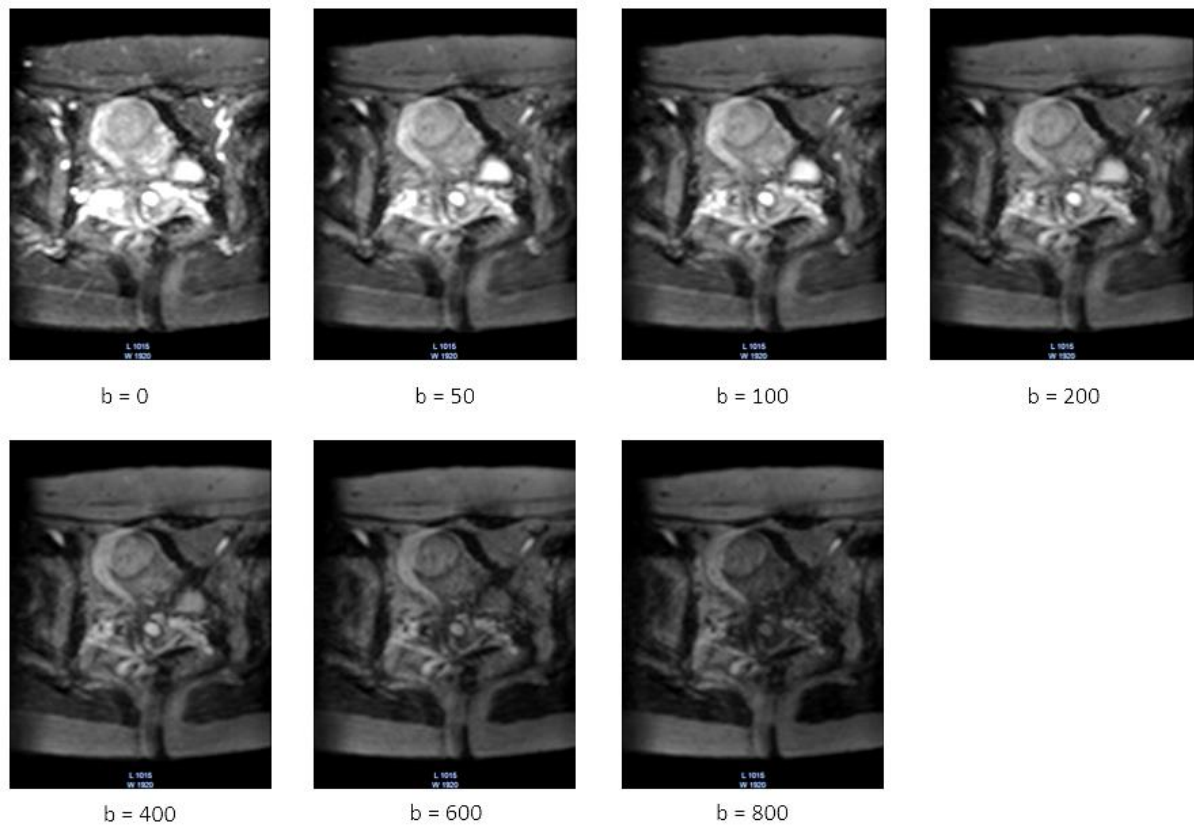


Figure 5: Diffusion Weighted Images with different weightings of the diffusion. B-values are expressed in s/mm^2 . The higher the b-value (i.e. the diffusion weighting), the greater the signal loss.

Normally, a short echo time (TE) is recommended to reduce the influence of the T₂ relaxation time and to keep the signal-to-noise ratio high. However, using a long TE (200 ms), it has been shown that malignant tumours can be distinguished from benign tumours. Both malignant and benign tumours can show a high signal intensity on DWI. A study by Tamura et al.¹⁵ shows that malignant tumours can be differentiated from benign by using long-TE DWI. The possibility to emphasize less-restricted water diffusion by using long-TE DWI, brings possibilities in further characterization of uterine fibroid tissue. Therefore, two different DWI sequences with different echo times are part of this study.

3.4.2.2 T₂ mapping

T₂ mapping is a quantitative tool to determine the T₂ relaxation time of tissue. The T₂ relaxation time characterizes the signal decay (or the relaxation) of the transverse magnetization, also called the spin-spin relaxation. Different biological tissues have different T₂ relaxation times. The T₂ relaxation time reflects the possibility of a molecule to move. Every variation inside the tissue (collagen, water, muscle cells, etc.) can induce differences in the T₂ time. By quantifying this T₂ relaxation time, tissue properties, in particular blood fraction, of the fibroid can be quantified. In figure 6, the signal decay using different echo times is shown. This decay is visually presented in an example of a uterine fibroid in figure 7.

Because T₂ weighted images with 12 different echo times have to be acquired, the speed of MRI scanning has to be improved, while maintaining a good signal-to-noise ratio and resolution. For this several imaging techniques are available such as echo-planar imaging (EPI) and turbo spin echo (TSE) imaging to acquire multiple lines of k-space. In this study we combined these two techniques by using GRASE (gradient and spin echo) imaging. This technique uses a train of refocusing 180° radio-

frequency pulses, as TSE imaging. However, for each spin echo, additional echoes are generated, namely gradient recalled echoes as in SE-EPI.

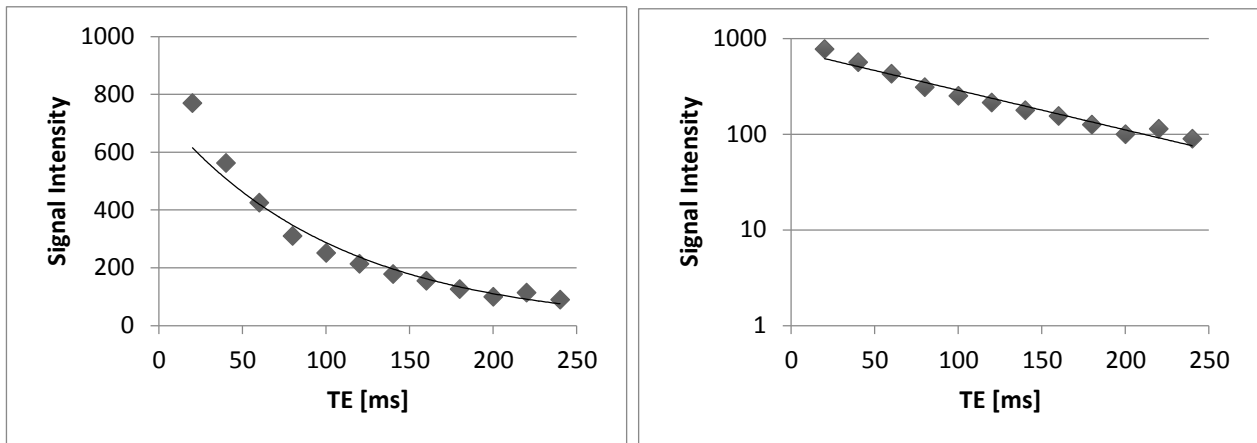


Figure 6: Example of a measured T2 relaxation time curve in uterine fibroid tissue. Left and right represent the same curve. On the right a logarithmic scale was used to emphasize the exponential behavior.

The gain in acquisition time as compared to conventional SE is the TSE echo train length times the EPI factor in single slice imaging. If for example the echo train length is four, the scan time is reduced by $\frac{1}{4}$. When using GRASE imaging with an additional EPI factor of three, then the scan time is roughly reduced to $(\frac{1}{4} \times \frac{1}{3})$ or $\frac{1}{12}$. This makes it possible to acquire T₂ weighted images with 12 different echo times.

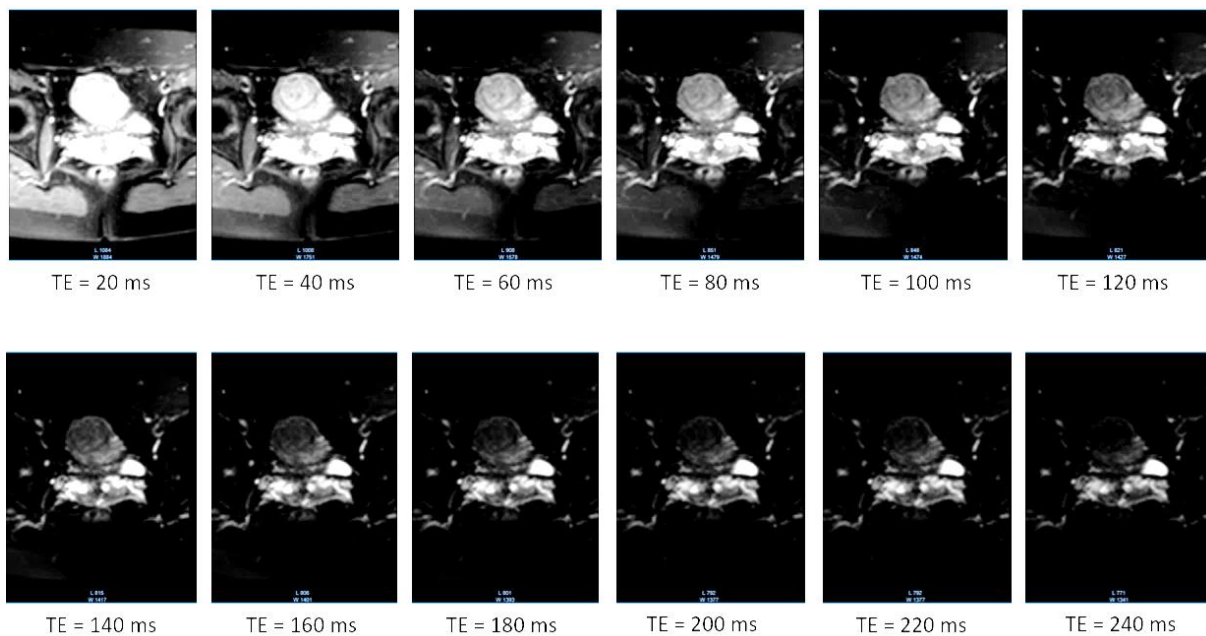


Figure 7: Example of a uterine fibroid images with different echo times (20-240 ms). This picture shows that different tissues have different signal decays.

4 In depth analysis of biological tissue characteristics of uterine fibroids using new MRI techniques

This chapter describes the MASS I study, conducted in the Isala Clinics, Zwolle. This is an ongoing research project and will be continued. Therefore results presented in this chapter are not final results but give an impression about the findings.

4.1 Introduction and rationale

Uterine fibroids are the most common benign gynaecological tumors affecting a high percentage of women. The life-time prevalence of fibroids lies between 70 to 80%. Symptoms of uterine fibroids are abnormal uterine bleeding and pelvic discomfort due to the existing mass, leading to a significant negative effect on the quality of life. Treatment possibilities include drug therapy, hysterectomy, myomectomy, uterine artery embolization and magnetic resonance guided “High Intensity Focused Ultrasound” (MR-HIFU). The choice of treatment and rate of success is dependent on the morphology and tissue characterization of the uterine fibroids².

From literature it is known that the histopathology of uterine fibroids varies among patients. Biological characterization of fibroid tissue is useful knowledge in the choice and suitability of treatment options^{2,16}. Without biopsy it is in current practice impossible to characterize tissue characteristics before treatment. However, we hypothesize it can be possible to make use of advanced MRI parameters that can be used as *non-invasive* biomarkers. This set of new MRI parameters can be used to visualize and gain knowledge about the biological variation and characterization of uterine fibroids.

MR-HIFU is a minimally invasive procedure. In this procedure, a tightly focused, high intensity ultrasound beam is sent into the fibroid tissue. The acoustic energy of the ultrasound is converted into thermal energy at the focal point which leads to thermal coagulation. The areas outside the focal point are unaffected.

4.1.1 Current patients selection for MR-HIFU treatment

Patient selection for MR-HIFU is done at the gynaecology department and based on a screening MRI. Current imaging-based biological characterizations of uterine fibroids are limited. The signal intensity on T₂-weighted MRI images is the main parameter of characterization. This parameter is described by the Funaki classification, defined by Funaki et al. in 2007⁷. In the Funaki classification, patients are stratified in 3 groups based on T₂ signal intensity in selection of MR HIFU treatment. Details of the Funaki classification are listed in appendix 1. This subdivision of uterine fibroids is correlated with pathohistologic structures, demonstrated by Zhao et al. in 2014¹³.

4.1.2 In depth analysis of uterine fibroid characteristics

Novel MRI techniques can potentially provide additional, more specific information about the biological characterization of uterine fibroids. These sequences have been specifically developed (by Philips healthcare, Eindhoven) for the use in uterine fibroids.

4.1.2.1 Diffusion weighted imaging

Diffusion weighted MRI for uterine fibroids is used in multiple studies to characterize the fibroid tissue^{17,18}. With the use of different b-values, perfusion and diffusion of the fibroid can both be imaged. Diffusion weighted imaging provides a quantitative parameter, called the apparent diffusion coefficient (ADC). The ADC is an indicator of the movement of water within the tissue and depends on the cellularity. This allows tissue characterization. This technique is also used in the minimal

invasive uterine artery embolization¹⁹. With respect to MR HIFU treatments, these parameters are important in treatment outcome. Jacobs et al.²⁰ found that DWI and ADC mapping are feasible for identification of ablated tissue after MR-HIFU treatment. Liapi et al. found that DWI and ADC maps provide functional information at a cellular level in uterine fibroids²¹. The recent work of Ikink et al.¹⁸ also shows DWI can be used to evaluate treatment results. We hypothesize DWI and ADC maps can also be used before HIFU treatment, as they give functional information about the uterine fibroid tissue on a cellular level.

4.1.2.2 *T2 relaxometry maps*

T2 mapping is a new technique for the specific use in uterine fibroids and not previously described for this application. The Funaki classification⁷ is also based on T₂ relaxation times. T₂ mapping quantifies this parameter, making it more reliable and more widely applicable. The T₂ relaxation time reflects the possibility of a molecule to move. Every variation inside the tissue (collagen, water, muscle cells, etc.) can induce differences in the T₂ time. By quantifying this T₂ relaxation time, tissue properties, in particular blood fraction, of the fibroid can be quantified.

We hypothesize that the above described MRI techniques give useful information about the uterine fibroid characteristics. These characteristics are important in determining patient suitability for MR-HIFU treatment, besides the currently used Funaki classification. The Funaki classification alone is a limited characterization parameter based on the average value of the full fibroid. Further, it classifies the entire patient population in only 3 groups. Recent clinical insights show that the uterine fibroid tissue can be very heterogeneous. This heterogeneity is also seen inside the Funaki groups. This gives grounds for the introduction of new MRI parameters for refinement of the fibroid stratification. To refine the stratification, more information is needed on tissue level. Therefore, in this study we want to analyze a completely new MRI protocol for the non-invasive characterization of uterine fibroid tissue using multi-parametric MRI.

Another future benefit of using novel MRI techniques is to reduce the use of a contrast agent for assessing treatment results after MR-HIFU treatment. Because the non-perfused-volume (the most used treatment outcome measure in HIFU treatment) is calculated after injecting a contrast agent it can only be determined after treatment. In order to be able to assess treatment results during the HIFU treatment, one of the novel MRI sequences presented in this study could serve as a treatment outcome parameter. This could be studied in a subsequent treatment study.

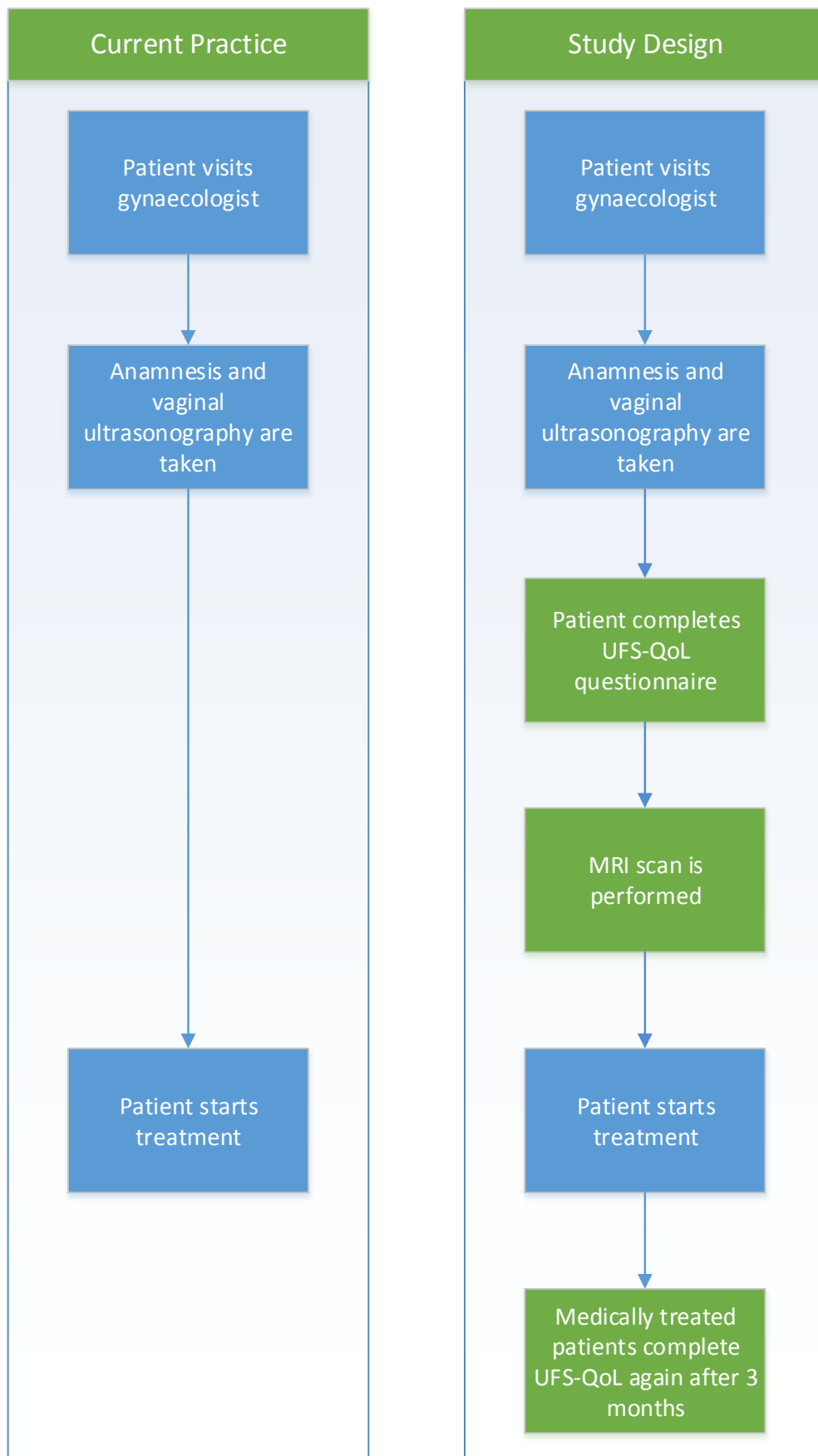


Figure 8: Study design and course of events. On the left, the conventional patient route is shown. On the left the additional study elements are shown in green.

4.2 Materials & Methods

4.2.1 Study Design

The study is a single-center, explorative research. Data collection and experiments are performed in the Isala Hospital Zwolle at the gynaecology and radiology department. This study was approved by the local ethics committee.

The course of events is visually presented in figure 8. On the left, the course of events in current practice is shown. The green fields at the right are the additional study events. All new patients presenting with uterine fibroid related symptoms at the gynaecologist's office are registered in the study database. Based as on the in-/exclusion criteria as further described the following section, only a selective patient group will undergo the screening MRI. Informed consent is obtained before MRI screening. During the screening MRI, performed at the radiology department, biological tissue parameters are visualized with a new MRI protocol, further elaborated in section 4.2.3. These parameters are tested for a correlation to the existing tissue classification method (Funaki classification) and the clinical findings. Clinical symptoms are examined by means of the uterine fibroid symptom and health related quality of life questionnaire (UFS-QoL). The treatment of the patients is not influenced by the additional questionnaire and MRI. The patients will undergo the MRI scan for scientific reasons. The goal of this study is not to improve patients treatment at this stage.

The main study parameter is the distribution and variation of MRI parameters (ADC map, K^{trans} map, v_e , v_p and T_2 map) and the correlation between the MRI parameters and the Funaki classification and the clinical UFS-QoL score.

4.2.2 Patient population: inclusion and exclusion criteria

In the Isala hospital Zwolle, annually 210 patients are presenting in the Isala with complains that are attributed to uterine fibroids. Patients with a diagnosed uterine fibroid (based on anamnesis, physical examination and vaginal ultrasonography) and uterine fibroid related symptoms are included in the study. To determine whether a patient would be an eligible candidate for MR-HIFU treatment after screening MRI, in- and exclusion criteria based on the guidelines are maintained. All in- and exclusion criteria are listed in table 1.

Sample size is determined based on the objective of the study: the distribution and variation in quantitative MRI parameters. From literature it is known, type 3 uterine fibroids (based on the Funaki classification) are difficult to treat. Therefore, distribution and variation is especially of added value in type 3 uterine fibroids. When looking at literature about the incidence of type 3 uterine fibroids, an average incidence of 20-25% is seen. For a reliable comparison between patients with type 3 uterine fibroids, an inclusion of 80 patients is desirable. In this case, we can include about 20 patients with type 3 uterine fibroids.

4.2.3 Study procedures: MRI protocol

MR scans were performed on a clinical 1.5-T MRI system (Achieva; Philips Healthcare, Best, the Netherlands). The patients are scanned in a supine position. The MRI protocol consists of different sequences as shown in table 2. The first 2 sequences are part of the conventional MRI protocol for patients undergoing pelvic MRI with a uterine fibroid indication. T_2 -weighted images are used to identify anatomical structures within the pelvis in the sagittal and transversal orientations. TR was 6407ms, TE 125ms, matrix size 256x256, slice thickness 3mm and slice gap 0.3mm. In order to visualize hemodynamic characteristics and to assess the viability of the uterine fibroid, a gadolinium-based contrast agent was injected. T_1 images were obtained in the transversal orientation before administration of gadolinium with TR of 663ms, TE 14ms, matrix size 256x256, slice thickness 3.5mm and a slice gap of 0.3mm. T_1 contrast enhanced images were obtained in the sagittal and transversal

orientations. All images in the transversal plane are angulated coronal to the uterine fundus. The additional multiparametric protocol consists of T₂ mapping and diffusion-weighted MRI, all elaborated below. All multiparametric imaging sequences were acquired in the transversal orientations and before the administration of contrast agent.

Table 1: In- and exclusion criteria

	Inclusion Criteria	Exclusion Criteria
Gynaecologist In-/exclusion	Diagnosed, symptomatic uterine fibroids Aged 18-55 Pre-menopausal patients	Post-menopausal patients Pregnancy Calcifications in uterine fibroid Severe abdominal obesity Uterine artery embolization in medical history MRI contra-indications
MRI In-/exclusion	Funaki Type 1 and 2 uterine fibroids Diameter 1-10 cm of dominant fibroid	Funaki type 3 uterine fibroids Calcified or pedunculated fibroids Close to the sciatic nerve or sacrum Interposition of bowel or ovary Diameter <1 or >10 cm in diameter Distance skin – midpoint of fibroid > 12 cm

4.2.3.1 MRI assessment and acquisition of T₂ mapping

For the calculation of the T₂ map, T₂-weighted images with different echo times are acquired by using the GRASE sequence, combining the TSE and EPI methods. TR was 2433ms, matrix size 128x128, slice thickness 7.0mm without a slice gap. We used 12 different echo times ranging from 20 to 240ms. In order to validate this MRI protocol for measuring true T₂ relaxation times, a calibrated phantom was used. Measurements using the calculated T₂ map were compared to the calibration phantom values. The results of this phantom test are shown in a scatterplot in figure 10. Linear regression analysis shows a R² of > 0.99. The Intra Class Correlation Coefficient (ICC) between the calibration phantom and the GRASE T₂ relaxometry measurements was > 0.99. This shows an excellent agreement between both values. Therefore, we concluded the GRASE sequence can be used for measuring true T₂ values with proper accuracy.

4.2.3.2 MRI assessment and acquisition of Diffusion Weighted MRI

The diffusion weighted images (DWI) are performed in the transversal plane and angulated as described above. A spin-echo sequence was used to acquire 7 different b-values: 0, 50, 100, 200, 400, 600 and 800 s/mm². TR was 2673ms, TE 64ms, matrix size 128x128, slice thickness 7.0mm without a slice gap. For the long-TE DWI, a spin-echo sequence with 4 different b-values (0, 50, 100, 200 s/mm²) were acquired. For long-TE DWI, TR was 6715ms, TE 140ms, matrix size 128x128, slice thickness 7.0mm without a slice gap.

ADC maps are created in IntelliSpace Portal (Philips). Briefly explained, an ADC map is calculated by plotting signal intensities of a specific pixel against the different b-values. The exponential decay indicates the apparent diffusion coefficient (ADC). An example of such an exponential signal decay is presented in figure 9. The ADC value is calculated by the following formula:

$$S_b = S_0 \times e^{-b \times ADC}$$

$$ADC = \frac{\ln(S_b/S_0)}{b_0 - b_b}$$

In general, an ADC value is calculated using two or more different image acquisitions, each with a different diffusion weighting (i.e. b-value). The faster the signal decay between the different b-values, the more water diffusion is measured. This fast exponential decay will result in a high ADC value. For the DWI sequence, three different ADC maps are calculated. The first ADC map is calculated with all measured b-values (0, 50, 100, 200, 400, 600, 800 s/mm²), the second ADC map is calculated with only low b-values (0, 50 and 100 s/mm²) and the last ADC map is calculated with only high b-values (400, 600, 800 s/mm²).

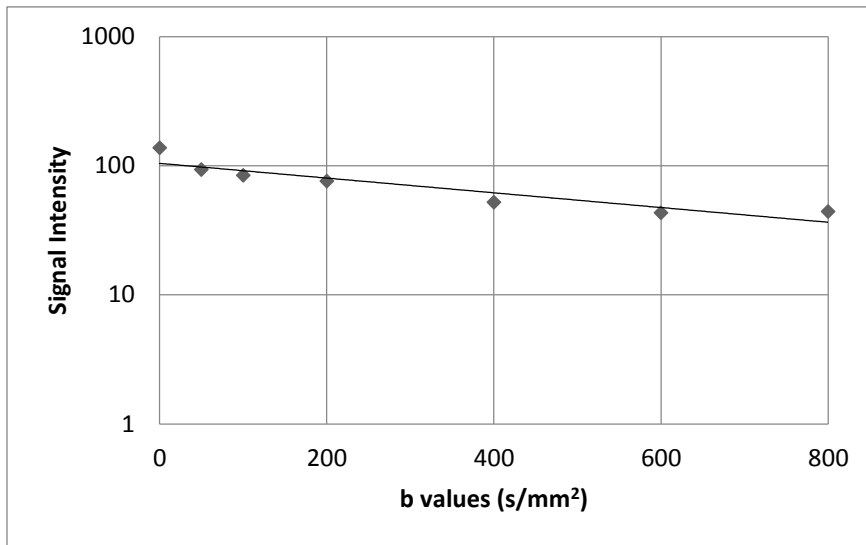


Figure 9: Example of the calculation of the ADC value for a pixel. Signal intensity decay is shown for 7 different b-values. A logarithmic scale is used to emphasize the exponential decay.

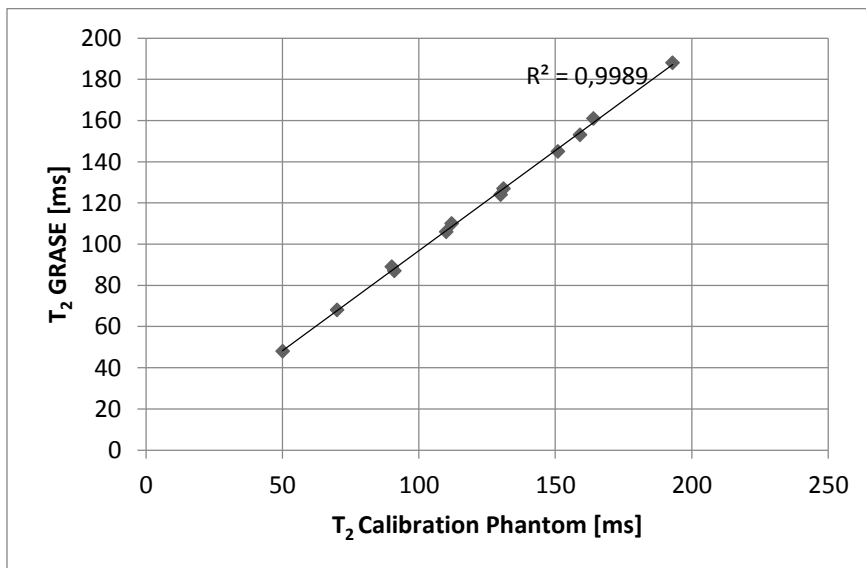


Figure 10: Calibrated T2 relaxation times versus Measured relaxation times using the GRASE technique. Linear regression shows a r2 of > 0.99.

Table 2: MRI sequences designed for the MASS 1 study

	Sequences	Objective
Conventional protocol	T ₂ -weighted imaging	Anatomic information about the size, location, position and type of uterine fibroid. Also the Funaki classification is based on T2 weighted Imaging.
	T ₁ -weighted imaging	Additional anatomic information.
	CE-T ₁ weighted imaging	Gives information about the contrast enhancement patterns. Evident non-perfused areas cannot be treated.
Multi-parametric Imaging protocol	T2 relaxometry	The T2 relaxation time reflects the possibility of a molecule to move. Every variation inside the tissue (collagen, water, muscle cells, etc.) can induce differences in the T ₂ time. Important parameter: T2 relaxation time
	MR-DWI	The quantitative ADC (determined by MR-DWI) is an indicator of the movement of water within the tissue and depends on the cellularity. This allows tissue characterization. Important parameter: ADC values
	MR-DWI with long TE	MR-DWI with a long echo time, gives the possibility to emphasize less-restricted water diffusion. This gives further possibilities of characterization of uterine fibroid tissue. Important parameter: ADC values long TE

4.2.4 Multiparametric analysis

In order to analyze MRI parameters of different tissues, Volumes-Of-Interest (VOIs) are manually drawn in the T₂ maps. VOIs are drawn in the uterine fibroid tissue and the normal myometrium. This is done in Matlab software, developed for this specific use. The user specifies the slices in which the uterine fibroid is present. In all of these slices, a region-of-interest (ROI) is manually drawn. In this way, the whole uterine fibroid volume is covered. From this selection, a mask is created. This process is repeated for normal myometrium. The masking is done on the T₂ relaxometry maps. The masks are used for all research MRI sequences. When calculating T2 values and ADC values of the uterine fibroid tissue and myometrium, the mean value of the masked area is calculated. All calculations are done in Matlab developed software (version 2015b, Mathworks, Natick, Massachusetts). The volume of the fibroid is calculated with use of the above described fibroid mask and the slice thickness and voxel size.

All MRI parameters (T2 values, ADC and Long-TE ADC) will be analyzed on variation inside a patient and between patients. This will give us information about the inter- and intra-patient variability. Also the relationship between the new MRI parameters and the Funaki classification are analyzed. The relationship between clinical UFS-QoL score and MRI parameters is also described.

4.2.5 Statistical analysis

All MRI parameters are statistically described and tested for normality. To describe variation of the MRI parameters, the coefficient of variation is used for all parameters. Because all MRI parameters have different units, a standardized measure of dispersion is used to compare the variation of each parameter.

To **describe** the relationship between the MRI parameters and the Funaki classification, scatterplots and boxplots are made for each MRI parameter (ADC maps and T₂ map). To **statistical test** the relationship between each MRI parameter and the Funaki classification, the Spearman's rank correlation coefficient is used. This uni-variate analysis is performed for each MRI parameter in relation to the Funaki classification.

To **describe** the relationship between the MRI parameters and the baseline UFS-QoL, scatterplots and boxplots are made for each MRI parameter and the Funaki classification. To **statistical test** which parameter is the best predictor for clinical symptoms, regression analysis on the UFS-QoL will be performed. If the UFS-QoL variable has a normal distribution, linear regression is used for the correlation between the UFS-QoL score and the MRI parameter. If the distribution of the variable is skewed, a logistic transformation is performed. When the distribution is normalized after this transformation, linear regression is used. When the variable is still skewed after the transformation, the UFS-QoL is dichotomized, based on the median. After dichotomization, logistic regression is used for the association between the UFS-QoL score and the MRI parameters.

4.3 Results

During the study period, 12 patients were included in the study. Two patients did not undergo MRI examination because they withdrew themselves from the study. In total, 10 patients successfully underwent a screening MRI. Mean age of the patients was 41.8 ± 6.1 in a range of 30-49. The characteristics of the patients and fibroids are summarized in table 3.

Table 3: Patient Characteristics

Age		41.8 ± 6.1 (30 - 49)
Weight (kg)		78.2 ± 11.2 (61 – 120)
Patient Symptoms	Heavy menstrual bleeding	80%
	Bowel pressure	20%
	Pain during menstruation	50%
	Pain during intercourse	10%
	Other	60%
Symptom Severity Score		37.1 ± 20.3 (6.2 – 65.6)

All MRIs are assessed and evaluated by an experienced radiologist. Based on the screening MRI, basic fibroid characteristics are determined. Five patients had single fibroids, four of the patients had multiple fibroids of which 1 patients had more than 15 fibroids. The mean diameter of the uterine fibroids was 55.4 mm. All fibroid characteristics are summarized in table 4. All MRI sequences of one patient are shown in figure 9.

All patients completed the UFS-QoL questionnaire. In this manuscript, the transformed Symptom Severity Score (SSS) is used, for which 100 points represents maximal symptom severity. The mean SSS $\pm$ std. sev. was 37.1 ± 20.3 (ranging from 6.2 to 65.6).

Fibroid volumes are ranging from 10.7 mL to 303.3 mL with a mean volume of 101.8 mL. The most frequent uterine fibroid type was Funaki type 2, as seen in 70% of the patients. The scaled signal intensity is calculated using the following formula²²:

$$\text{Scaled Signal Intensity (SSI)} = \frac{\text{SI of UF} - \text{SI of Skeletal Muscle}}{\text{SI of Subcutaneous Fat} - \text{SI of Skeletal Muscle}}$$

The scaled signal intensity (SSI) ranged from 6.9 to 73.2 with a mean of 23.9. When comparing the Funaki classification to the SSI, we found the following results. For Funaki type I patients, SSI ranged from 6.9 to 10.3. For Funaki type II patients, mean SSI was 20.5 ranging from 10.4 to 43.2. For the Funaki type 3 patient, SSI was 73.2. These results are shown in figure 11.

Quantitative T₂ values are measured separate for the uterine fibroid tissue and normal myometrium. For the uterine fibroid tissue, mean T2 relaxation time $\pm$ std. dev. is 150.6 ± 41.6 (ranging from 75.6 to 221.9). For the normal myometrium mean T2 relaxation time $\pm$ std. dev. is 224.4 ± 39.4 (ranging from 168.7 to 284.5). Quantitative T2 values were also compared to the scaled signal intensity. These results are shown in figure 12.

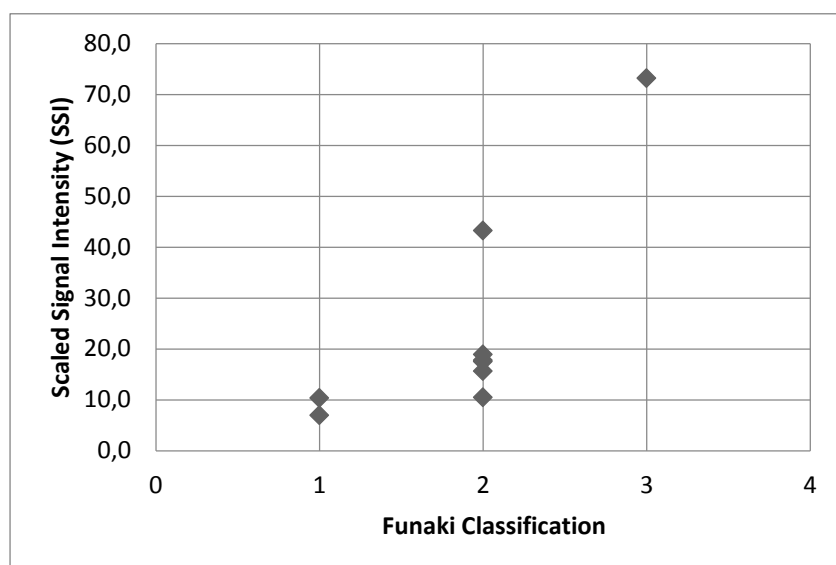


Figure 11: Scaled Signal Intensity vs. the Funaki Classification

ADC values representing perfusion and diffusion are measured separate for the uterine fibroid tissue and normal myometrium. When using all b-values, mean ADC values for uterine fibroid tissue were $1152.9 \pm 393.8 \times 10^{-6} \text{ mm}^2/\text{s}$ and for normal myometrium $1410.0 \pm 550.4 \times 10^{-6} \text{ mm}^2/\text{s}$. When only using low b-values, mean ADC values for uterine fibroid tissue were $446.6 \pm 118.1 \times 10^{-6} \text{ mm}^2/\text{s}$ and for normal myometrium $651.2 \pm 231.2 \times 10^{-6} \text{ mm}^2/\text{s}$. With high b-values, mean ADC values for uterine fibroid tissue were $818.4 \pm 314.6 \times 10^{-6} \text{ mm}^2/\text{s}$ and for normal myometrium $909.0 \pm 377.7 \times 10^{-6} \text{ mm}^2/\text{s}$. These results are shown in figure 13. For all used b-value combinations, the ADC is higher in the normal myometrium than in the uterine fibroid tissue, indicating a higher diffusion in the myometrium.

We also measured ADC values when using a diffusion weighted sequence with long TE. This emphasizes less-restricted water diffusion. These ADC values are also measured separately for uterine fibroid tissue and normal myometrium. Mean ADC values for uterine fibroid tissue were $465.5 \pm 80.4 \cdot 10^{-6} \text{ mm}^2/\text{s}$. For normal myometrium, ADC values with long TE were $657.1 \pm 150.1 \cdot 10^{-6} \text{ mm}^2/\text{s}$. This means that the less-restricted water diffusion is larger in the normal myometrium tissue than in the fibroid tissue. These results are shown in figure 13.

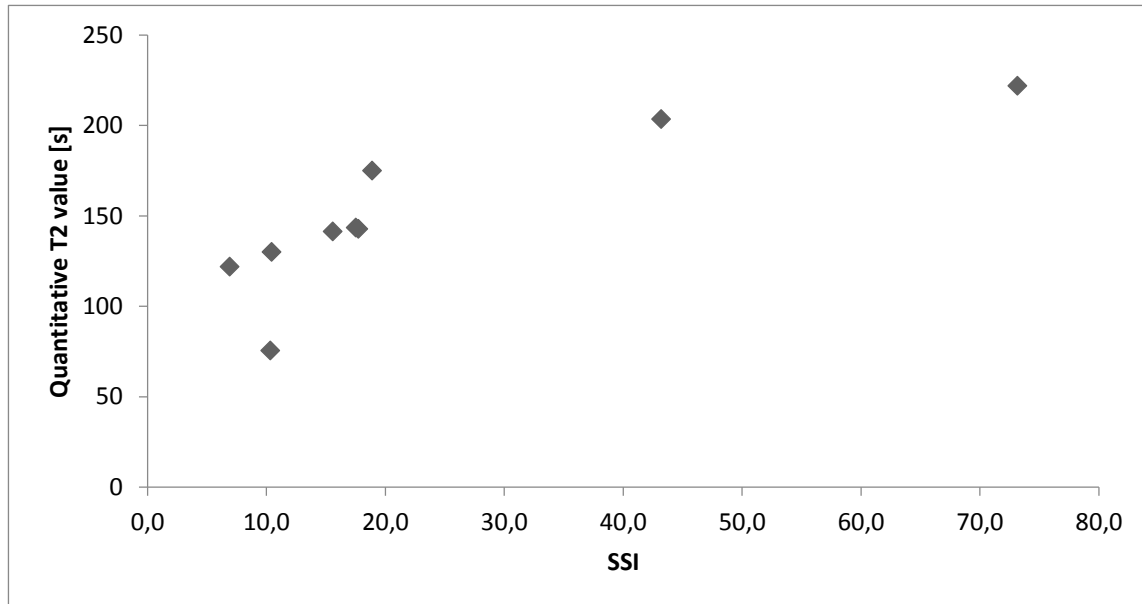


Figure 12: Quantitative T2 values (s) plotted against the scaled signal intensity (SSI). Both measures are based on the T2 relaxation time. The quantitative T2 values are measured by T2 relaxometry. The scaled signal intensity is calculated based on the signal intensity of different tissues. It is a normalized measure.

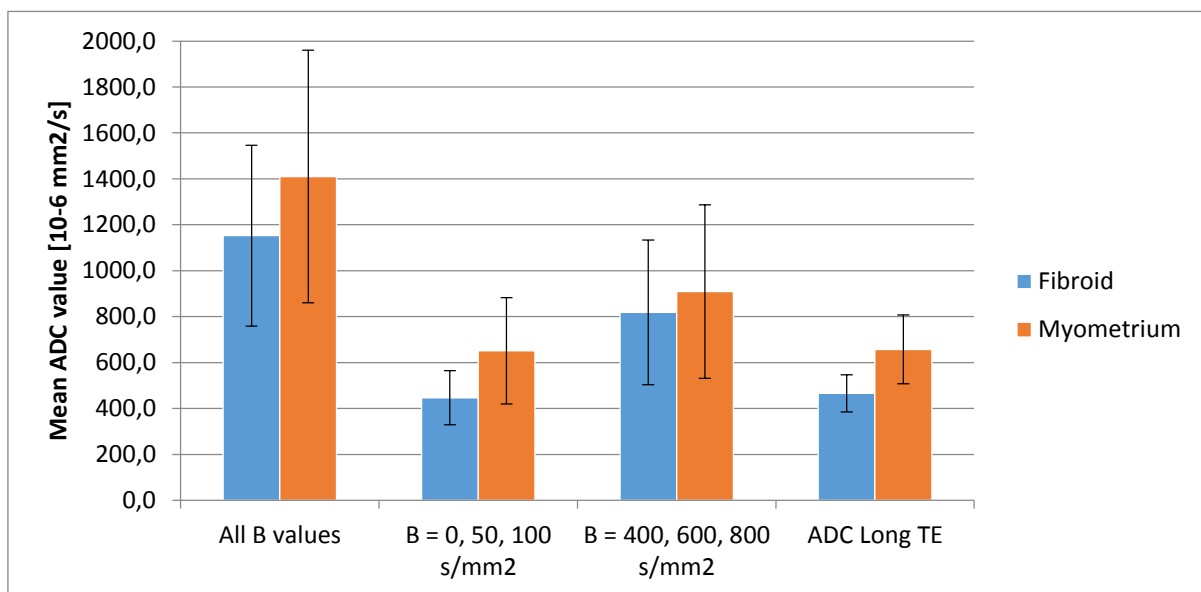


Figure 13: Results of diffusion weighted imaging (DWI) in uterine fibroid tissue and normal myometrium. Measurements are done in different ADC maps. The first three measurements are based on different sets of b-values. The last measurement on the right is based on diffusion weighted imaging using long echo times.

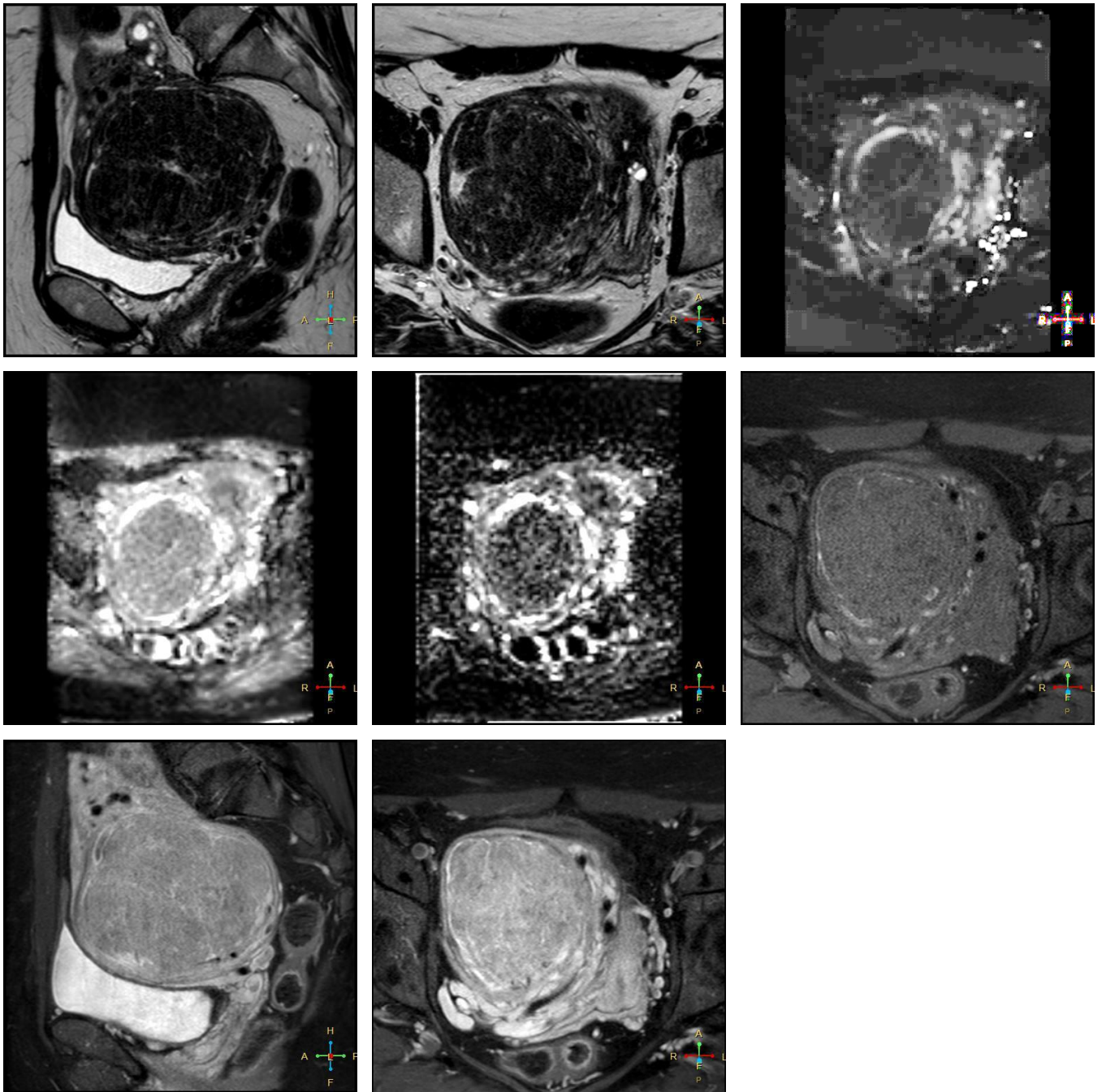


Figure 14: MRI imaging of one of the patients. From the left upper corner: T2 weighted sagittal image, T2 weighted transversal image, T2 relaxometry map, ADC map, ADC map with long TE, T1 weighted transversal image, T1 weighted sagittal image after administration of contrast agent and T1 weighted transversal image after administration of contrast agent.

Table 4: Fibroid Characteristics

Number of fibroids	1	6 (60%)
	2-4	2 (20%)
	5-10	1 (10%)
	>10	1 (10%)
Dominant Fibroid Diameter (mm)		55.4 (18.4 – 87.9)
Dominant Fibroid Volume (mL)		101,8 (10,7 – 303,3)
Dominant Fibroid Funaki Classification	Type 1	2 (20%)
	Type 2	7 (70%)
	Type 3	1 (10%)
Scaled Signal Intensity ¹		23.9 (6.9 – 73.2)
Fibroid location	Anterior Wall	1
	Posterior Wall	2
	Side Wall	4
	Fundus	3
T ₂ relaxation time (ms)	Uterine Fibroid	150.6 ± 41.6 (75.6 – 221.9)
	Myometrium	224.4 ± 39.4 (168.7 – 284.5)
ADC value (mm ² /s * 10 ⁻⁶)	Uterine Fibroid	1152.9 ± 393.8 (586.2 – 1643.4)
	Myometrium	1410.0 ± 550.4 (625.8 – 2176.2)
ADC values long TE	Uterine Fibroid	465.5 ± 80.4 (380.9 – 641.0)
	Myometrium	657.1 ± 150.1 (511.5 – 897.1)

¹ Scaled Signal Intensity (SSI) = $\frac{SI \text{ of UF} - SI \text{ of Skeletal Muscle}}{SI \text{ of Subcutaneous Fat} - SI \text{ of Skeletal Muscle}}$

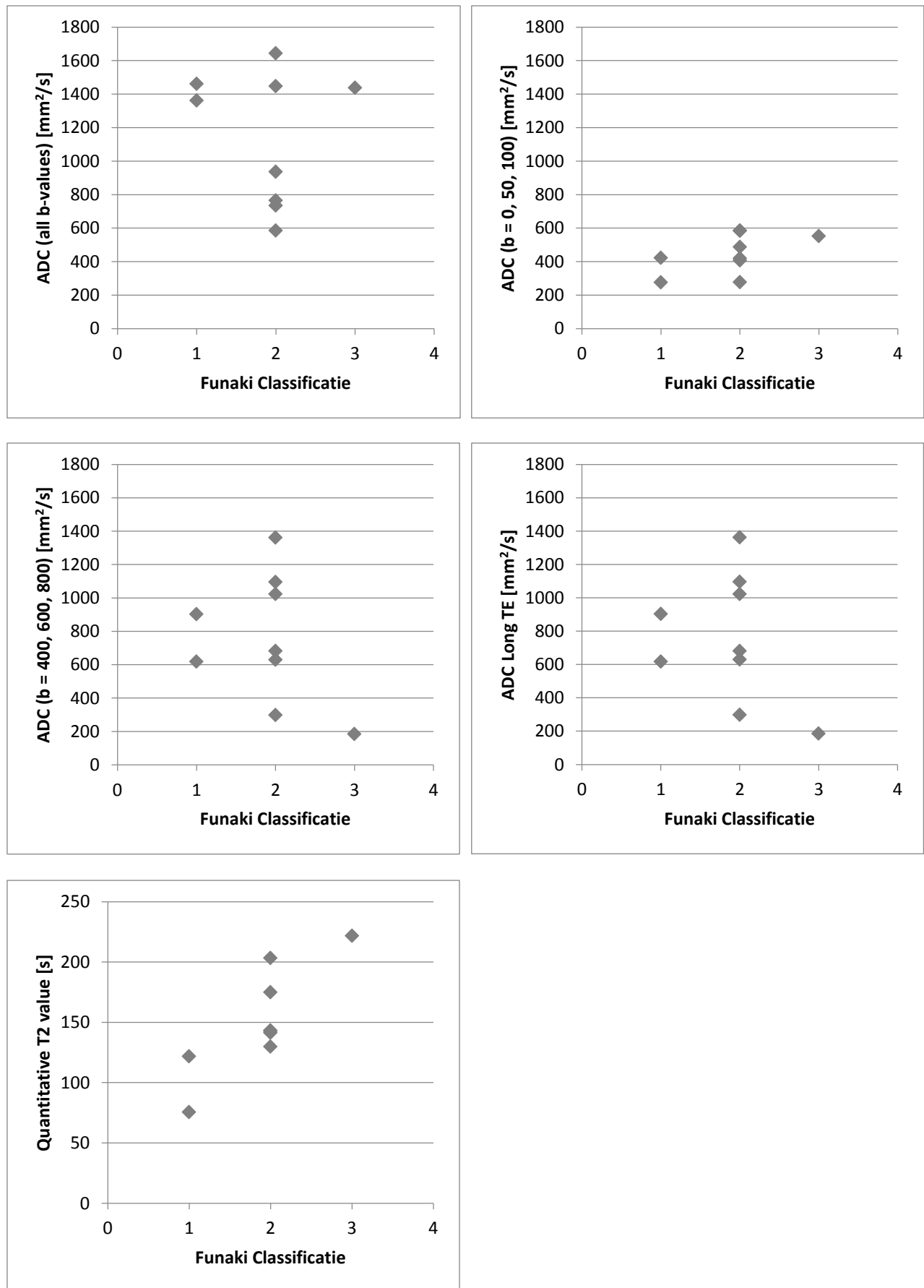


Figure 15: Scatterplots visualizing the relationship between the different MRI parameters and the Funaki Classification

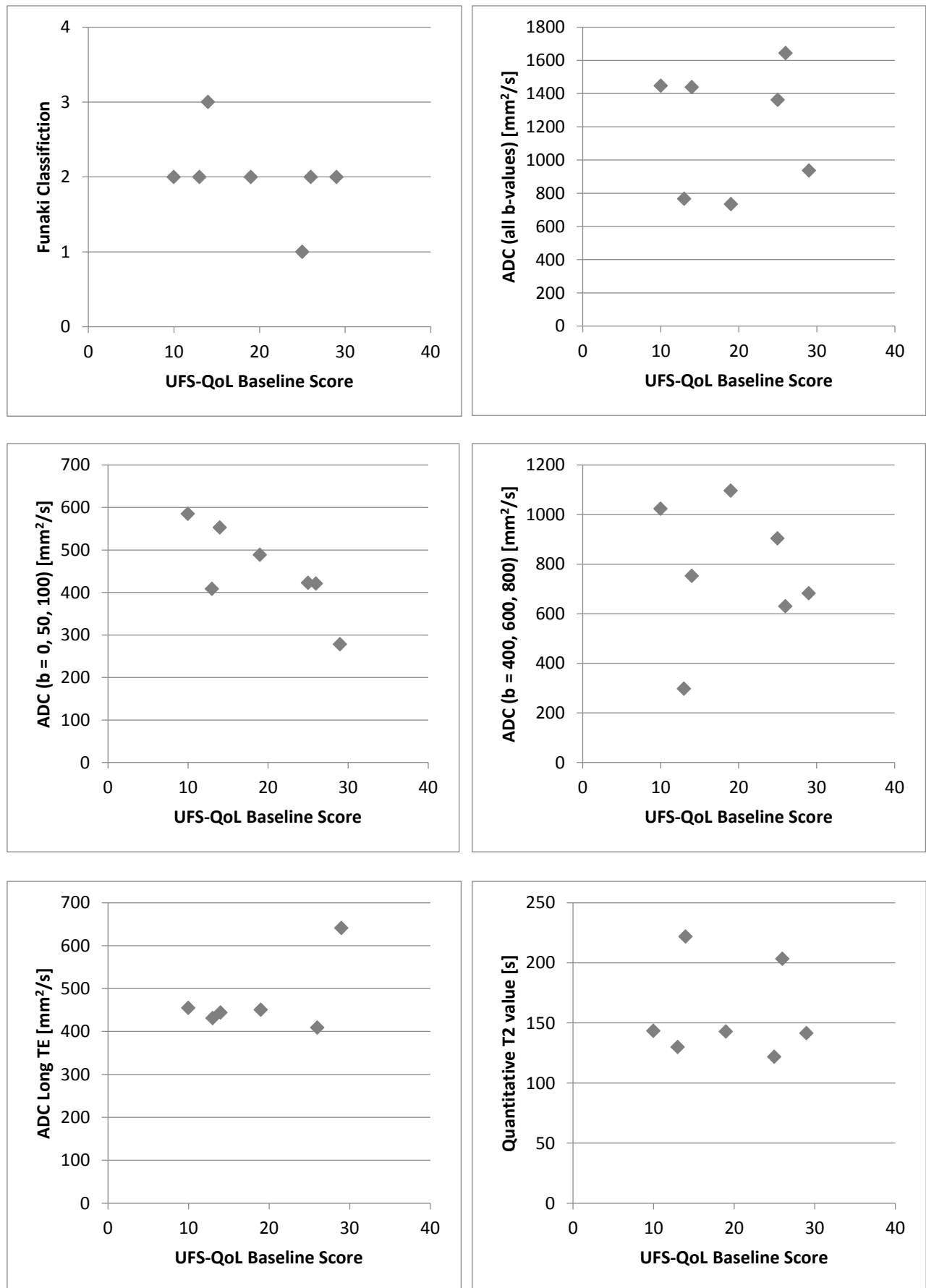


Figure 16: Scatterplots visualizing the relationship between the different MRI parameters and the UFS-QoL score

The relationship between the different MRI parameters (multiple ADC values and quantitative T2 values) and the Funaki classification, a scatterplot for each of the parameters is made. Those can be found in figure 15. On the next page, in figure 16, scatterplots are shown visualizing the relationship between the MRI parameters and the baseline UFS-QoL score.

To look at the variation between uterine fibroids for the different MRI parameters, the coefficient of variation is calculated for each parameter measured in the fibroid tissue. For T2 mapping, the coefficient of variation is 29%. The different ADC measurements gave the following coefficients: for all b-values it was 34%, for low b-values (0, 50, 100 s/mm²) 26%, for high b-values (400, 600, 800 s/mm²) and for ADC measurements with long TE 17%. We are looking for a parameter which can distinguish uterine fibroid tissue. Therefore, we need a parameter with a high coefficient of variations. However, based on these results of 10 patients, we cannot yet draw conclusions out of these results.

4.4 Discussion

The results of this study demonstrate a wide variety in uterine fibroid characteristics among patients, based on the wide range of new MRI parameters. Based on the Funaki classification⁷, we included patients from all classification groups. Also, fibroid volumes and baseline symptom severity scores were variable among patients. All these findings prove the hypothesis that uterine fibroid tissue is variable among patients.

Funaki classification and Scaled Signal Intensity

The Funaki classification is a simple characterization tool, dividing the whole patient population in three groups. Funaki group 1 has a low signal intensity, whereas Funaki classification 3 shows a high T2 signal intensity caused by a high vascularity. This high vascularity is disadvantageous for MR-HIFU treatment, because of the rapid drainage of heat. Various studies have shown a correlation between T₂ signal intensity and MRI-HIFU treatment outcome^{7,10}. The Funaki classification is based on T₂ values of the fibroid compared to skeletal muscle and abdominal fat. Therefore, we compared the Funaki classification to the scaled signal intensity (SSI). The SSI is also based on T₂ relaxation times, but in contrast to the Funaki classification it is a continuous variable, allowing a more precise inclusion tool. The scatterplot comparing Funaki to SSI shows group formation: the higher the Funaki classification, the higher the SSI. We found a mean SSI of 23.9 ± 21.3. This value cannot be compared to the findings in literature, because we included Funaki classification 3 patients, which are generally excluded from other studies. Park et. al. found that fibroids with a SSI value of 16.0 or less can be expected to have optimal results²². When using this criterion in our patient population, it would result in an inclusion of 4 people based on SSI value. When using the Funaki classification, 8 patients would have been included. This indicates a possible over-inclusion of patients.

Quantitative T₂ values

We found a mean T₂ value in the uterine fibroid tissue of 150.6 ms, which is considerably lower than the T₂ value in normal myometrium (224.4 ms). This suggests uterine fibroid tissue has a lower vascularity than normal myometrium i.e. the uterine fibroid tissue is less hydrated than normal myometrium. Sipola et al. did a preinterventional quantitative MRI study for the embolization treatment of uterine fibroids²³. They found a mean T₂ time before embolization treatment in the fibroid of 220 ms in 48 patients. When comparing the quantitative T2 value with the Scaled Signal Intensity, we see a linear trend between the two measurements. This is in line with our expectations, since both measurements are based on T₂ relaxation times.

Diffusion Weighted Imaging

The results of the ADC measurements show that there is a difference in ADC values between the normal myometrium and the uterine fibroid tissue. In all different ADC maps, the mean ADC value of the myometrium is higher than the ADC value of the uterine fibroid tissue. This indicates less diffusion in the uterine fibroid tissue compared to the myometrium. This finding corresponds to the finding of the quantitative T_2 values, mentioned above.

Furthermore, when looking at the results, we see a strong influence of the choice of b-values on the mean ADC. This finding suggests that the signal decay displays non-mono-exponential behavior. This conclusion is also seen in the work of Ikink et al.¹⁸. Therefore it can be stated that DWI in uterine fibroids reflects the effects of both diffusion and perfusion¹⁸. When only using low b-values, rapid incoherent motion of water molecules is accentuated. This could be the arterial vascular component^{18,24}. Blood flow is an important parameter of treatment effect prediction and therefore is important in screening MRI. The principle of MR-HIFU is to send acoustic energy into uterine fibroid tissue, which is converted into thermal energy. If the uterine fibroid tissue is strongly perfused with a high blood flow, the thermal energy is rapidly drained. For this reason, fibroids with a low perfusion give better treatment results. In addition to the treatment prediction, when treating the uterine fibroid tissue, this parameter is useful in determining treatment effects without the use of a contrast agent. This is also done in the study of Ikink et al.¹⁸. They suggest that the destruction of vessels and therefore change in perfusion during MR-HIFU treatment can be best emphasized with ADC maps using only low b-values (0, 200 s/mm²). A big advantage of this technique is that it can be used during treatment to visualize interim treatment effects. Looking at the coefficients of variation, ADC maps using all b-values (0, 50, 100, 200, 400, 600, 800 s/mm²) gave the highest variation suggesting that these ADC maps can distinguish uterine fibroid types best.

Multiple studies earlier demonstrated the use of diffusion MRI in treatment monitoring of MR-HIFU treatments. Jacobs et al.²⁵ found a decrease in ADC value after treatment. Pilatou et al.²⁶ also investigated DWI after MR-HIFU treatment. They found that when using DWI for determining NPV, an underestimation was made. Also, the change in ADC values was unpredictable, as in some patients the value increased (19/45) and in others decreased (26/45). This could be due to the effect that when using both low (0 – 200 s/mm²) and high b-values (400 – 800 s/mm²), two effects are measured namely perfusion and diffusion. By creating ADC maps with only low b-values and only high-values, the effects of these different processes can be split. A technique to achieve this, would be IVIM (intravoxel incoherent motion) imaging. In an IVIM analysis, a bi-exponential function is used to describe the DWI data instead of a mono-exponential fit. In this way, both diffusion and perfusion are taken into account.

Above described studies use diffusion weighted imaging in measuring treatment effect. However, by completing and expanding this study with MR-HIFU treatments, we want to prove that DWI techniques can also be used in accurate patient selection. Especially ADC maps calculated from low b-values (0 – 200 s/mm²) are interesting because they visualize the tissue perfusion.

In this research, we compare different MRI techniques in the same patient population. To our knowledge, this is the only study comparing T1 and T2 MRI sequences with different DWI techniques and T2 relaxometry. The aim of this study is to include 80 patients. Compared to other MRI studies about quantitative measurements of the fibroid tissue, this is a large number. This advantage allows us to map the whole, heterogeneous patient population suffering from uterine fibroids in a high volume, non-academic setting. Also the developed MRI protocol and the in Matlab developed software is tested in an extensive patient population.

There are some limitations to this study. At time of writing, we only included 10 patients in the study. The preliminary results therefor are difficult to interpret, as there can be no statistical evidence given. Technical weaknesses to this study include the possible artifacts in the MRI images because of physiologic motion. These motions can be due to respiratory motion or bowel movement. Also, we saw in some patients that bladder filling significantly increased during the MRI scan. For the evaluation of the MRI scans, we only placed the volume-of-interest once. The actual volume of interest however can change during the different sequences. Because we haven't performed a scan-and-resect study, we have no correlation of the non-invasive MRI-based parameters with the histopathology.

It is important to further include patients up to the desirable sample size of 80 patients. In the small patient population of 10 patients included in this study, we found coefficients of variation for the different parameters. It is important that these numbers are based on a larger, and therefore more representative patient population. We then can map the whole patient population and visualize the difference in uterine fibroids tissues among patients. Also, we get an evidence-based assessment of patient eligibility for MR-HIFU treatment in the Isala. More awareness will be created in the treatment of uterine fibroids, as in which MR-HIFU treatment will take in a potential minimal-invasive treatment choice.

The next step in this study is to treat patients with MR-HIFU and track the tissue changes during and after treatment with the MRI protocol developed in this study. This subsequent study is currently set up in the Isala. The research protocol can be found in chapter 5. In the ideal situation, an MRI screening is made with the extensive protocol, the patient will be treated with MR-HIFU after which the extensive protocol is repeated and after that the fibroid is resected to correlate with histopathologic characteristics.

4.5 Conclusion

In conclusion, we developed and clinically implemented, by means of a METC approved research protocol, a MRI protocol for patients suffering from uterine fibroids. This protocol uses non-invasive biomarkers to characterize the uterine fibroid tissue. The different functional imaging parameters show a difference between normal myometrium tissue and uterine fibroid tissue. The functional parameters also show a difference between patients. Quantitative T2 mapping shows a visual correlation with the currently used Funaki classification. When using the scaled signal intensity, which can be compared to the Funaki classification, a more accurate cut-off value could be identified. By including more patients in this study, the whole range of heterogeneous uterine fibroid tissue can be imaged, described and analyzed.

5 Treatment of Uterine Fibroids with Magnetic Resonance guided High Intensity Focused Ultrasound: the search for revealing Imaging Parameters

Part of the graduation assignment was designing a study protocol for the subsequent study in which patients will be treated with MR-HIFU. This study protocol is submitted at the local ethics committee.

5.1 Introduction

Uterine fibroids are the most common benign gynaecological tumors affecting a high percentage of women. Symptoms of uterine fibroids are abnormal uterine bleeding and pelvic discomfort due to the existing mass, leading to a significant negative effect on the quality of life. Treatment possibilities include drug therapy, hysterectomy, myomectomy, uterine artery embolization and recently magnetic resonance guided “High Intensity Focused Ultrasound” (MR-HIFU).

Surgical approaches often go along with complications, longer hospital stays and longer recovery times. The advantages of the minimally invasive MR-HIFU treatment are a lower morbidity, no need for general anesthesia, no radiation exposure, low probability of bleeding and infection and short recovery times. Patients return to work approximately 24 hours after treatment⁹.

Thermal ablation using MR-HIFU allows for a non-invasive treatment of uterine fibroids by selective tissue heating. The heat generating possibilities of ultrasound are combined with the imaging capabilities of MRI. The MRI facilitates in treatment planning, thermometry for real-time temperature feedback during the treatment and for evaluation of treatment outcome. The energy of the focused ultrasound causes heating of the tissue on the focal spot to locally induce coagulative necrosis, while the surrounding tissue remains unaffected.

Result assessment of the MR-HIFU treatment is mostly done by means of two important outcome parameters: NPV (non-perfused volume) ratio and the UFS-QoL (Uterine Fibroid Symptom and Health related Quality of Life) improvement after a follow-up of 6-12 months. The NPV is defined as the non-perfused areas of gadolinium-enhanced images immediately after treatment. The NPV ratio gives the percentage of treated uterine fibroid tissue.

Many studies are published on the safety, efficacy and quality of life improvements. The systematic review of Gizzo et al.⁵ gives a good summary. A NPV ratio of >50% was achieved in 60% of the studies (16-98%), with a mean UFS-QoL improvement after 12 months of 31 points (15-66).

Among all treated patients, a large variety is seen in volume and symptom reduction, despite of clear exclusion criteria. There already has been some research on predictors of success of the MR-HIFU treatment. In these studies, the signal intensity of the fibroid on T₂-weighted images was the main parameter of characterization. T₂ signal intensity (SI) of the uterine fibroid is compared with the SI of skeletal muscle. In 2007, Funaki et al.²⁷ defined a classification system based on the T₂ signal intensity : type 1, a very low-intensity uterine fibroid image comparable to skeletal muscle (hypo-intense); type 2, an image intensity lower than that of the myometrium and higher than that of skeletal muscle (iso-intense); and type 3, an image intensity equal or higher than that of myometrium. Multiple studies show that type 1 and 2 fibroids are associated with a higher chance of treatment success than type 3 fibroids^{10,14,28}. However, this classification parameter may be too limited as a predictor of treatment outcome. Yoon et al. show a case report of a successfully treated hyper-

intense type 3 uterine fibroid. This suggests that an additional screening parameter is required to make a subpopulation of uterine fibroid patients suitable for successful MR-HIFU treatment.

Tissue perfusion is an important parameter for the characterization of uterine fibroids prior to MR-HIFU treatment because blood flow deprives heat from the fibroid. For this reason, quantification of the tissue perfusion could serve as a predictor of success in the MR-HIFU treatment. Both diffusion weighted MRI (MR-DWI) and dynamic contrast enhanced MRI (DCE MRI) can provide information about fibroid perfusion. The addition of these MRI sequences could give a more extensive and more complete picture of the fibroid. By introducing this more extensive MRI screening protocol, an inter- and intra-patient spread in uterine fibroid characteristics could be identified. Knowledge of MRI-based predictors of success prior to treatment, may contribute to an optimization of patient selection, treatment planning and treatment outcomes.

5.2 Objectives

We are looking for MRI determinants to better determine patient suitability for a successful treatment of symptomatic uterine fibroids using MR-HIFU. A successful treatment is determined as a symptom reduction of minimal 10 points on the UFS-QoL questionnaire.

The possible MRI determinants are that can predict treatment outcome are:

- ADC value
- Quantitative T2 value
- Ktrans

Because we want to eliminate the use of an intravenous contrast agent, we are looking for a functional MRI parameter that is capable of measuring the treated tissue. Therefore, the secondary objective of this study is to determine the feasibility of ADC mapping, T2 mapping and DCE in monitoring thermal ablation effects from MR-HIFU in the treatment of uterine fibroids.

5.3 Study Design

Data collection and experiments are performed in the Isala Zwolle. Patient inclusion is the responsibility of the gynaecology department. Imaging and treatment are performed on the radiology department.

The flow-chart in figure 17 shows the course of events. All new patients presenting with fibroid related symptoms on the gynaecology department are registered in the database with the aim to chart the total patient population in the Isala. Based on the in-/exclusion criteria as further described in section 4.2 and 4.3, only a selective patient group will undergo the screening MRI. Patients excluded for the screening MRI can start directly with the conventional treatment path on the gynaecology department.

Patients that meet the inclusion criteria, undergo a screening MRI. After this screening MRI, again in- and exclusion criteria apply to further determine HIFU treatment eligibility. Patients that meet all criteria will be informed about the MR HIFU treatment and once given informed consent will undergo treatment. At baseline ($t=0$), the UFS-QoL score is measured by means of an online questionnaire. Follow-up consists of visits at the gynaecologist's office, and MRI and the UFS-QoL questionnaire is repeated.

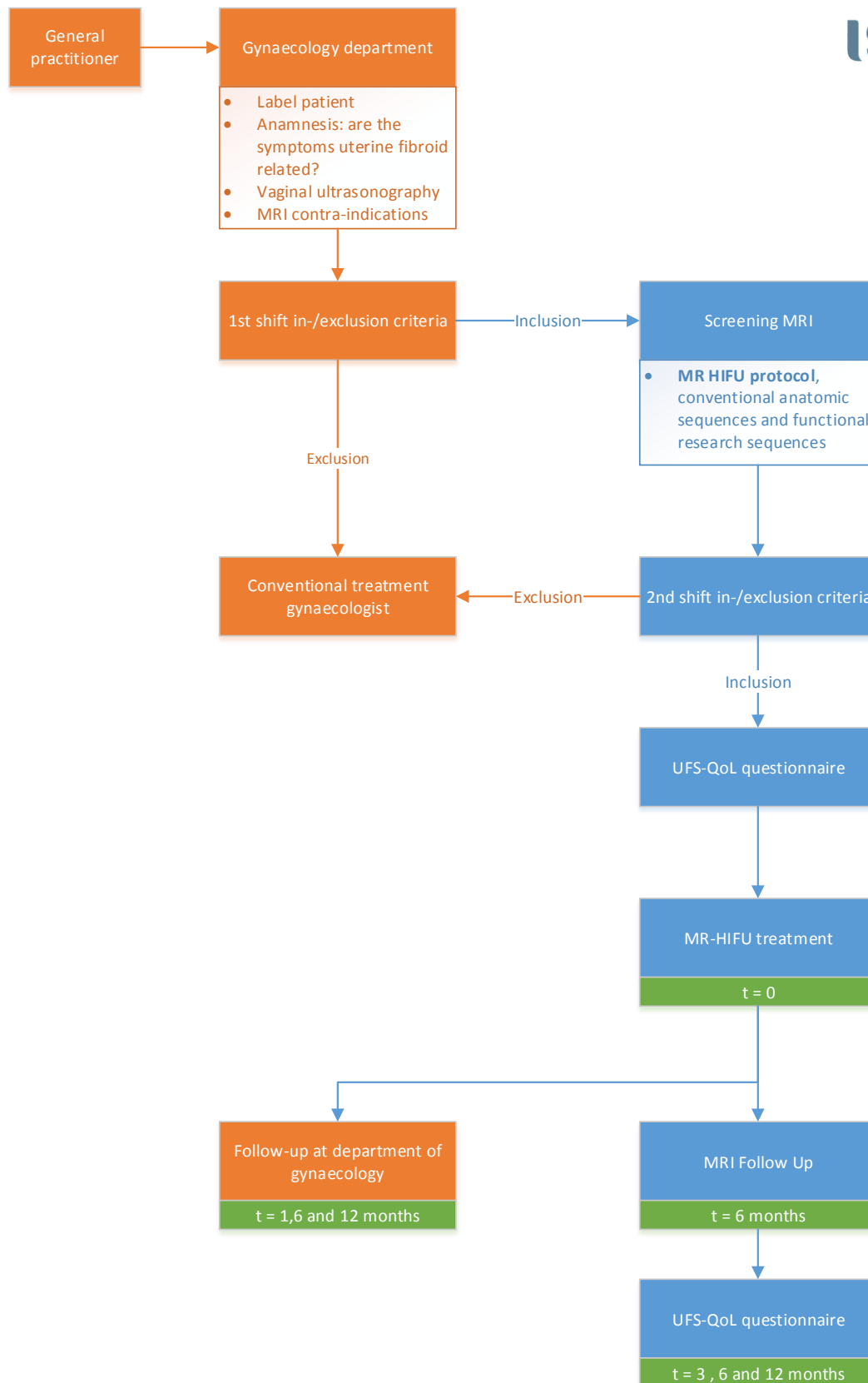


Figure 17: Flow chart of the course of events in the MASS II study

Because the MR-HIFU treatment is a new introduced treatment option in the Isala Hospital, patient satisfactory surveys are held after treatment. The survey is included in the appendix. It will be digitally send to the treated patients by Research Manager.

Relation to the MASS I study

In the MASS I study, a screenings MRI is made of patients potentially suitable for MR-HIFU treatment. This screening MRI is the same as the protocol that will be used in the MASS II study. Therefore, patients can be included in the MASS I as well as the MASS II study. The MASS I study does not have to be completed in order to start the MASS II study.

5.4 Study population

All new patients presenting with uterine fibroid related symptoms on the department of gynaecology at Isala are included in the study database. In most cases, these patients will be referred by their general practitioner for a consult on the gynaecology department. For MRI screening in preparation of the MR-HIFU treatment, only patients meeting the in-/exclusion criteria based on physical examination and vaginal ultrasonography are concerned as candidates.

For MRI screening inclusion, the following criteria are applied based on anamnesis, physical examination and vaginal ultrasonography:

- 18 – 59 years old
- Uterine fibroid related symptoms
- Pre- or perimenopausal

To determine whether the patient is eligible for the MR-HIFU treatment *after* the screening MRI, the following inclusion criteria are used.

- Type 1 & 2 uterine fibroids (based on Funaki classification)
- Diameter of 1-10 cm of dominant fibroid

Exclusion criteria for the MRI screening, based on anamnesis, physical examination and vaginal ultrasonography, are defined as follows:

- Post-menopausal
- Wish for future fertility
- Pregnancy
- Severe abdominal obesity or BMI > 40
- Uterine artery embolization in medical history
- MRI contra-indications
- Calcifications in uterine fibroid

To determine whether the patient is eligible for the MR-HIFU treatment *after* screening MRI, the following exclusion criteria are used.

- Type 3 uterine fibroids (based on Funaki classification)

- Calcified or pedunculated uterine fibroids
- Close to sciatic nerve or sacrum
- Interposition of bowel or ovary
- Diameter of < 1 cm or > 10 cm
- Distance skin – uterine fibroid > 12 cm

5.5 Study Parameters

The main study endpoint is the symptom reduction measured with the Uterine Fibroid Symptom and Health related Quality of Life questionnaire (UFS-QoL) to measure the clinical efficacy of the MR-HIFU treatment. Since uterine fibroids are benign, symptom reduction is the most important outcome in the treatment of uterine fibroids. The UFS-QoL is a standardized, worldwide accepted and used questionnaire to measure the specific symptoms caused by uterine fibroids. Based on literature, a symptom reduction of 10 points on the UFS-QoL is proven clinically efficient. The number of treated patients minimally compassing this 10 point reduction are seen as clinical efficient treatments.

Second study parameter is the non-perfused volume (NPV) ratio. Just like the UFS-QoL symptom reduction score, this parameter assesses the clinical efficacy of the MR-HIFU treatment. The NPV represent the tissue undergoing successful ablation, measured directly after the total treatment.

5.6 Study Procedures

Patients presenting with uterine fibroid related symptoms at the department of gynaecology will undergo standard consultation, physical examination and vaginal ultrasonography. When the inclusion criteria (section 4.4) are met, the screening MRI will be scheduled as soon as possible. After informed consent, patients also are asked to complete the Uterine Fibroid Symptom and Health related Quality of Life questionnaire (UFS-QoL). The UFS-QoL is a validated, disease-specific questionnaire.

The screening MRI is performed at the radiology department in the Isala. The MRI protocol consists of multiple sequences, schematically visualised below. This MRI protocol is currently researched in the MaSS study (NL52739.075.15, METC number 15.0580). A small overview of the protocol is given in table 5.

Since the multi-parametric imaging protocol is still being studied, patient's eligibility is determined on the conventional MR images. In-/exclusion criteria are given in section 4.2 and 4.3. In table 6, an explanation of the different multiparametric MRI parameters is given.

Table 5: Overview of the extensive MRI protocol used in the MASS 1 and MASS 2 study.

Conventional Imaging	Multi-parametric Imaging
T2-weighted Imaging	T2 mapping
T1-weighted Imaging	MR-DWI
CE-T1 weighted Imaging	Long-TE MR-DWI

Table 6: Multiparametric MRI parameters

MRI Sequence	Obtained Parameter	Unit	
Diffusion Weighted Imaging	Apparent Diffusion Coefficient (ADC)	mm ² /s	A measure for the magnitude of diffusion of water in the fibroid tissue.
T₂ mapping	Quantitative T ₂ value	s	Gives the quantitative T ₂ value of the fibroid tissue, which reflects the amount of moisture (and therefore blood) in the tissue.
Dynamic Contrast Enhanced Imaging	k _{trans}	min ⁻¹	Transfer constant, characterizes the diffusive transport of the contrast agent across the endothelium.

All parameters described in table 6, are measured by drawing a volume-of-interest (VOI) in the uterine fibroid. The mean value in the VOI is calculated and used for further analyses.

MR-HIFU treatment is performed when the patient is found eligible and after accurate informing the patient about the treatment and possible complications. The MR-HIFU treatment will be performed in the MRI scanner. After treatment, patients normally return home the same day of the treatment. Follow-up is conducted 3, 6 and 12 months after treatment at the gynaecologists office. A follow-up MRI is performed after 6 months. During this MRI, the same protocol will be used as before the treatment. Also, the UFS-QoL questionnaire is taken after 3, 6 and 12 months to monitor symptom relief.

5.7 Statistical Analysis

The primary study parameter are the possible MRI determinants, summarized below:

- Mean ADC values, measured by diffusion weighted imaging. It is a continuous parameter, measure in mm²/s/
- Quantitative T₂ values, measured by T₂ mapping. It is a continuous parameter measured in seconds.
- Mean k_{trans}, measured by dynamic contrast enhanced imaging. It is a continuous parameter, measured in min⁻¹

Each study parameter described above is a continuous variable. All parameters will be tested for normality with the Shapiro-Wilk test. If the parameter has a normal distribution, it will be described with the mean ± standard deviation. If the distribution is skewed, the parameter will be described with the median and the minimum/maximal values.

For each possible determinant, a regression analysis is performed. This is a logistic regression analysis, because the endpoint (a clinical relevant successful treatment with a symptom reduction of minimal 10 points) is dichotome. From this regression analysis, the regression coefficient, p-value and confidence intervals are calculated. If the p-value is smaller than 0.05, a dependency between the determinant and a clinical relevant successful treatment is confirmed. This is done for all 3 determinants.

If one or more of the determinants is confirmed, a multivariable regression is performed. This could result in a useful algorithm. However, we are aware that this algorithm has to be cross validated before use in clinical practice.

The NPV ratio and the three MRI determinants are continuous variables. All parameters will be tested for normality with the Shapiro-Wilk test. If the parameter has a normal distribution, it will be described with the mean $\pm$ standard deviation. If the distribution is skewed, the parameter will be described with the median and the minimum/maximal values.

To validate of a MRI parameter can be used in order to measure the NPV, the following steps are taken. This is done for each of the three MRI parameters.

- Scatterplot, in which the MRI parameter is plotted against the golden standard (NPV)
- Bland-Altman plot
- The ICC is calculated

6 Overall Conclusion and Future Perspectives

This study focused on the characterization of uterine fibroid tissue in preparation of Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU) treatment. Because of the diversity in fibroid tissue and the varying, but promising, results of MR-HIFU treatment, we want to chart the heterogeneity of uterine fibroid tissue in an extensive patient population.

In the MASS 1 study we explored several functional MRI parameters as well as the clinically and often used Funaki classification. In a collaboration with Philips, we set-up an extensive MRI protocol. We also focused on clinical symptom scores, as these are the most important reason to treat people with uterine fibroids. The MRI protocol, now performed in 10 patients, shows great variety between patients in size, location and tissue characteristics of the fibroid. We showed that functional MRI parameters, such as ADC maps and quantitative T2 mapping, can be measured in uterine fibroids. All parameters showed a difference between normal myometrium and uterine fibroid tissue. By including more patients in the study, we can identify different uterine fibroid tissue. We found that the inclusion of patients and the existence of MR-HIFU in the treatment possibilities is an ongoing and time consuming process. The design for the MASS 1 study, as presented in this report, will be continued until 80 patients are included in the study. The MASS I study gives a great stepping stone to the treatment studies of uterine fibroids with MR-HIFU in the Isala.

In the near future, we start with the MASS 2 study, in which patients will be screened with the extensive MRI protocol and treated with MR-HIFU. Directly after treatment, the MRI protocol is repeated. By performing this study, our aim is to find imaging predictors of success for the MR HIFU treatment. This contributes to a better patient selection and inclusion and as a result better treatment effects. At long last, we are aiming for reimbursement of MR-HIFU treatment for uterine fibroids, as this minimally invasive treatment should belong in the treatment possibilities for women suffering from uterine fibroids.

7 Abbreviations

ADC	Apparent Diffusion Coefficient
CE	Contrast Enhanced
CT	Computed Tomography
DCE	Dynamic Contrast Enhanced
DWI	Diffusion Weighted Imaging
EPI	Echo-planar Imaging
FIGO	Federation of Gynaecology and Obstetrics
GRASE	Gradient and Spin Echo
ICC	Intra Class Correlation Coefficient
IVIM	Intra Voxel Incoherent Motion
MASS	Myoma Screening Study
MR-HIFU	Magnetic Resonance guided High Intensity Focused Ultrasound
MRI	Magnetic Resonance Imaging
NPV	Non-perfused Volume
SE	Spin Echo
TE	Echo Time
TR	Repetition Time
TSE	Turbo Spin Echo
TVS	Transvaginal ultrasonography
UFS-QoL	Uterine Fibroid Symptom and Health related Quality of Life questionnaire

8 References

1. Wilde S, Scott-Barrett S. Radiological appearances of uterine fibroids. *Indian J Radiol Imaging*. 2009;19(3):222-231.
2. Murase E, Siegelman ES, Outwater EK, Perez-Jaffe LA, Tureck RW. Uterine leiomyomas: Histopathologic features, MR imaging findings, differential diagnosis, and treatment 1. *Radiographics*. 1999;19(5):1179-1197.
3. Flake GP, Andersen J, Dixon D. Etiology and pathogenesis of uterine leiomyomas: A review. *Environ Health Perspect*. 2003;111(8):1037-1054.
4. Crow J. 3 pathology of uterine fibroids. *Baillière's clinical obstetrics and gynaecology*. 1998;12(2):197-211.
5. Gizzo S, Saccardi C, Patrelli TS, et al. Magnetic resonance-guided focused ultrasound myomectomy: Safety, efficacy, subsequent fertility and quality-of-life improvements, a systematic review. *Reprod Sci*. 2014;21(4):465-476.
6. Yoon SW, Lee C, Kim KA, Kim SH. Contrast-enhanced dynamic MR imaging of uterine fibroids as a potential predictor of patient eligibility for MR guided focused ultrasound (MRgFUS) treatment for symptomatic uterine fibroids. *Obstet Gynecol Int*. 2010;2010:10.1155/2010/834275. Epub 2010 Aug 16.
7. Funaki K, Fukunishi H, Funaki T, Sawada K, Kaji Y, Maruo T. Magnetic resonance-guided focused ultrasound surgery for uterine fibroids: Relationship between the therapeutic effects and signal intensity of preexisting T2-weighted magnetic resonance images. *Obstet Gynecol*. 2007;196(2):184. e1-184. e6.
8. Tempany CM, Stewart EA, McDannold N, Quade BJ, Jolesz FA, Hynynen K. MR Imaging-guided focused ultrasound surgery of uterine leiomyomas: A feasibility study 1. *Radiology*. 2003;226(3):897-905.
9. Stewart EA, Gostout B, Rabinovici J, Kim HS, Regan L, Tempany CM. Sustained relief of leiomyoma symptoms by using focused ultrasound surgery. *Obstet Gynecol*. 2007;110(2 Pt 1):279-287.
10. Lénárd ZM, McDannold NJ, Fennessy FM, et al. Uterine leiomyomas: MR Imaging-guided focused ultrasound Surgery—Imaging predictors of success 1. *Radiology*. 2008;249(1):187-194.
11. Harding G, Coyne KS, Thompson CL, Spies JB. The responsiveness of the uterine fibroid symptom and health-related quality of life questionnaire (UFS-QOL). *Health Qual Life Outcomes*. 2008;6(1):99.
12. Dueholm M, Lundorf E, Hansen ES, Ledertoug S, Olesen F. Accuracy of magnetic resonance imaging and transvaginal ultrasonography in the diagnosis, mapping, and measurement of uterine myomas. *Obstet Gynecol*. 2002;186(3):409-415.
13. Zhao W, Chen J, Chen W. Effect of biological characteristics of different types of uterine fibroids, as assessed with T2-weighted magnetic resonance imaging, on ultrasound-guided high-intensity focused ultrasound ablation. *Ultrasound Med Biol*. 2014.
14. Funaki K, Fukunishi H, Sawada K. Clinical outcomes of magnetic resonance-guided focused ultrasound surgery for uterine myomas: 24-month follow-up. *Ultrasound in Obstetrics & Gynecology*. 2009;34(5):584-589.
15. Tamura A, Tanaka H, Ishii C, et al. Long echo time diffusion-weighted imaging (long TE-DWI) in diagnosing female pelvic tumors. . 2009.
16. Crow J. 3 pathology of uterine fibroids. *Baillière's clinical obstetrics and gynaecology*. 1998;12(2):197-211.
17. Shimada K, Ohashi I, Kasahara I, et al. Differentiation between completely hyalinized uterine leiomyomas and ordinary leiomyomas: Three-phase dynamic magnetic resonance imaging (MRI) vs. diffusion-weighted MRI with very small b-factors. *Journal of Magnetic Resonance Imaging*. 2004;20(1):97-104.

18. Ikink ME, Voogt MJ, van den Bosch, Maurice AAJ, et al. Diffusion-weighted magnetic resonance imaging using different b-value combinations for the evaluation of treatment results after volumetric MR-guided high-intensity focused ultrasound ablation of uterine fibroids. *Eur Radiol*. 2014;24(9):2118-2127.
19. Faye N, Pellerin O, Thiam R, et al. Diffusion-weighted imaging for evaluation of uterine arterial embolization of fibroids. *Magnetic resonance in medicine*. 2013;70(6):1739-1747.
20. Jacobs MA, Herskovits EH, Kim HS. Uterine fibroids: Diffusion-weighted MR imaging for monitoring therapy with focused ultrasound Surgery—Preliminary study 1. *Radiology*. 2005;236(1):196-203.
21. Liapi E, Kamel IR, Bluemke DA, Jacobs MA, Kim HS. Assessment of response of uterine fibroids and myometrium to embolization using diffusion-weighted echoplanar MR imaging. *J Comput Assist Tomogr*. 2005;29(1):83-86.
22. Park H, Yoon S, Sokolov A. Scaled signal intensity of uterine fibroids based on T2-weighted MR images: A potential objective method to determine the suitability for magnetic resonance-guided focused ultrasound surgery of uterine fibroids. *Eur Radiol*. 2015:1-4.
23. Sipola P, Ruuskanen A, Yawu L, et al. Preinterventional quantitative magnetic resonance imaging predicts uterus and leiomyoma size reduction after uterine artery embolization. *Journal of Magnetic Resonance Imaging*. 2010;31(3):617-624.
24. Lemke A, Laun FB, Simon D, Stieltjes B, Schad LR. An in vivo verification of the intravoxel incoherent motion effect in diffusion-weighted imaging of the abdomen. *Magnetic resonance in medicine*. 2010;64(6):1580-1585.
25. Jacobs MA, Gultekin DH, Kim HS. Comparison between diffusion-weighted imaging, T2-weighted, and postcontrast T1-weighted imaging after MR-guided, high intensity, focused ultrasound treatment of uterine leiomyomata: Preliminary results. *Med Phys*. 2010;37(9):4768-4776.
26. Pilatou MC, Stewart EA, Maier SE, et al. MRI-based thermal dosimetry and diffusion-weighted imaging of MRI-guided focused ultrasound thermal ablation of uterine fibroids. *Journal of Magnetic Resonance Imaging*. 2009;29(2):404-411.
27. Funaki K, Fukunishi H, Funaki T, Kawakami C. Mid-term outcome of magnetic resonance-guided focused ultrasound surgery for uterine myomas: From six to twelve months after volume reduction. *Journal of minimally invasive gynecology*. 2007;14(5):616-621.
28. Machtinger R, Inbar Y, Cohen-Eylon S, Admon D, Alagem-Mizrachi A, Rabinovici J. MR-guided focus ultrasound (MRgFUS) for symptomatic uterine fibroids: Predictors of treatment success. *Human Reproduction*. 2012;27(12):3425-3431.

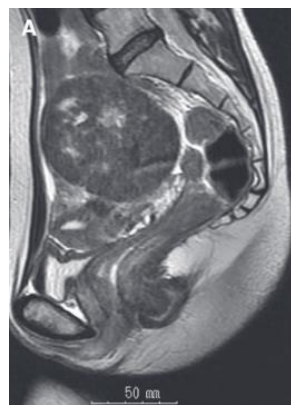
Appendix I – Funaki Classification

In 2007, Funaki et al²⁷ defined a classification system based on the T_2 signal intensity. This classification system divides the patient population in three groups as follows:



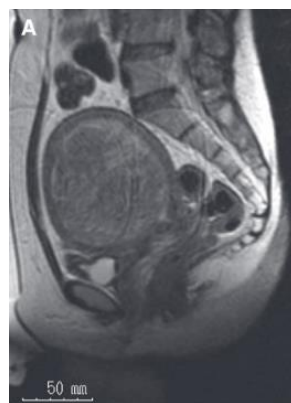
Funaki type I

The fibroid tissue has a very low T_2 signal intensity compared to skeletal muscle. The fibroid is hypo-intense.



Funaki type II

The fibroid tissue has a T_2 signal intensity lower than that of the myometrium, but higher than skeletal muscle. The fibroid is iso-intense.



Funaki type III

The fibroid has a T_2 signal intensity equal or higher than that of the myometrium. The fibroid is hyper-intense. These uterine fibroid types are associated with poor MR-HIFU treatment results.

Appendix II: Theoretical MRI background

Diffusion Weighted Imaging

In the magnetic field, all hydrogen atoms will precess with the Larmor frequency. The Larmor frequency depends on the magnetic field:

$$\omega = \gamma \times B$$

With ω as the Larmor frequency, γ the gyromagnetic ratio (42.6 MHz/T for hydrogen atoms) and B the magnetic field strength. When working in a uniform magnetic field, all the atoms precess with the same frequency. However, when applying a magnetic field gradient pulse, the Larmor frequency will lower for the atoms in a lower magnetic field, and increase for the atoms in a higher magnetic field. They will return to a uniform frequency when the gradient pulse is stopped, but the phase is changed. The result of the gradient pulse is therefore a phase difference of the atoms, depending of the position on the gradient axis. This dephasing is counteracted by applying a new gradient, but with the opposite polarity. This will lead to refocusing of the atoms.

In case of movement of the hydrogen atoms, the refocusing will not succeed resulting in a lower signal. So, diffusion weighted imaging is based on T2 weighted images with inserted additional gradients to measure the stochastic molecular motion as signal attenuation. The b-value (s/mm²) means how sensitive the images are to diffusion-based contrast. The b-value depends on the timing and the amplitude of the diffusion gradients.

$$b = \gamma^2 \times G^2 \times \delta^2 \times (\Delta - \delta/3)$$

δ is the duration of each diffusion gradient, Δ is the interval between the gradients, G the amplitude of the diffusion gradient and γ is the gyromagnetic ratio (42.6 MHz/T for hydrogen atoms). For example: the longer the time between the two gradient pulses, the more time the molecules have to diffuse. To calculate the diffusivity in a quantitative manner, at least two acquisitions with different b-values are needed. Always a measurement without the influence of diffusion is needed. This is done by using a b-value of 0 s/mm². Thereafter, one or multiple other acquisitions with different b-values are performed. The apparent diffusion coefficient (ADC) is calculated from the different images:

$$S_b = S_0 \times e^{-b \times ADC}$$

$$ADC = \frac{\ln(S_b/S_0)}{b_0 - b_b}$$

Appendix III: METC Application MASS 1 Study

RESEARCH PROTOCOL

**In depth analysis of biological tissue characteristics of
uterine fibroids using new MRI techniques**

MaSS

May 2015

PROTOCOL TITLE

In depth analysis of biological tissue characteristics of uterine fibroids using new MRI techniques

Protocol ID	NL53499.075.15
Short title	MaSS study
Simplified, dutch title	Studie naar het in beeld brengen van vleesbomen in de baarmoeder met behulp van een MRI scan
Version	1
Date	May 2015
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
ADC	Apparent Diffusion Coefficient
AE	Adverse Event
CE	Contrast Enhanced
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DCE-MRI	Dynamic Contrast Enhanced Magnetic Resonance Imaging
GCP	Good Clinical Practice
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MR-DWI	Magnetic Resonance, Diffusion Weighted, Imaging
MRI	Magnetic Resonance Imaging
MR-HIFU	Magnetic Resonance guided High Intensity Focused Ultrasound
(S)AE	(Serious) Adverse Event
SI	Signal Intensity
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
UFS-QoL	Uterine Fibroid Symptom and Health related Quality of Life
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgegevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

SUMMARY

Rationale:

Recent clinical evidence shows that uterine fibroid tissue can be very heterogeneous. To refine the stratification of uterine fibroids, more biological information is needed on tissue level. Therefore, in this study we want to analyze a completely new MRI protocol for the characterization of uterine fibroid tissue using multiparametric MRI techniques.

Objective:

The primary objective is to determine new biological properties of uterine fibroid tissue using advanced MRI image sequences and to correlate these properties with the currently used simple Funaki classification and with clinical symptoms.

Study design:

The proposed research will concern a single-center, explorative research, performed on the departments of gynecology and radiology.

Study population:

The study population consists of women, diagnosed with a uterine fibroid and presenting uterine fibroid related symptoms.

Main study parameters/endpoints:

The main exploratory study parameter is the distribution and variation of MRI parameters (ADC map, K^{trans} map, v_e , v_p and T_2 map), and the correlation between the MRI parameters and the Funaki score and clinical UFS-QoL score.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness

Patients included in this study will undergo an MRI examination before proceeding to treatment. During this MRI, use is made of a gadolinium-based MRI contrast agent (Dotarem). This macro-cyclic coated, intra-venous contrast agent is routinely used in current practice and adverse effects are scarce. Besides, patients are asked to complete the UFS-QoL questionnaire.

1. INTRODUCTION AND RATIONALE

Uterine fibroids are the most common benign gynecological tumors affecting a high percentage of women. The life-time prevalence of fibroids lies between 70 to 80%. Symptoms of uterine fibroids are abnormal uterine bleeding and pelvic discomfort due to the existing mass, leading to a significant negative effect on the quality of life. Treatment possibilities include drug therapy, hysterectomy, myomectomy, uterine artery embolization and magnetic resonance guided “High Intensity Focused Ultrasound” (MR-HIFU). The choice of treatment and rate of success is dependent on the morphology and tissue characterization of the uterine fibroids¹.

Histopathology of uterine fibroids

From literature it is known that the histopathology of uterine fibroids varies among patients. Based on the histopathology, multiple subtypes of uterine fibroids are recognized. Uterine fibroids are composed of smooth muscle fiber bundles, surrounded by collagenous fibrous connective tissue within which are blood vessels. Also variable numbers of mast cells are present. The density of each of these cell types is different for the various types of uterine fibroids². Biological characterization of fibroid tissue is useful knowledge in the choice and suitability of treatment options^{1,2}. For example, the treatment effects of drug therapy are lower in patients with a high proportion of collagenous tissue because of its unresponsiveness². MRI can also provide information about the suitability for a uterine artery embolization¹.

Without biopsy it is in current practice impossible to characterize tissue characteristics before treatment. However, recent new, multiparametric MRI parameters are developed by Philips Healthcare (Eindhoven) that can be used as *non-invasive* biomarkers. This set of new MRI parameters can be used to visualize and gain knowledge about the biological variation and characterization of uterine fibroids. This could be important in the choice of the most appropriate minimal invasive therapy.

Current imaging-based characterization

Current imaging-based biological characterizations of uterine fibroids are limited. The signal intensity on T₂-weighted MRI images is the main parameter of characterization. This parameter is described by the Funaki classification, defined by Funaki et al. in 2007³. In the Funaki classification, patients are stratified in 3 groups based on T2 signal intensity in selection of MR HIFU treatment. Details of the Funaki classification are listed in appendix 1. This subdivision of uterine fibroids is correlated with pathohistologic structures, demonstrated by Zhao et al. in 2014⁴.

Novel MRI techniques for imaging-based biological characterization

Three novel MRI techniques can potentially provide extra information about the biological characterization of uterine fibroids. These sequences have been specifically developed (by Philips healthcare, Eindhoven) for the use in uterine fibroids and will be eluded separately below.

1. Dynamic contrast enhanced MRI (DCE)

Shows the vascular distribution and perfusion within the uterine fibroid⁵. This vascular distribution is quantified by the **transfer constant K^{trans}**

- 2. Diffusion weighted MRI (DWI)** This parameter visualizes the *perfusion* (microcirculation of blood in the capillaries) and *diffusion* of free water molecules in the uterine fibroid. The **ADC parameters** are used as quantitative values.
- 3. T₂ mapping** Quantitative T2 measurement, reflecting the blood fraction in the fibroid. The **T2 relaxation time** is the quantitative parameter of this sequence.

From literature, it is known that dynamic contrast enhanced MRI (DCE) is one of the most commonly adopted perfusion MR techniques in the field of oncologic imaging, because it can quantitatively measure important perfusion parameters. The use of DCE MRI in uterine fibroids is described in some case reports^{5,6} and one small study consisting of 10 patients⁷. These studies correlate the vascular distribution to treatment outcome of MR-HIFU treatment.

Diffusion weighted MRI (DWI) for uterine fibroids is used in multiple studies to characterize the uterine fibroid tissue^{8,9}. With the use of different b-values, perfusion and diffusion can both be imaged.

T₂ mapping is a, not previously described, new technique for the specific use in uterine fibroids. The Funaki classification³ is also based on T₂ relaxation times. T₂ mapping quantifies this parameter, making it more reliable and more widely applicable.

The knowledge from the literature, as described above, is gained from small patient groups and the combination of the different techniques is not described. With this study, we want to create further insight in these techniques, which could be of importance later in choosing the most appropriate minimal invasive therapy for each individual patient. The new sequences could give the opportunity for treatment selection of patients, provided that the parameters have a distinctive character.

Rationale

The Funaki classification alone is a limited characterization parameter based on the average value of the full fibroid. Further, it classifies the entire patient population in only 3 groups. Recent clinical insights shows that the uterine fibroid tissue can be very heterogeneous. To refine the stratification, more information is needed on tissue level. Therefore, in this study we want to analyze a completely new MRI protocol for the non-invasive characterization of uterine fibroid tissue using multiparametric MRI.

In Isala Hospital, on average 210 patients present annually with physical complains that can be attributed to uterine fibroids. This makes the Isala an ideal hospital for this research proposal.

2. OBJECTIVES

Primary Objective:

To determine new biological properties of uterine fibroid tissue using advanced MRI image sequences and to correlate these properties with the currently used simple Funaki classification and with clinical symptoms.

Secondary Objective(s):

To determine the correlation of symptom reduction after 3 months of medically treated patients with the in this study evaluated new, extra MRI parameters.

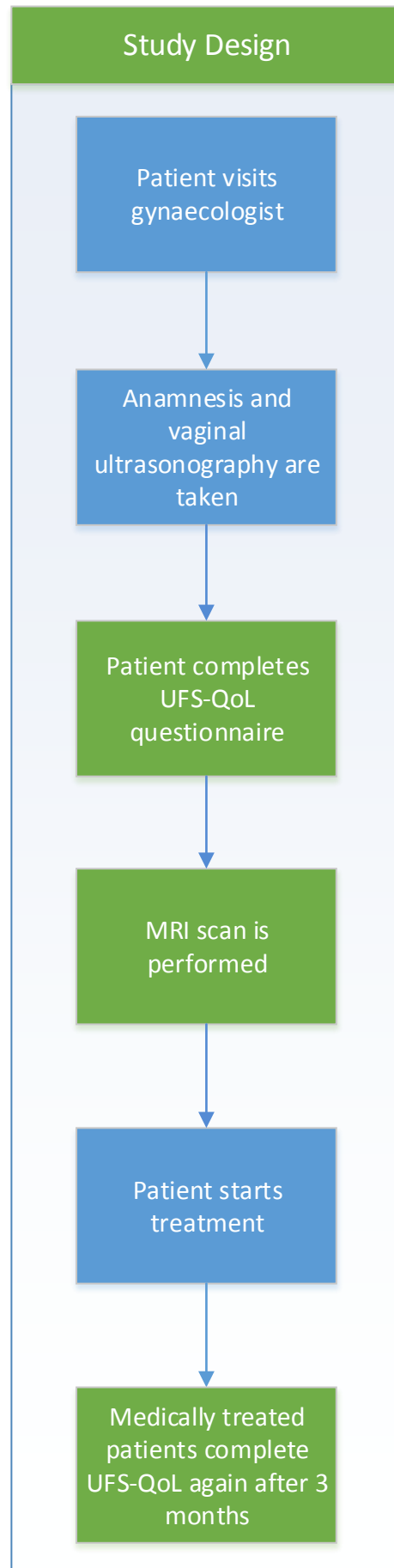
3. STUDY DESIGN

The proposed research will concern a single-center, explorative research. Data collection and experiments are performed in the Isala Zwolle at the gynecology and radiology department.

The flow-chart on page 12 shows the course of events. On the left, the course of events in current practice is shown. The green fields on the right are the extra study events.

All new patients presenting with fibroid related symptoms in the gynecology department are registered in the study database. Based on the in-/exclusion criteria as further described in section 4.2 and 4.3, only a selective patient group will undergo the screening MRI.

With the screening MRI, biological tissue parameters are determined with a new MRI protocol. These tissue parameters are correlated to the existing tissue classification method (Funaki classification) and clinical findings. Clinical findings are examined by means of the Uterine Fibroid Symptom and health related Quality of Life questionnaire (UFS-QoL). Medically treated patients will be asked to complete the UFS-QoL questionnaire again after 3 months.



4. STUDY POPULATION

4.1 Population (base)

210 new patients per year present themselves in Isala with complains that can be attributed to uterine fibroids.

4.2 Inclusion criteria

Patients with a diagnosed uterine fibroid (based on anamnesis, physical examination and vaginal ultrasonography) and uterine fibroid related symptoms are included in the study.

4.3 Exclusion criteria

Exclusion criteria for the MRI screening, based on anamnesis, physical examination and vaginal ultrasonography, are defined as follows:

- Post-menopausal patients
- Pregnant patients
- Calcified uterine fibroids
- Severe abdominal obesity
- Uterine artery embolization in medical history
- MRI contra-indications

4.4 Sample size calculation

Sample size is determined based on the objective of the study: the distribution and variation in quantitative MRI parameters. From literature it is known, type 3 uterine fibroids (based on the Funaki classification) are difficult to treat. Therefore, distribution and variation is especially of added value in type 3 uterine fibroids. When looking at literature about the incidence of type 3 uterine fibroids, an average incidence of 20-25% is seen. For a reliable comparison between patients with type 3 uterine fibroids, an inclusion of 80 patients is desirable. In this case, we can include about 20 patients with type 3 uterine fibroids.

5. METHODS

5.1 Study parameters/endpoints

5.1.1 Main study parameter/endpoint

Distribution and variation of MRI parameters (ADC map, K^{trans} map, v_e , v_p and T_2 map), and the correlation between the MRI parameters and the Funaki score and clinical UFS-QoL score.

5.1.2 Secondary study parameters/endpoints

Correlation between UFS-QoL improvement after 3 months and the MRI parameters (ADC map, K^{trans} map, v_e , v_p and T_2 map) in medically treated patients

5.2 Randomisation, blinding and treatment allocation

Not applicable

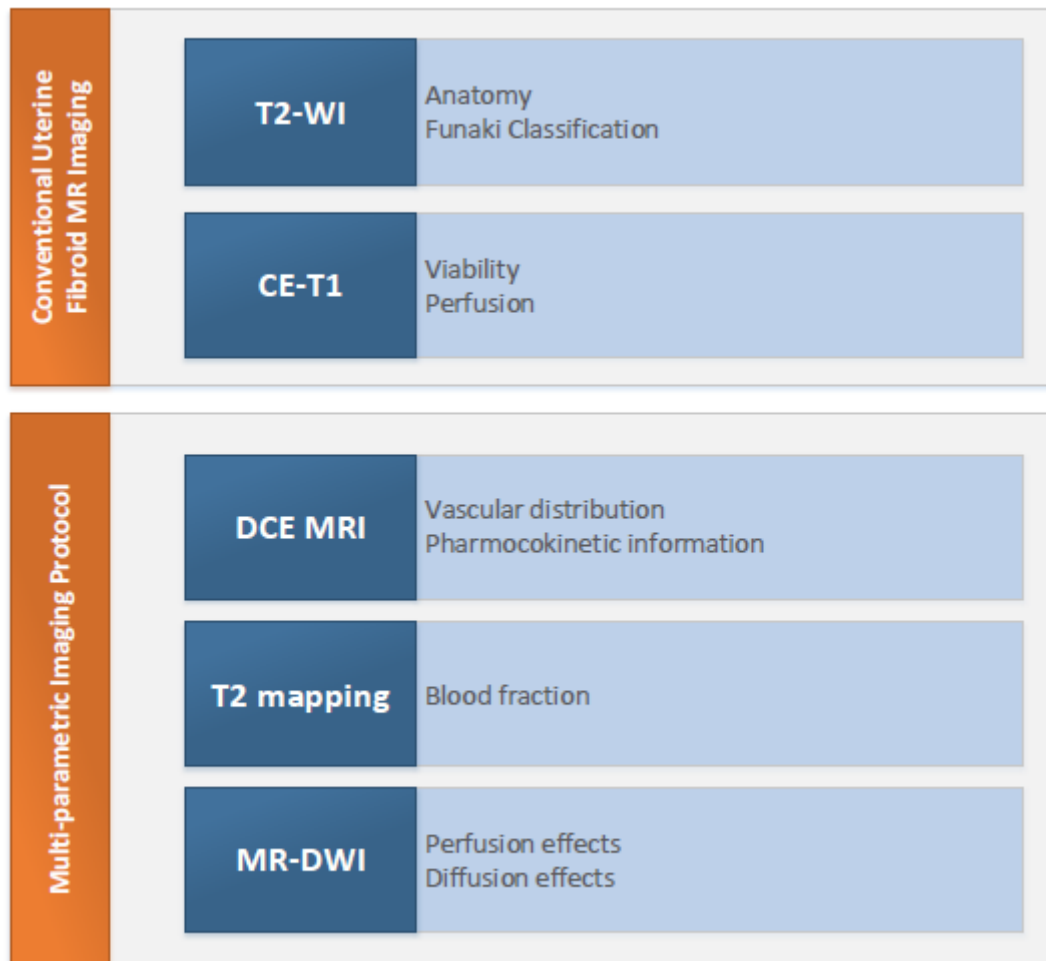
5.3 Study procedures

Patients presenting with uterine fibroid related symptoms at the department of gynaecology will undergo standard consultation, physical examination and vaginal ultrasonography.

When the inclusion and exclusion criteria (section 4.2 and 4.3) are met, the patient will be asked informed consent and the screening MRI will be scheduled as soon as possible. Patients also are asked to complete the Uterine Fibroid Symptom and Health related Quality of Life questionnaire (UFS-QoL). The UFS-QoL is a validated, disease-specific questionnaire.

The screening MRI is performed at the radiology department in the Isala. The MRI protocol consists of multiple sequences, schematically visualised below. The first two sequences are part of the conventional protocol for patients undergoing pelvic MRI with an uterine fibroid indication. Three sequences are added to this protocol for evaluation of the new, multi-parametric classification approach.

Medically treated patients will be asked to complete the UFS-QoL questionnaire again after 3 months.



5.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

5.5 Replacement of individual subjects after withdrawal

Not applicable

5.6 Follow-up of subjects withdrawn from treatment

Not applicable

6. SAFETY REPORTING

6.1 Section 10 WMO event

In accordance with section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

6.2 Adverse events (AEs) and Serious adverse events (SAEs)

6.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the screening MRI as described in this protocol. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

6.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse events.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

6.3 Annual safety report

Not applicable

6.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

7. STATISTICAL ANALYSIS

7.1 Primary study parameter(s)

Descriptive statistics

The different variables are statistically described as follows:

- MRI parameters (ADC map, K^{trans} map, v_e , v_p and T_2 map) are quantitative continuous variables. Each parameter will be tested for normality with the Shapiro-Wilk test. If the parameter has a normal distribution, it will be described with the mean $\pm$ standard deviation. When the distribution is skewed, the parameter will be described with the median and the minimum/maximal value.
- The Funaki classification is a categorical variable (values 1, 2 and 3). This variable will be described by the number of patients within each group and the corresponding percentages.
- The UFS-QoL questionnaire score is a discrete variable (range 0-100). It will be described with the median and minimum/maximal value. The Shapiro-Wilk test is used to test the normality.

Variation in MRI parameter

To describe the variation in each MRI parameter (ADC map, K^{trans} map, v_e , v_p and T_2 map), the coefficient of variation is used for all parameters. Because all MRI parameters have different units, a standardized measure of dispersion is used to compare the variation of each parameter.

MRI parameters versus Funaki classification

To **describe** the relationship between the MRI parameters and the Funaki classification, scatterplots and boxplots are made for each MRI parameter (ADC map, K^{trans} map, v_e , v_p and T_2 map).

To **statistical test** the relationship between each MRI parameter and the Funaki classification, the Spearman's rank correlation coefficient is used. This univariate analysis is performed for each MRI parameter (ADC map, K^{trans} map, v_e , v_p and T_2 map) in relation to the Funaki classification.

UFS-QoL score on t_0 versus MRI parameters and Funaki classification

To **describe** the relationship between the MRI parameters and the UFS-QoL score on t_0 , scatterplots and boxplots are made for each MRI parameter (ADC map, K^{trans} map, v_e , v_p and T_2 map) and the Funaki classification.

To **statistical test** which parameter is the best predictor for clinical symptoms, regression analysis on the UFS-QoL will be performed. If the UFS-QoL variable has a normal distribution, linear regression is used for the correlation between the UFS-QoL score and the MRI parameter. If the distribution of the variable is skewed, a logistic transformation is performed. When the distribution is normalized after this transformation, linear regression is used. When the variable is still skewed after the transformation, the UFS-QoL is dichotomized, based on the median. After

dichotomization, logistic regression is used for the association between the UFS-QoL score and the MRI parameters.

7.2 Secondary study parameter(s)

Descriptive statistics

The difference in UFS-QoL score after 3 months (Δ UFS-QoL) will be described with the means ($\pm$ sd) or median (minimum-maximal) value, in the case of skewed distribution. The Shapiro-Wilk test is used to test normality.

Correlation between Δ UFS-QoL and the MRI parameters

The association between the MRI parameters (ADC map, K^{trans} map, v_e , v_p , T_2 map and Funaki classification) and the difference in UFS-QoL score after 3 months (Δ UFS-QoL) is determined univariate as well as multivariable. If the Δ UFS-QoL variable has a normal distribution, linear regression is used for the correlation between the Δ UFS-QoL score and the MRI parameter. If the distribution of the variable is skewed, a logistic transformation is performed. When the distribution is normalized after this transformation, linear regression is used. When the variable is still skewed after the transformation, the Δ UFS-QoL is dichotomized, based on the median. After dichotomization, logistic regression is used for the association between the Δ UFS-QoL score and the MRI parameters.

8. ETHICAL CONSIDERATIONS

8.1 Regulation statement

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki. The study will be conducted in compliance with the protocol. The protocol and any Amendments and the subjects Informed Consent will receive Institutional Review Board (IRB/Independent Ethics Committee (IEC) approval/favorable opinion prior to initiation of the study.

8.2 Recruitment and consent

Freely written informed consent will be obtained from every subject prior to study participation, including informed consent for any screening procedures conducted to establish subject eligibility for the study.

8.3 Benefits and risks assessment, group relatedness

Subjects meeting all inclusion criteria without meeting any exclusion criterion will be eligible for the screening MRI. Patients entering the screening MRI study do not directly benefit from study participation. Risks associated with study participation are negligible. The contrast agent used in this study (Dotarem) is a monocyclic coated gadolinium-based contrast agent. The occurrence of Nephrogenic systemic fibrosis (NSF) is according to the FDA not recurred. Also allergic reactions are very rare.

8.4 Compensation for injury

Statutory obligation to provide insurance is at time of writing under construction.

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 Handling and storage of data and documents

Research Manager is used in this study for the storage of data. For each module, specific rights are assigned. All gynaecologists can include patients in this database. At inclusion, all patients will be assigned a unique study number. The database which links the study number to the Isala patients numbers is only available to coordinating investigators.

9.2 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

9.3 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

9.4 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the latest patient inclusion and MRI scan.

In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

REFERENCES

1. Murase E, Siegelman ES, Outwater EK, Perez-Jaffe LA, Tureck RW. Uterine leiomyomas: Histopathologic features, MR imaging findings, differential diagnosis, and treatment 1. *Radiographics*. 1999;19(5):1179-1197.
2. Crow J. 3 pathology of uterine fibroids. *Baillière's clinical obstetrics and gynaecology*. 1998;12(2):197-211.
3. Funaki K, Fukunishi H, Funaki T, Sawada K, Kaji Y, Maruo T. Magnetic resonance-guided focused ultrasound surgery for uterine fibroids: Relationship between the therapeutic effects and signal intensity of preexisting T2-weighted magnetic resonance images. *Obstet Gynecol*. 2007;196(2):184. e1-184. e6.
4. Zhao W, Chen J, Chen W. Effect of biological characteristics of different types of uterine fibroids, as assessed with T2-weighted magnetic resonance imaging, on ultrasound-guided high-intensity focused ultrasound ablation. *Ultrasound Med Biol*. 2014.
5. Liu J, Keserci B, Yang X, et al. Volume transfer constant (K trans) maps from dynamic contrast enhanced MRI as potential guidance for MR-guided high intensity focused ultrasound treatment of hypervascular uterine fibroids. *Magn Reson Imaging*. 2014;32(9):1156-1161.
6. Yoon SW, Lee C, Kim KA, Kim SH. Contrast-enhanced dynamic MR imaging of uterine fibroids as a potential predictor of patient eligibility for MR guided focused ultrasound (MRgFUS) treatment for symptomatic uterine fibroids. *Obstet Gynecol Int*. 2010;2010:10.1155/2010/834275. Epub 2010 Aug 16.
7. Kim YS, Lim HK, Kim JH, et al. Dynamic contrast-enhanced magnetic resonance imaging predicts immediate therapeutic response of magnetic resonance-guided high-intensity focused ultrasound ablation of symptomatic uterine fibroids. *Invest Radiol*. 2011;46(10):639-647.
8. Shimada K, Ohashi I, Kasahara I, et al. Differentiation between completely hyalinized uterine leiomyomas and ordinary leiomyomas: Three-phase dynamic magnetic resonance imaging (MRI) vs. diffusion-weighted MRI with very small b-factors. *Journal of Magnetic Resonance Imaging*. 2004;20(1):97-104.
9. Ikink ME, Voogt MJ, van den Bosch, Maurice AAJ, et al. Diffusion-weighted magnetic resonance imaging using different b-value combinations for the evaluation of treatment results after volumetric MR-guided high-intensity focused ultrasound ablation of uterine fibroids. *Eur Radiol*. 2014;24(9):2118-2127.

Studie naar het in beeld brengen van vleesbomen in de baarmoeder met behulp van een MRI scan

Inleiding

Geachte mevrouw,

U bent gevraagd om mee te doen aan de hierboven genoemde medisch-wetenschappelijke studie (zie titel).

Om te beoordelen of u mee wilt doen, is goede voorlichting van onze kant nodig, en een zorgvuldige afweging van uw kant. Vandaar dat u deze schriftelijke informatie ontvangt. U kunt deze rustig (her)lezen en in eigen kring bespreken. Ook daarna kunt u nog altijd vragen stellen aan de artsen/onderzoekers die aan het eind van deze informatie staan genoemd.

1. Wat is het doel van het onderzoek?

Het doel van dit onderzoek is om vleesbomen in de baarmoeder af te beelden met een MRI scan. Met dit onderzoek wordt gekeken of de MRI scan meer informatie kan geven over vleesbomen.

2. Welk product, geneesmiddel, medisch hulpmiddel of behandeling wordt onderzocht?

Het extra MRI onderzoek wordt in deze studie onderzocht.

3. Hoe wordt het onderzoek uitgevoerd?

Nadat bij u is vastgesteld dat u een vleesboom in uw baarmoeder heeft, zal u gevraagd worden aan het onderzoek deel te nemen. Als u dit wilt, zal een afspraak worden gemaakt voor het MRI onderzoek. Het MRI onderzoek heeft geen invloed op uw behandeling.

4. Wat wordt er van u verwacht?

Van u wordt verwacht een extra MRI scan te ondergaan. Voor deze MRI scan zijn thuis geen extra voorbereidingen nodig. Tijdens het onderzoek krijgt u een koptelefoon met muziek om het kloppende geluid van de MRI te dempen. Ook krijgt u een infuus in uw arm. Door dit infuus wordt contrastmiddel in uw bloedvaten ingebracht. Voor uitgebreide informatie over een MRI scan, kunt u kijken op de website van de Isala: <http://www.isala.nl/patienten/folders/5707-mri>

Ook wordt u gevraagd om een vragenlijst in te vullen. Deze krijgt u digitaal in via e-mail. Mocht u liever een vragenlijst op papier ontvangen, dan is dit ook mogelijk. Wanneer u door de gynaecoloog medicijnen krijgt voorgeschreven, vragen we u de vragenlijst nogmaals na 3 maanden in te vullen.

5. Wat is meer of anders dan de reguliere behandelingen die u ontvangt?

Uw reguliere behandeling zal niet anders zijn.

6. Welke bijwerkingen kunt u verwachten?

De arts zal met u een vragenlijst doornemen om uw veiligheid in de MRI te garanderen. Het contrastmiddel wat in uw bloedvaten wordt gespoten, kan bij sommige patiënten een allergische reactie oproepen. Dit zou een warm gevoel, hoofdpijn of misselijkheid kunnen geven. Deze reacties zijn zeer zeldzaam.

7. Wat zijn de mogelijke voor- en nadelen van deelname aan dit onderzoek?

Dit onderzoek kan nuttige wetenschappelijke gegevens voor de toekomst leveren. Er zitten geen nadelen aan deelname aan dit onderzoek.

8. Wat gebeurt er als u niet wenst deel te nemen aan dit onderzoek?

Deelname aan dit onderzoek is geheel vrijwillig. Als u niet wilt deelnemen, hoeft u daarvoor geen reden op te geven. Als u besluit niet mee te doen, geeft dat geen enkele verandering in uw verdere behandeling of begeleiding. Ook als u nu toestemming geeft, kunt u die te allen tijde zonder opgave van redenen weer intrekken. Het onderzoek zal zo nauwkeurig mogelijk volgens plan verlopen. Als uw veiligheid of welbevinden in gevaar zijn, beëindigt de onderzoeker uw deelname aan het wetenschappelijk onderzoek direct.

9. Bent u verzekerd wanneer u aan het onderzoek meedoet?

Voor de deelnemers aan dit onderzoek is een verzekering afgesloten bij Landschot en Chabot. Deze verzekering dekt schade door letsel of overlijden als gevolg van deelname aan het onderzoek, en die zich gedurende de deelname aan het onderzoek openbaart, of binnen vier jaar na beëindiging van de deelname aan het onderzoek.

Zie voor verzekerde bedragen, de uitsluiting gronden en adresgegevens van de verzekeraar, de bijlage.

10. Wat gebeurt er met uw gegevens?

Tot uw persoon herleidbare gegevens kunnen slechts met uw toestemming (aan te geven op het toestemmingsformulier) door derden worden ingezien. En dan ook alleen als zij hiertoe bevoegd zijn, zoals medewerkers van het onderzoeksteam, medewerkers van de Inspectie voor de Gezondheidszorg (IGZ) of bevoegde inspecteurs van een buitenlandse overheid, en leden van de Medisch Ethische Toetsingscommissie. Inzage kan nodig zijn om de betrouwbaarheid en kwaliteit van het onderzoek na te gaan. Onderzoeksgegevens worden gehanteerd met inachtneming van de Wet Bescherming Persoonsgegevens.

Persoonsgegevens die tijdens de studie worden verzameld, worden vervangen door een codenummer. Alleen dat nummer wordt gebruikt voor studiedocumentatie, in rapporten of publicaties over dit onderzoek. Slechts degene die de sleutel van de code heeft (de onderzoeker/ behandelend arts) weet wie de persoon achter het codenummer is.

Uw persoonsgegevens worden bewaard gedurende het onderzoek en na afloop vernietigd, of (indien van toepassing) de gegevens worden gedurende vijftien jaar bewaard.

11. Zijn er extra kosten wanneer u besluit aan dit onderzoek mee te doen?

Voor dit onderzoek dient u een keer extra naar het ziekenhuis te komen. Voor eventuele reiskosten kunt u contact met ons opnemen. Wanneer u al een andere afspraak in de Isala heeft staan, proberen we deze te combineren. De MRI scan brengt voor u geen extra kosten met zich mee.

12. Is het onderzoek goedgekeurd door een medisch-ethische toetsingscommissie?

Voor dit onderzoek is goedkeuring verkregen van de Medisch Ethische Toetsingscommissie van de Isala klinieken te Zwolle. De voor dit onderzoek geldende nationale en internationale richtlijnen worden nauwkeurig in acht genomen.

13. Wilt u verder nog iets weten?

Voor het stellen van vragen en het inwinnen van nadere informatie voor, tijdens en na het onderzoek is een niet bij het onderzoek betrokken arts beschikbaar. Indien u behoefte heeft aan een onafhankelijk advies over dit onderzoek, kunt u terecht bij Walter Kuchenbecker (w.k.h.kuchenbecker@isala.nl, 038 424 7010).

Voor inhoudelijke vragen kunt u ook altijd contact op nemen met de één van de hoofdonderzoekers:

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038 424 7011

RESEARCH PROTOCOL

Minimal invasive treatment of Uterine Fibroids with Magnetic Resonance guided High Intensity Focused Ultrasound – the search for revealing imaging parameters

January 2016

PROTOCOL TITLE

Protocol ID	
Short title	MASS II
Simplified, dutch title	Minimaal-invasieve behandeling van vleesbomen met MR-HIFU
Version	1.0
Date	19/02/2016
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
ADC	Apparent Diffusion Coefficient
AE	Adverse Event
CE	Contrast Enhanced
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DCE-MRI	Dynamic Contrast Enhanced Magnetic Resonance Imaging
GCP	Good Clinical Practice
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MR-DWI	Magnetic Resonance, Diffusion Weighted, Imaging
MRI	Magnetic Resonance Imaging
MR-HIFU	Magnetic Resonance guided High Intensity Focused Ultrasound
mp-MRI	Multi-parametric Magnetic Resonance Imaging
NPV	Non-perfused Volume
ROI	Region of Interest
(S)AE	(Serious) Adverse Event
SI	Signal Intensity
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
UFS-QoL	Uterine Fibroid Symptom and Health related Quality of Life
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgegevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

ENGLISH SUMMARY

Rationale:

Patient selection and inclusion for treatment of uterine fibroids with MR-HIFU is worldwide still in progress. Results of the MR-HIFU treatment show a large variety in volume- and symptom-reduction. Knowledge of MRI based predictors of success prior to treatment, may contribute to an optimization of patient selection, treatment planning and treatment outcomes.

Objective:

We are looking for MRI determinants to determine patient suitability for a successful treatment of symptomatic uterine fibroids using MR-HIFU. Also, we want to eliminate the use of an intravenous contrast agent. Therefore we want to determine the feasibility of 3 MRI parameters to monitor thermal ablation effects.

Study design:

The proposed research will concern a single-center research, performed on the departments of gynecology and radiology. Fifty patients will be included in this study. Patients undergoing MR-HIFU treatment within the clinical trial, will additionally undergo three extra MRI sequences and are asked to fulfill the UFS-QoL four times. Also, a patient satisfactory survey is held for the new introduced MR-HIFU treatment.

Study population:

The study population consists of women, diagnosed with a uterine fibroid and presenting uterine fibroid related symptoms willing to undergo MR-HIFU treatment. All gynecological and MRI in-/exclusion criteria must be met.

Main study parameters/endpoints:

The main study parameter is regression analysis to find MRI determinants for a clinical successful treatment. MRI parameters are ADC values, quantitative T_2 value and k_{trans} values.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Patients included in this study will undergo three extra MRI sequences during MRI examination. Also the patients are asked to fulfill the UFS-QoL questionnaire four times. The additional MRI sequences have no additional risks compared to the standard MRI protocol.

NEDERLANDSE SAMENVATTING

Rationale:

Patiënt selectie en inclusie voor de behandeling van uterus myomen met MR-HIFU is wereldwijd een belangrijk onderzoeksgebied. De resultaten van de MR-HIFU behandeling laten een grote variëteit zien in volume- en symptoom reductie. Kennis van MRO gebaseerde succesvoorspellers vóór behandeling kunnen bijdragen aan een optimalisatie van patiënt selectie, behandelingsplanning en behandelresultaten.

Doelstelling:

We zijn op zoek naar MRI determinanten om de patiënt-geschiktheid voor een succesvolle MR-HIFU behandeling te bepalen. Ook willen we het gebruik van intraveneus contrastmiddel proberen te elimineren. Daarvoor bepalen we de mogelijkheden van drie MRI-parameters om de thermale ablatie effecten (en daarmee het behandel-effect) te monitoren.

Studie opzet:

Het voorgestelde onderzoek zal een single-center onderzoek zijn, uitgevoerd op de afdelingen gynaecologie en radiologie. Vijftig patiënten zullen worden geïnccludeerd. Patiënten die een MR-HIFU behandeling zullen ondergaan in studie verband, zullen drie extra MRI sequenties ondergaan. Ook worden ze gevraagd vier keer de UFS-QoL vragenlijst in te vullen en daarbij één keer een patiënt tevredenheidsonderzoek.

Studie populatie:

De studie populatie bestaat uit vrouwen, gediagnosticeerd met een uterus myoom, bekend met myoom gerelateerde klachten en bereid om een MR-HIFU behandeling te ondergaan. De patiënten moeten voldoen aan alle gynaecologische en MRI in-/exclusie criteria.

Belangrijkste studie parameter/eindpunt:

De belangrijkste studie parameter is de regressie analyse om zo MRI determinanten te vinden voor een klinisch succesvolle behandeling. De MRI parameters zijn: ADC, kwantitatieve T_2 en k_{trans} .

Aard en omvang van de lasten en risico's verbonden aan deelname:

Patiënten die worden geïnccludeerd zullen drie extra MRI sequenties ondergaan. Deze drie sequenties geven geen additionele risico's bovenop het standaard protocol. Ook wordt aan de patiënten gevraagd om de UFS-QoL vragenlijst viermaal in te vullen.

INTRODUCTION AND RATIONALE

Uterine fibroids are the most common benign gynecological tumors affecting a high percentage of women. Symptoms of uterine fibroids are abnormal uterine bleeding and pelvic discomfort due to the existing mass, leading to a significant negative effect on the quality of life. Treatment possibilities include drug therapy, hysterectomy, myomectomy, uterine artery embolization and recently magnetic resonance guided “High Intensity Focused Ultrasound” (MR-HIFU).

Surgical approaches often go along with complications, longer hospital stays and longer recovery times. The advantages of the minimally invasive MR-HIFU treatment are a lower morbidity, no need for general anesthesia, no radiation exposure, low probability of bleeding and infection and short recovery times. Patients return to work approximately 24 hours after treatment¹.

Thermal ablation using MR-HIFU allows for a non-invasive treatment of uterine fibroids by selective tissue heating. The heat generating possibilities of ultrasound are combined with the imaging capabilities of MRI. The MRI facilitates in treatment planning, thermometry for real-time temperature feedback during the treatment and for evaluation of treatment outcome. The energy of the focused ultrasound causes heating of the tissue on the focal spot to locally induce coagulative necrosis, while the surrounding tissue remains unaffected. In figure 1, a schematic view of the MR-HIFU system is shown. The ultrasound transducer is incorporated in the MRI table, on which the patients lies in prone position during the treatment.

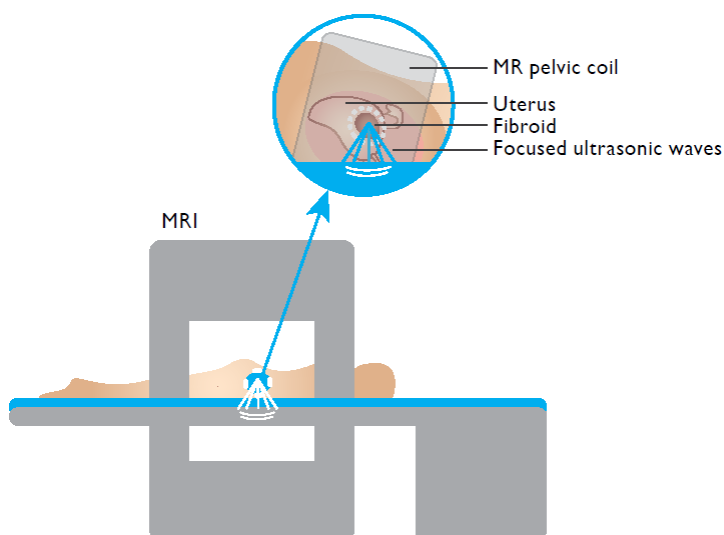


Figure 18: MR HIFU system

Result assessment of the MR-HIFU treatment is mostly done by means of two important outcome parameters: NPV (non-perfused volume) ratio and the UFS-QoL (Uterine Fibroid Symptom and Health related Quality of Life) improvement after a follow-up of 6-12 months. The NPV is defined as the non-perfused areas of gadolinium-enhanced images immediately after treatment. The NPV ratio gives the percentage of treated uterine fibroid tissue.

Many studies are published on the safety, efficacy and quality of life improvements. The systematic review of Gizzo et al.² gives a good summary. A NPV ratio of >50% was achieved in 60% of the studies (16-98%), with a mean UFS-QoL improvement after 12 months of 31 points (15-66).

Among all treated patients, a large variety is seen in volume and symptom reduction, despite of clear exclusion criteria. There already has been some research on predictors of success of the MR-HIFU treatment. In these studies, the signal intensity of the fibroid on T₂-weighted images was the main parameter of characterization. T₂ signal intensity (SI) of the uterine fibroid is compared with the SI of skeletal muscle. In 2007, Funaki et al.³ defined a classification system based on the T₂ signal intensity : type 1, a very low-intensity uterine fibroid image comparable to skeletal muscle (hypo-intense); type 2, an image intensity lower than that of the myometrium and higher than that of skeletal muscle (iso-intense); and type 3, an image intensity equal or higher than that of myometrium. Multiple studies show that type 1 and 2 fibroids are associated with a higher chance of treatment success than type 3 fibroids⁴⁻⁶. However, this classification parameter may be too limited as a predictor of treatment outcome. Yoon et al. show a case report of a successfully treated hyper-intense type 3 uterine fibroid. This suggests that an additional screening parameter is required to make a subpopulation of uterine fibroid patients suitable for successful MR-HIFU treatment.

Tissue perfusion is an important parameter for the characterization of uterine fibroids prior to MR-HIFU treatment because blood flow deprives heat from the fibroid. For this reason, quantification of the tissue perfusion could serve as a predictor of success in the MR-HIFU treatment. Both diffusion weighted MRI (MR-DWI) and dynamic contrast enhanced MRI (DCE MRI) can provide information about fibroid perfusion. The addition of these MRI sequences could give a more extensive and more complete picture of the fibroid. By introducing this more extensive MRI screening protocol, an inter- and intra-patient spread in uterine fibroid characteristics could be identified. Knowledge of MRI-based predictors of success prior to treatment, may contribute to an optimization of patient selection, treatment planning and treatment outcomes.

OBJECTIVES

Primary Objective:

We are looking for MRI determinants to better determine patient suitability for a successful treatment of symptomatic uterine fibroids using MR-HIFU. A successful treatment is determined as a symptom reduction of minimal 10 points on the UFS-QoL questionnaire.

The possible MRI determinants are that can predict treatment outcome, are:

- ADC value
- Quantitative T2 value
- ktrans

Secondary Objective(s):

Because we want to eliminate the use of an intravenous contrast agent, we are looking for a functional MRI parameter that is capable of measuring the treated tissue. Therefore, the secondary objective of this study is to determine the feasibility of ADC mapping, T2 mapping and DCE in monitoring thermal ablation effects from MR-HIFU in the treatment of uterine fibroids.

STUDY DESIGN

Data collection and experiments are performed in the Isala Zwolle. Patient inclusion is the responsibility of the gynecology department. Imaging and treatment are performed on the radiology department.

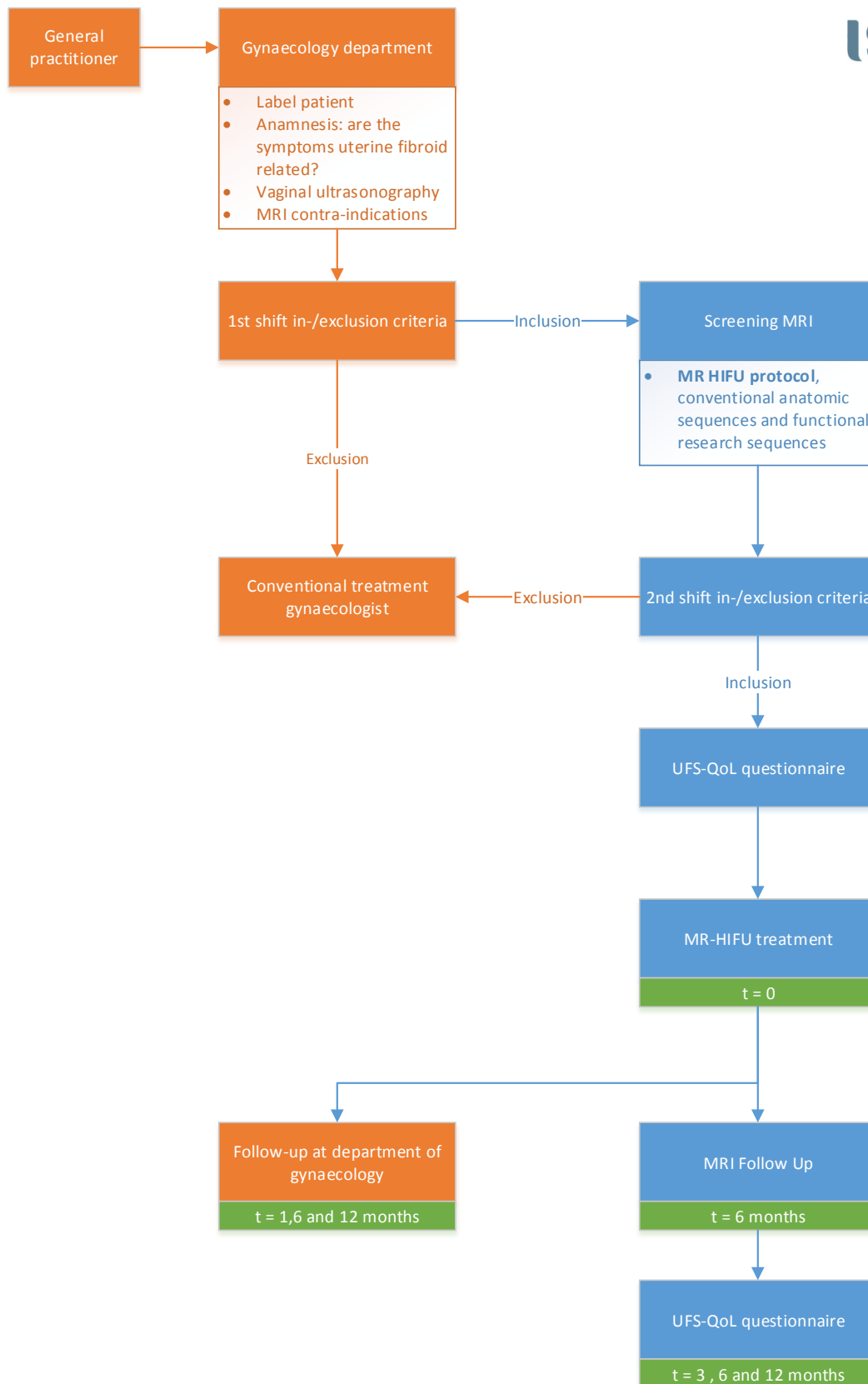
The flow-chart on page 12 shows the course of events. All new patients presenting with fibroid related symptoms on the gynecology department are registered in the database with the aim to chart the total patient population in the Isala. Based on the in-/exclusion criteria as further described in section 4.2 and 4.3, only a selective patient group will undergo the screening MRI. Patients excluded for the screening MRI can start directly with the conventional treatment path on the gynecology department.

Patients that meet the inclusion criteria, undergo a screening MRI. After this screening MRI, again in- and exclusion criteria apply to further determine HIFU treatment eligibility. Patients that meet all criteria will be informed about the MR HIFU treatment and once given informed consent will undergo treatment. At baseline ($t=0$), the UFS-QoL score is measured by means of an online questionnaire. Follow-up consists of visits at the gynecologist's office, and MRI and the UFS-QoL questionnaire is repeated.

Because the MR-HIFU treatment is a new introduced treatment option in the Isala Hospital, patient satisfactory surveys are held after treatment. The survey is included in the appendix. It will be digitally send to the treated patients by Research Manager.

Relation to the MASS I study

In the MASS I study, a screenings MRI is made of patients potentially suitable for MR-HIFU treatment. This screening MRI is the same as the protocol that will be used in the MASS II study. Therefore, patients can be included in the MASS I as well as the MASS II study. The MASS I study does not have to be completed in order to start the MASS II study.



STUDY POPULATION

Population (base)

All new patients presenting with uterine fibroid related symptoms on the department of gynecology at Isala are included in the study database. In most cases, these patients will be referred by their general practitioner for a consult on the gynecology department. For MRI screening in preparation of the MR-HIFU treatment, only patients meeting the in-/exclusion criteria based on physical examination and vaginal ultrasonography are concerned as candidates.

Inclusion criteria

For MRI screening inclusion, the following criteria are applied based on anamnesis, physical examination and vaginal ultrasonography:

- 18 – 59 years old
- Uterine fibroid related symptoms
- Pre- or perimenopausal

To determine whether the patient is eligible for the MR-HIFU treatment *after* the screening MRI, the following inclusion criteria are used.

- Type 1 & 2 uterine fibroids (based on Funaki classification)
- Diameter of 1-10 cm of dominant fibroid

Exclusion criteria

Exclusion criteria for the MRI screening, based on anamnesis, physical examination and vaginal ultrasonography, are defined as follows:

- Post-menopausal
- Wish for future fertility
- Pregnancy
- Severe abdominal obesity or BMI > 40
- Uterine artery embolization in medical history
- MRI contra-indications
- Calcifications in uterine fibroid

To determine whether the patient is eligible for the MR-HIFU treatment *after* screening MRI, the following exclusion criteria are used.

- Type 3 uterine fibroids (based on Funaki classification)

- Calcified or pedunculated uterine fibroids
- Close to sciatic nerve or sacrum
- Interposition of bowel or ovary
- Diameter of < 1 cm or > 10 cm
- Distance skin – uterine fibroid > 12 cm

METHODS

Study parameters/endpoints

Main study parameter/endpoint

The main study endpoint is the symptom reduction measured with the Uterine Fibroid Symptom and Health related Quality of Life questionnaire (UFS-QoL) to measure the clinical efficacy of the MR-HIFU treatment. Since uterine fibroids are benign, symptom reduction is the most important outcome in the treatment of uterine fibroids. The UFS-QoL is a standardized, worldwide accepted and used questionnaire to measure the specific symptoms caused by uterine fibroids. Based on literature, a symptom reduction of 10 points on the UFS-QoL is proven clinically efficient. The number of treated patients minimally compassing this 10 point reduction are seen as clinical efficient treatments.

Secondary study parameters/endpoints

Second study parameter is the non-perfused volume (NPV) ratio. Just like the UFS-QoL symptom reduction score, this parameter assesses the clinical efficacy of the MR-HIFU treatment. The NPV represent the tissue undergoing successful ablation, measured directly after the total treatment.

Randomisation, blinding and treatment allocation

Not applicable

Study procedures

Patients presenting with uterine fibroid related symptoms at the department of gynaecology will undergo standard consultation, physical examination and vaginal ultrasonography.

When the inclusion criteria (section 4.2) are met, the screening MRI will be scheduled as soon as possible. After informed consent, patients also are asked to complete the Uterine Fibroid Symptom and Health related Quality of Life questionnaire (UFS-QoL). The UFS-QoL is a validated, disease-specific questionnaire.

The screening MRI is performed at the radiology department in the Isala. The MRI protocol consists of multiple sequences, schematically visualised below. This MRI protocol is currently researched in the MaSS study (NL52739.075.15, METC number 15.0580).

Conventional MR Imaging	Multi-parametric Imaging
• T2 TSE sagittal • T2 TSE transversal • T1 SPIR Clear transversal • T1 SPIR Gado transversal • T1 SPIER Gado sagittal	• T2 mapping • DWI UMCU • DWI lange TE • DCE MRI

Since the multi-parametric imaging protocol is still being studied, patient's eligibility is determined on the conventional MR images. In-/exclusion criteria are given in section 4.2 and 4.3. In table 1, an explanation of the different multiparametric MRI parameters is given.

Table 7: Multiparametric MRI parameters

MRI Sequence	Obtained Parameter	Unit	
Diffusion Weighted Imaging	Apparent Diffusion Coefficient (ADC)	mm ² /s	A measure for the magnitude of diffusion of water in the fibroid tissue.
T₂ mapping	Quantitative value	T ₂ s	Gives the quantitative T ₂ value of the fibroid tissue, which reflects the amount of moisture (and therefore blood) in the tissue.
Dynamic Contrast Enhanced Imaging	k _{trans}	min ⁻¹	Transfer constant, characterizes the diffusive transport of the contrast agent across the endothelium.

All parameters described in table 1, are measured by drawing a volume-of-interest (VOI) in the uterine fibroid. The mean value in the VOI is calculated and used for further analyses.

MR-HIFU treatment is performed when the patient is found eligible and after accurate informing the patient about the treatment and possible complications. The MR-HIFU treatment will be performed in the MRI scanner. A detailed description of the MR-HIFU on the Sonalleve system from Philips can be found in the appendix.

After treatment, patients normally return home the same day of the treatment. Follow-up is conducted 3, 6 and 12 months after treatment at the gynaecologists office. A follow-up MRI is performed after 6 months. During this MRI, the same protocol will be used as before

the treatment. Also, the UFS-QoL questionnaire is taken after 3, 6 and 12 months to monitor symptom relief.

Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

Replacement of individual subjects after withdrawal

Not applicable

Follow-up of subjects withdrawn from treatment

Not applicable

Premature termination of the study

We do not expect a premature termination of the study, as the MR-HIFU Sonalleve system is a dedicated technique with a CE mark for the treatment of uterine fibroids. Also, MRI with the use of a contrast agent has no serious expected complications or adverse events.

SAFETY REPORTING

Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

Adverse events (AEs) and Serious adverse events (SAEs)

Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the screening MRI as described in this protocol. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse events.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

Annual safety report

Not applicable

Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

STATISTICAL ANALYSIS

Primary study parameter(s)

The primary study parameter are the possible MRI determinants, summarized below:

- Mean ADC values, measured by diffusion weighted imaging. It is a continuous parameter, measure in mm^2/s
- Quantitative T_2 values, measured by T_2 mapping. It is a continuous parameter measured in seconds.
- Mean k_{trans} , measured by dynamic contrast enhanced imaging. It is a continuous parameter, measured in min^{-1}

Each study parameter described above is a continuous variable. All parameters will be tested for normality with the Shapiro-Wilk test. If the parameter has a normal distribution, it will be described with the mean $\pm$ standard deviation. If the distribution is skewed, the parameter will be described with the median and the minimum/maximal values.

For each possible determinant, a regression analysis is performed. This is a logistic regression analysis, because the endpoint (a clinical relevant successful treatment with a symptom reduction of minimal 10 points) is dichotome. From this regression analysis, the regression coefficient, p-value and confidence intervals are calculated. If the p-value is smaller than 0.05, a dependency between the determinant and a clinical relevant successful treatment is confirmed. This is done for all 3 determinants.

If one or more of the determinants is confirmed, a multivariable regression is performed. This could result in a useful algorithm. However, we are aware that this algorithm has to be cross validated before use in clinical practice.

Secondary study parameter(s)

The NPV ratio and the three MRI determinants are continuous variables. All parameters will be tested for normality with the Shapiro-Wilk test. If the parameter has a normal distribution, it will be described with the mean $\pm$ standard deviation. If the distribution is skewed, the parameter will be described with the median and the minimum/maximal values.

To validate of a MRI parameter can be used in order to measure the NPV, the following steps are taken. This is done for each of the three MRI parameters.

- Scatterplot, in which the MRI parameter is plotted against the golden standard (NPV)
- Bland-Altman plot
- The ICC is calculated

ETHICAL CONSIDERATIONS

Regulation statement

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki. The study will be conducted in compliance with the protocol. The protocol and any Amendments and the subjects Informed Consent will receive Institutional Review Board (IRB/Independent Ethics Committee (IEC) approval/favorable opinion prior to initiation of the study.

Recruitment and consent

Freely written informed consent will be obtained from every subject prior to study participation, including informed consent for any screening procedures conducted to establish subject eligibility for the study.

Benefits and risks assessment, group relatedness

Subjects meeting all inclusion criteria without meeting any exclusion criteria will be eligible for the MR-HIFU treatment. Patients entering the MR-HIFU study do not directly benefit from study participation in comparison to the standard MR-HIFU treatment. Risks associated with study participation are negligible, as the additional MRI sequences have no additional risks compared to the standard protocol.

Compensation for injury

A dispensation from the statutory obligation to provide insurance is requested.

ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

Handling and storage of data and documents

Research Manager is used in this study for the storage of data. For each module, specific rights are assigned. All gynaecologists can include patients in this database. At inclusion, all patients will be assigned a unique study number. The database which links the study number to the Isala patients numbers is only available to coordinating investigators.

Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the latest patient inclusion and MRI scan.

In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

10. REFERENCES

1. Stewart EA, Gostout B, Rabinovici J, Kim HS, Regan L, Tempany CM. Sustained relief of leiomyoma symptoms by using focused ultrasound surgery. *Obstet Gynecol.* 2007;110(2 Pt 1):279-287.
2. Gizzo S, Saccardi C, Patrelli TS, et al. Magnetic resonance-guided focused ultrasound myomectomy: Safety, efficacy, subsequent fertility and quality-of-life improvements, a systematic review. *Reprod Sci.* 2014;21(4):465-476.
3. Funaki K, Fukunishi H, Funaki T, Kawakami C. Mid-term outcome of magnetic resonance-guided focused ultrasound surgery for uterine myomas: From six to twelve months after volume reduction. *Journal of minimally invasive gynecology.* 2007;14(5):616-621.
4. Funaki K, Fukunishi H, Sawada K. Clinical outcomes of magnetic resonance-guided focused ultrasound surgery for uterine myomas: 24-month follow-up. *Ultrasound in Obstetrics & Gynecology.* 2009;34(5):584-589.
5. Machtinger R, Inbar Y, Cohen-Eylon S, Admon D, Alagem-Mizrachi A, Rabinovici J. MR-guided focus ultrasound (MRgFUS) for symptomatic uterine fibroids: Predictors of treatment success. *Human Reproduction.* 2012;27(12):3425-3431.
6. Lénárd ZM, McDannold NJ, Fennessy FM, et al. Uterine leiomyomas: MR imaging-guided focused ultrasound Surgery—Imaging predictors of success 1. *Radiology.* 2008;249(1):187-194.
7. Kim YS, Lim HK, Kim JH, et al. Dynamic contrast-enhanced magnetic resonance imaging predicts immediate therapeutic response of magnetic resonance-guided high-intensity focused ultrasound ablation of symptomatic uterine fibroids. *Invest Radiol.* 2011;46(10):639-647.
8. Yoon SW, Lee C, Kim KA, Kim SH. Contrast-enhanced dynamic MR imaging of uterine fibroids as a potential predictor of patient eligibility for MR guided focused ultrasound (MRgFUS) treatment for symptomatic uterine fibroids. *Obstet Gynecol Int.* 2010;2010:10.1155/2010/834275. Epub 2010 Aug 16.
9. Liu J, Keserci B, Yang X, et al. Volume transfer constant (K trans) maps from dynamic contrast enhanced MRI as potential guidance for MR-guided high intensity focused ultrasound treatment of hypervascular uterine fibroids. *Magn Reson Imaging.* 2014;32(9):1156-1161.
10. Ikink ME, Voogt MJ, van den Bosch, Maurice AAJ, et al. Diffusion-weighted magnetic resonance imaging using different b-value combinations for the evaluation of treatment results after volumetric MR-guided high-intensity focused ultrasound ablation of uterine fibroids. *Eur Radiol.* 2014;24(9):2118-2127.