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Master Thesis

Optimisation of Treatment Time in Ultrasound guided High Intensity Focused Ultrasound (HIFU) in the Treatment of Breast Fibroadenomata.



Mirjam Peek, BSc Master Thesis Technical Medicine Faculty of Science and Technology December 5th, 2014

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Preface

In December 2013 I started my Technical Medicine graduation on the HIFU-F project at Guy's and St. Thomas' hospitals after a previous three month internship in London. This thesis reports the results of the work I performed in the last 12 months for the master in Technical Medicine.

Firstly, I would like to thank my medical supervisor, Michael Douek, for giving me the opportunity to do my graduation in London. You trusted in me and gave me the responsibility of co-running the HIFU-F trial. You challenged me to get the best out of me and this resulted in three articles, three oral and three poster presentations and a Young Investigators Award. I am looking forward in working with you in the next three years during my MPhil/PhD.

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And last but not least, I would like to thank the patients for participating in the trial, without them this thesis would not have been possible and we would not have had these results.

Finally, I would like to thank my family, friends (both in London and in the Netherlands), colleagues and housemates for their support during the last year. You all created a safe environment in which I was able to work on this project fulltime and you made me realise that relaxing every now and then is important as well and you played a great part in this. Without all the people mentioned above this thesis would not have been made possible.

Abstract

Introduction

Breast fibroadenomata (FAD) are the most common breast lesions in woman. High intensity focused ultrasound (HIFU) is a promising non-invasive ablative technique for the treatment of these FAD. In HIFU, an ultrasound (US) beam propagates through tissue as a high-frequency pressure wave elevating the temperature within a few seconds without causing damage to the direct adjacent tissues. Two systematic reviews were performed evaluating the current evidence of HIFU and minimally invasive ablative techniques in the treatment of breast cancer. In the "HIFU in the treatment of breast Fibroadenomata" (HIFU-F) trial, circumferential HIFU treatment was performed to isolate the FAD from its blood supply. Outcome measures were volume decrease on US, short-term complication rate, decrease in treatment time and patient recorded outcome measures.

Methods

Two systematic reviews were conducted following the Cochrane Handbook and STROBE statement. Patients (age \geq 18 years) were recruited with symptomatic palpable FAD which had to be visible on US (graded either benign or indeterminate). Patients were treated using the US-guided - Echopulse device (Theraclion Ltd, Malakoff, France) under local anaesthesia. Two circumferential rings of pulses were applied by deselecting the centre of the FAD. Patients were followed-up at two weeks, three, six and 12 months.

Results

The systematic review demonstrated that very small studies have been conducted to HIFU and other ablative techniques. From December 2013, 25 patients with symptomatic palpable FAD underwent circumferential HIFU treatment. Nine patients opted for HIFU due to pain or discomfort. Average treatment time was 38.5 minutes (SD 12.0 minutes). Circumferential treatment significantly reduced treatment time by an average of 36.4% (SD 18.9%) (T-test, P=0.0001, two tailed). At two weeks short-term complications were erythema (n=6), ecchymosis (n=8), numbness of the skin (n=1), hypo-pigmentation (n=1), dimpling of the skin (n=1), a first-degree skin burn (n=1) and reduced pain in 5/8 patients with resolution in two patients. At three months all local complications had resolved apart from hyper-pigmentation (n=6). Reduction of pain was seen in 7/8 patients with resolution of pain in six. Two weeks post-treatment a volume reduction of 14.3% (SD 24.9%) was seen on US and at three months the reduction was 41.7% (SD 28.6%).

Conclusion

Circumferential HIFU ablation of FAD is feasible with a significant reduction in treatment time. Further patient follow-up and large prospective trials are needed to demonstrate consistent tumour and margin necrosis, reliable follow-up imaging and establish the effect of HIFU treatment.

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List of Abbreviations

AJCC	American joint committee on cancer
ASTRO	American society of radiation oncology
BCS	Breast conserving surgery
BRCA	Breast cancer gene
CNB	Core needle biopsy
CRF	Clinical report form
DCIS	Ductal carcinoma in situ
ER	Oestrogen
FAD	Fibroadenoma
FNAC	Fine needle aspiration cytology
FUS	Focused ultrasound
HEM	Hyper-echoic mark
HER2	Human epidermal growth factor type 2
HIFU	High intensity focused ultrasound
HIFU-F	High intensity focused ultrasound in the treatment of breast fibroadenomata
H&E	Haematoxylin and eosin
ISI	Increase in signal intensity
MDCT	Multi detector computed tomography
MDM	Multi disciplinary meeting
MDF	Maximum difference function
MRI	Magnetic resonance imaging
NADH	Nicotinamide adenine dinucleotide
PEI	Positive enhancement integral
PET-CT	Positron emission tomography – computed tomography
PIS	Patient information sheet
PR	Progesterone
RCT	Randomized control trial
RFA	Radiofrequency ablation
SD	Standard deviation
SPECT	Single positron emission computer tomography
STROBE	Strengthening the reporting of observational studies in epidemiology
TNM	Tumour, node and metastasis classification
US	Ultrasound
VAB	Vacuum assisted biopsy
VAM	Vacuum assisted mammotomy
VTU	Visualisation and treatment unit

1. Introduction

1.1 Fibroadenomata

Breast fibroadenomata (FAD) are the most common breast lesions in woman and can develop at any age but most often during the second and third decades of their life. They are also not uncommon in post-menopausal women and arise more often after hormone replacement therapy. Breast FAD occur in about 10% of all woman and account for about 50% of performed breast biopsies. Studies revealed that up to 59% of FAD will regress or completely resolve within five years. [1] The average lifetime of a FAD is about 15 years. FAD have been shown to be more common in patients of higher socio-economic classes and in population with darker skin. Age of menarche, menopause and hormonal therapy, including oral contraceptives were shown not to influence the risk of developing these lesions. [2] A negative correlation was found between the risk of developing FAD and the body mass index and number of full-term pregnancies. Consumption of large quantities of vitamin C and cigarette smoking were found to reduce the risk of developing FAD. [2] Transformation of a FAD into a malignant lesion is considered exceptionally rare (0.002 - 0.0125%). [2-4] There is a 1.3 - 2.1 increased risk of developing breast cancer in women with FAD compared with to general population. [2]

FAD are benign lesions that are encapsulated from their surrounding tissues. They can be considered as an aberration of normal development rather than a true neoplasm. On histology, FAD consist of combined proliferation of epithelial and fibroblastic tissue elements which are oestrogen (ER) dependent and slowly growing. [4, 5] FAD are considered to be a benign mixed tumour but recent studies have revealed that only the fibroblastic element is neoplastic, while the epithelial is reactive. Epithelial proliferation appears in a single terminal ductal unit and describes duct-like areas surrounded by fibroblastic stroma. Depending on the ratio between these two elements there are two main histological types: intracanalicular and pericanalicular. Both types are often found within the same FAD. In intracanalicular FAD stromal proliferation pedominates and compresses the ducts and in pericanalucular FAD the fibrous stroma proliferates around the ductal spaces. [5] FAD develop from a lobular origin which explains the high incidence in woman between their twenties and thirties, at their maximum lobular development of the ductal system of the breast. [2] It also explains why the rare cases of cancer developed from FAD are of the lobular type (lobular carcinoma in situ). [2, 4]

Patients with FAD usually present themselves in the clinic with a palpable lesion detected during self- or medical examination. FAD are normally solitary, non-tender, smooth, mobile masses of about 1 - 3 cm. [2, 4] The majority are located in the upper outer quadrant of the breast. [2] In 20% of cases, the FAD are multiple or 4 cm or larger (5% of all FAD are 5 cm or larger). In the case of multiple FAD, there is often a strong family history of these lesions. [2] FAD stay at the same size or increase until about 2 - 3 cm, in 15% the FAD regresses spontaneously and in 5 - 10% the lesion progresses. [2, 4]

Diagnosis

The diagnosis of a FAD can be made by triple assessment. The first step is physical examination, however in only 50 - 67% of cases the lesion identified as a FAD is actually a FAD due to the similar characteristics with other benign diseases. [2, 4] Therefore, more accurate

diagnostic methods are needed to get the correct diagnosis. The second step is imaging. Ultrasound (US) is the main diagnostic imaging method used for the diagnosis of FAD. FAD are visible as oval smooth solid masses with even low-level internal echoes. [2] However, not all FAD have the same characteristics and not all FAD are visible on US images. In 25% of FAD, features like an irregular border are suggestive that the lesion might be malignant. [2, 4] In mammography, FAD are often visible as homogenous well-circumscribed lesions in which calcifications are often observed. [2] This technique is not always used in the diagnosis of breast FAD but can be useful in older woman especially in patients with non-palpable lesions. [4]

A fine needle aspiration cytology (FNAC) or core needle biopsy (CNB) is the third step and can be used to get final confirmation of the diagnosis. In FNAC, a thin needle is used to obtain cells of the lesion. This technique is used both in young patients and in patients with needle phobia. The drawback of this technique is that there is a high rate of insufficient obtained tissue material to be examined. CNBs are more reliable but multiple re-insertions are needed and in the case of dense breasts, insufficient breast tissue could be obtained. A third option is vacuum assisted biopsy (VAB), in this technique no re-insertion is needed and a larger amount of breast tissue is obtained. [4] On cytology, FAD are recognised as clusters of spindle cells without inflammatory fat cells; aggregates of cells with a papillary configuration resembling antler horn clusters and/or uniform cells with well-defined cytoplasm lying in rows and columns, these can be found in 96%, 93% and 95% of all FAD, respectively. In one study, it was found that only 82% of CNB proven FAD could be visualised with US. [2] In general, FNAC, CNB and VAB could be performed in all patients, however in woman <25 years no biopsy is performed when US reveals a solid lesion which has benign characteristics due to the low incidence of breast cancer in woman <25 years. [6, 7]

The overall diagnostic efficacy of this triple assessment is approximately 70-80% but an accurate differentiation between a benign and a malignant lesion is provided in 95%. [2, 4]

Management

The management of non-palpable lesions is reassurance with or without a follow-up period of one to three years after diagnosis by CNB or FNAC. For palpable lesions, there are currently three main treatment options available: reassurance (with or without follow-up), vacuum assisted mammotomy (VAM), which officially is not licensed for the treatment of FAD only to obtain the diagnosis of a lesion, or surgical excision. In the case of reassurance, it is advised for patients up to 35 years to use a follow-up protocol in which the patient comes back every six months to determine if the lesion has changed in size. [2]

Intervention is normally offered to patients with large FAD, fast growing lesions or to patients requesting for removal of the lesion due to anxiety or discomfort. Patients with a family history of breast cancer are also advised to get interventional treatment. [4] In other cases, surgical removal might involve unnecessary excisions of benign lesions and unbecoming cosmesis. [2] In about 30% of all patients which underwent surgical excision of a lesion diagnosed as a FAD the lesion is found to be another type of benign disease. [2]

VAM is performed in an outpatient setting under US guidance with subcutaneous local anaesthetic and is less invasive with a better cosmetic outcome compared to surgical excision. Complete resection of the FAD is reported in 75 - 100% of all cases. Disadvantages of VAM are the reduced visibility due to blood, air, local anaesthesia and/or soft tissue oedema. Lesions close to the skin (<0.5 cm) and of a size larger than 3 cm are not suitable for treatment. In some lesions close to the pectoralis major and/or skin local anaesthesia can be injected between the lesion and the skin to increase the distance. Possible complications after VAM are hematoma, skin defect and/or a pneumothorax. [8]

In surgical excision the lesion is removed under general anaesthesia. This can be the best option in the case of large or multiple FAD or lesions that have the appearance of a phyllodes tumour (a relatively fast growing potentially malignant tumour). [2] The main advantage is that the whole FAD is completely removed. Possible disadvantages are the scarring, damage to the ducts, cosmetic outcome and chances of anaesthetic and/or operative complications. [2, 4]

A new technique in the treatment of FAD is high intensity focused ultrasound (HIFU) ablation. This a non-invasive ablative technique in which the FAD is treated with focused consecutive repeated US pulses, while surrounding tissues are not damaged.

1.2 Breast Cancer

Breast cancer is the most common cancer in women in the United Kingdom. In 2010, just under 50,000 women were diagnosed with invasive breast cancer and with 11,684 deaths in 2011, it is the second most common cause of death from cancer in women. [9] In men, breast cancer diagnosis and death due to breast cancer accounts for about 1%. [10]

With the wider use of mammographic screening, breast cancers are diagnosed at an increasingly earlier stage. [10-12] Increasing age is the most important risk factor for breast cancer, other risk factors are family health history, major inheritance susceptibility, alcohol intake, breast tissue density, ER level, hormone therapy history, obesity, lack of physical exercise, personal history of breast cancer, personal history of proliferative forms of benign breast disease, race and radiation exposure to the breast. [10, 13] Of all female breast cancers, about 5-10 % may have germ line mutation of the breast cancer 1 and 2 genes (BRCA). Patients carrying the BRCA 1 or 2 gene, also have an increased risk of developing ovarian or other primary cancers. [10, 14] Protective factors for breast cancer are oestrogens use, exercising, early pregnancy, breast feeding, risk reducing mastectomy and oophorectomy or ovarian ablation. [10, 13] When a patient is suspected to have breast cancer the plan is as followed: the diagnosis is confirmed, the stage of the disease is evaluated and the therapy is selected. For the diagnosis the following modalities can be used or a combination of them: mammography, US, magnetic resonance imaging (MRI) and/or breast biopsy. [10]

Breast cancer can be treated by various combinations of breast surgery, radiotherapy, chemotherapy and hormone therapy. The following clinical and pathological features influence the prognosis and choice of treatment: menopausal status of the patient, stage of the disease, grading of the primary tumour, ER and progesterone (PR) status of tumour, human epidermal growth factor type 2 (HER2) receptor over-expression and / or amplification, and histological type. [10]

Breast cancer can present itself in a invasive or non-invasive or intraductal form. Infiltrating or invasive ductal cancer is the most common breast cancer histology type and accounts for 70-80% of all cases. Ductal carcinoma in situ (DCIS) is a non-invasive condition. DCIS can progress to become invasive cancer, but the likelihood of this happening is very extensive. Staging is determined according to the tumour size, lymph node status, ER and PR expression levels, HER 2 status, menopausal status and the general health of the patient. The American Joint Committee on Cancer (AJCC) has stratified breast cancer according to the tumour, regional nodal status and distant metastasis (TNM) classification. [10]

Surgery in the form of either breast conservation (BCS) or mastectomy followed by adjuvant therapy constitutes the main stay of treatment for early stage breast cancer. [11, 12] In BCS the cancer is removed without removing other breast tissue, this could be done with a lumpectomy, in which the lesion is removed along with a small margin of normal tissue or with a partial mastectomy in which the breast segment with the cancer is removed. Patients who undergo BCS might also have their sentinel lymph node (first lymph node to receive lymphatic drainage from a tumour) removed under the arm to determine if cancer cells have spread to the lymphatic system. [10] In mastectomy, all breast tissue is removed, patients could opt for breast reconstruction with either their own tissue (autologous reconstruction) or an implant (implant based reconstruction). Adjuvant therapy consists of one or a combination of: radiotherapy, in which radiation is used to kill cancer cells and keep them from growing; chemotherapy, in which hormones are removed or blocked, which prevents the cancer cells from growing. [10]

BCS has proved to be effective and well accepted by patients. However, BCS can be associated with alterations in the size and symmetry of the treated breast and this could lead to a reduced patient quality of life. [15] This would make non-surgical techniques without the removal of breast tissue more attractive. Breast conservation is dependent upon clear margins - defined as no ink visible on resected tumour according to recent American Society for Radiation Oncology (ASTRO) guidelines. [16] However, during surgery the surgeon is unable to visualize the tumour to aid in determining clear margins. This lack of intra-operative target definition results in higher re-operation rates aimed in an attempt to excise residual tumour. There is a clinical need to develop minimally invasive ablative techniques to further reduce re-operation rates by defining the target and the tumour margins intra-operatively. These techniques potentially benefit from the absence of general anaesthesia, a reduced recovery time, because the treatment is under local anaesthesia, absence of scarring and consequently economic benefits. [17] Non-surgical techniques including HIFU; radiofrequency ablation (RFA); cryo-; laser and microwave ablation are under investigation for local treatment of breast tumours. [18]

1.3 Ablative Techniques

HIFU is a non-invasive ablative technique that has been used for the treatment of liver, kidney, prostate, brain, bone and breast tumours. [19, 20] During HIFU treatment, an US beam generated by a piezoelectric US transducer propagates through soft tissue as a high-frequency pressure wave. [20] The beam is focused onto the targeted tissue and with every pulse treats a volume of 0.2 cm by 0.9 cm. The energy from the beam elevates the temperature of the focused area to 60 - 95^oC within a few seconds, thereby leading to a very localised protein denaturation

and coagulative necrosis. [20, 21] The available HIFU devices are generally integrated with either MRI or US in order to plan treatment and monitor response in real-time. [19, 20] HIFU is capable of providing a completely non-invasive therapy, without causing damage to the direct adjacent tissues, avoiding discomfort and potential complications associated with general anaesthesia and surgery. [20, 21] Disadvantages are the long treatment times and the possible complications (e.g. local pain, redness of the skin, skin burn). [22]

In RFA, a needle electrode is inserted percutaneously under US guidance to deliver an alternating current that generates ionic agitation, localized tissue heating and cell death. [15, 23] The primary source of heat is the tissue surrounding the electrode and not the electrode itself. It is presumed that heating drives extracellular and intracellular water out of the tissue, resulting in coagulative necrosis. [24] Average treatment time is 10 - 30 minutes and moderate complications like discomfort and a skin burn could occur. The procedure can be performed under intravenous sedation, general or local anaesthesia. [22]

Cryo-ablation uses freezing instead of heating. It is accomplished by inserting a cryo-probe under US guidance into the targeted breast tissue. The freezing process involves two phases: freezing and thawing. Four mechanisms destroy the tumour cells: direct damage by (1) intracellular ice formation and (2) osmotic dehydration, indirect damage due to (3) ischemia and (4) immunologic response. The treatment has good precision and control because the formation of an ice ball can be clearly visualized with US. [25, 26] The treatment takes about 15 - 30 minutes with minimal discomfort under local anaesthesia. [22]

In laser ablation, tumours are destroyed using direct heating with low-power laser light energy delivered via thin optical fibers inserted percutaneously under US or MRI guidance. [27] Upon absorption in the tissue, heat is produced, inducing lethal thermal injury. [18] The size and shape of thermal lesions are difficult to predict, however owing to biologic variability, fiber tip charring and changing optical and thermal properties of the tissue during interstitial laser photocoagulation. [27] Treatment times are approximately 25 - 30 minutes and moderate complications like discomfort and skin burns could occur. The treatment can be performed under intravenous sedation, general and local anaesthesia. [22]

Microwave ablation uses localized heating with externally applied focused microwaves to cause tissue necrosis. Due to the varying effects, the water molecules move in the tissue and cause frictional heating. This heating is determined by power deposition and dielectric and thermal properties of the ablated tissue. This technique is promising because it can heat and damage high-water-content tumour cells, compared with the lesser degrees of heating that occur in lower-water-content adipose and breast glandular tissues. [28, 29] The electrode antenna is inserted percutaneously under US guidance. The treatment takes up to 20 - 60 minutes and possible complications are pain or skin burns. The treatment is performed under intravenous sedation. [22]

1.4 Aim of Thesis

In general, HIFU is capable of providing a completely non-invasive therapy, avoiding potential complications associated with general anaesthesia and surgery. [30]

HIFU has been evaluated for treatment of FAD in only a single clinical trial. Hynynen *et al.* [31] described a study in which 11 FAD with a volume of 1.9 cm^3 (0.7 - 6.5 cm³), were ablated in nine patients (median 29 years, range 19 - 38 years) to establish the feasibility, safety and effectiveness of HIFU ablation. A complete response was defined as a volume reduction \geq 90%, partial response was defined as a volume reduction between 50 - 90% and a volume reduction \geq 50% was defined as a minor response. T1-weighted images showed a partially or nearly completed ablation in 73% (8/11 FAD). Six months post-treatment, T2-weighted images showed a median volume decreased from 1.9 to 1.3 cm³. Pain was marked as slight in four patients, moderate in two and severe in one patient, and tenderness was common up to ten days and oedema was visible up to two days post-treatment.

Currently there are four trials in which US guided HIFU is used for the treatment of breast FAD. Kovatcheva *et al.* [32] recruited 27 patients between March 2011 and January 2014 to demonstrate the efficacy of HIFU in the treatment of FAD. Boulanger *et al.* [33] designed a multicentre trial for the observation of histological changes in FAD following HIFU and recruited 24 patients between October 2011 and February 2014. Hahn *et al.* [34] recruited 27 patients between December 2013 and January 2016 to evaluate the efficacy of HIFU in the treatment of FAD and Benin *et al.* [35] designed a feasibility study to determine the safety and efficacy of the HIFU device by recruiting 20 patients between April 2014 and May 2016. None of these trials have published their results thus far. (A review evaluating ablative techniques in the treatment of breast FAD is presented in appendix I.)

For breast cancer HIFU has the potential to improve cosmetic outcomes (by preventing scarring and excessive ductal damage) and allowing earlier administration of systemic therapies due to shorter postoperative recovery times. [30, 36] The treatment also benefits from being able to alter to the shape of the lesion. Two systematic reviews are performed evaluating the current evidence for the clinical outcomes (residual tumour, imaging treatment response and cosmesis) of HIFU and minimally invasive ablative techniques in the treatment of breast tumours. These systematic reviews are presented in chapters two and three. The HIFU technique is described in detail in chapter four.

Standard HIFU treatment is normally applied to the whole lesion. The limitation of this method is the prolonged treatment time. Since a single pulse generates a small lesion, a lot of these consecutive repeated pulses have to be applied with an adequate idle time in between to prevent overheating the tissue. [37] The High Intensity Focused Ultrasound in the treatment of breast Fibroadenomata trial (HIFU-F trial) is designed in which circumferential HIFU treatment is performed: two rings around the FAD are treated by deselecting the centre of the FAD. The materials and methods and results are presented in chapter five.

In the HIFU-F trial, the US-guided Echopulse device (Theraclion Ltd, Malakoff, France) is used due to its increased mobility and accessibility to clinics and operating rooms. Our goal is to isolate the tissue in the centre of the FAD from the blood supply. The tissue in the centre of the lesion will therefore die as well and the body will be able to remove the dead tissue from the body, thereby shrinking the FAD. The major benefit of this circumferential treatment is the decreased treatment time of the procedure. The aim of our trial is to evaluate circumferential HIFU treatment for the effective ablation of FAD, with a reduced treatment time as the most important outcome measure.

2. Systematic Review: High Intensity Focused Ultrasound (HIFU) Ablation in the Treatment of Breast Tumours

2.1 Material and Methods

Study selection

A systematic review of the literature was performed using Medline/PubMed library databases to identify all studies published up to December 2013 that evaluated the role of HIFU for the treatment of breast tumours. The MeSH search terms used were High Intensity Focused Ultrasound, HIFU, focused ultrasound ablation and FUS, all in combination with breast. Except for reports in the English language and human subjects, there were no further restrictions. The related articles function was used to broaden the search, and all abstracts, studies and citations obtained were reviewed. References of the articles acquired were also searched by hand. The last search was conducted on December 20th, 2013.

Inclusion criteria

Studies were considered eligible for the systematic review if they addressed the following; (1) studies performed on human subjects with breast tumours; and (2) studies objectively recorded at least one clinical outcome measure of response (cosmetic, imaging and/or histopathology) to HIFU treatment.

Exclusion criteria

Studies that failed to fulfil the inclusion criteria or studies in which the outcomes of interest were not reported or if it was not possible to analyse these from the published reports, were excluded. Conference abstracts, letters, editorials and case reports were also excluded.

Data extraction

Each study was initially evaluated for either inclusion or exclusion. The data extracted from the included studies were: first author, year of publication, study design, number of included patients, mean patient age, tumour type, tumour size, type of guided imaging, frequency, dose, treatment margin used, total treatment time, resection (yes/no), follow-up, cosmetic outcome, imaging outcome, histopathology staining, histopathological outcome, complications, retreatment of tumours and recurrence. One reviewer, (M.P) extracted data for all selected studies and a second reviewer (M.A) verified the accuracy of the extracted data. In case of a disagreement the senior author (M.D) made the final decision.

Risk of bias in individual studies

The "Risk of bias" tool presented in the Cochrane Handbook [38] was used to determine the suitability of randomised control trials (RCTs) selected for inclusion in the quantitative analysis. The quality of cohort studies was assessed according to the recommendations of the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement. [39] Seven items of the STROBE statement were considered relevant for quality evaluation. Studies with a score of less than four were excluded. Two reviewers (M.P and M.A) performed the assessment independently. In case of a disagreement, a consensual decision was made.

Statistical analysis

All extracted data was tabulated and presented as means and percentages. Numerators and denominators were provided to address outcomes of included studies. For continuous variables the mean (standard deviation (SD)), median and range are extracted and reported.

The mean proportion of patients with no residual tumour left after HIFU treatment was evaluated by calculating the pooled inverse variance-weighted proportion. Studies with a standard deviation of zero (i.e. in studies with 100% complete ablation) were excluded from the analysis. A random-effects analysis was performed following the suspected high heterogeneity of studies included. All statistical analyses were performed with STATA 12.0 (StataCorp 2011, College station, TX).

2.2 Results

Selected studies

A total of 140 articles published up to December 2013 were identified from the literature search (*figure 1*). After reviewing the abstracts, 101 articles were excluded (because they were not relevant) and 39 articles underwent full text examination. A total of nine articles matched the selection criteria of which six [12, 17, 40-43] were feasibility studies, one was a prospective cohort study [44] and one was a retrospective cohort study. [21] A single RCT [19] was identified in which HIFU followed by mastectomy was compared to mastectomy alone.



Figure 1: Results of systematic search of the literature.

Study characteristics

In total, nine studies with 167 patients (mean age of 58.0 ± 2.8 years) and 169 lesions were included in the systematic review. The included breast cancer types were: invasive ductal carcinoma (83.4% (106/127 patients)), DCIS (5.5% (7/127 patients)), adenocarcinoma (2.4% (3/127 patients)), invasive lobular carcinoma (2.4% (3/127 patients)), invasive lobular carcinoma (2.4% (3/127 patients)), invasive mucinous adenocarcinoma (0.8% (1/127 patients)) and unknown breast carcinoma (5.5% (7/127 patients)). In a further three studies [12, 42, 45] different grading systems were used. Characteristics of the studies are summarized in *table 1*.

Six studies (116 patients) used MRI [12, 17, 40-43] as the mode of guided imaging and three [19, 21, 44] (51 patients) used US as guided imaging for HIFU. After HIFU treatment, resection of the tumour was performed in six studies [12, 17, 19, 40, 42, 43], follow-up with biopsies in one [44] and follow-up by both scans and biopsies in two studies. [21, 41] The cosmetic outcome was described in four studies [19, 21, 42, 44], histopathology results were discussed in all nine studies [12, 17, 19, 21, 40-44] whilst the imaging results were reported in six studies. [19, 21, 40, 41, 43, 44] The outcomes are described in *tables 2 and 3*.

Study	N	Median age	Tumour size	Ablation margin (cm)	Ablative device	Image guiding modality**	Max. W / pulse*	Treatment time (min)	Complications and cosmetic results	
Gianfelice et al. (2003) [12]	12	60	2.8 cm ³ (0.1 - 8.8)	0.5	ExAblate 2000, HAIFA	MRI	400	80 (35-133)	Pain/discomfort: slight (4), moderate (8), tender: mild (1), moderate (2). 2nd degree burn (2).	
Gianfelice et al. (2003) [41]	24	74.2	1.51 cm (0.6 - 2.5)	-	ExAblate 2000, HAIFA	MRI	60	-	Pain: moderate (14), mild (10). 2nd degree skin burn (1).	
Gianfelice et al. (2003) [40]	17	61.2	2.5 cm ³ (0.1 - 8.8)	-	ExAblate 2000, HAIFA	MRI	-	-	-	
Wu et al. (2003)[19]	23	46.5	3.1 ± 0.8 cm (2.0 - 4.7)	1.5 - 2.0	JC HIFU, HAIFU	US	545	78 (45-210)	Minimal skin burn (1), mild local pain, warmth and sensation of heaviness in breast (14), oedema (unknown).	
Wu et al. (2005)[44]	22	48.6	3.4 cm (2.0 - 4.8)	1.5 - 2.0	ExAblate 2000, HAIFA	US	545	132 (60-180)	Local oedema (all pt.), mild local pain (14, oral analgesics (6)). Cosmesis: good - excellent (16/17), acceptable (1/17).	
Zippel et al. (2005)[42]	10	56	2.2 cm	0.5	ExAblate 2000, HAIFA	MRI	-	Max 240	2nd degree burn (2), pain during procedure. Cosmesis: good - excellent (9), acceptable (1).	
Furusawa et al. (2006) [17]	28	56.9	1.3 cm (0.5 - 2.5)	0.5	ExAblate 2000, HAIFA	MRI	400	140 (76-231)	3rd degree skin burn (1), minor adverse events (5) (claustrophobia, abdominal and breast skin redness, pain (2), shoulder pain).	
Khiat et al. (2006)[43]	25	61.3	3.3 cm ³ (0.1-11.2)	-	ExAblate 2000, HAIFA	MRI	-	-	-	
Kim et al. (2010)[21]	6	62.1	2.6 cm (1.2 - 3.7)	1.0	JC HIFU, HAIFU	US	240	171 (80-285)	Mammary oedema (6), pectoralis major muscle injury (6), skin and trabecular thickening.	

Table 1: Patient, tumour and treatment characteristics of the included studies.

* pre-operatively defined, W = power ** MRI = magnetic resonance imaging and US = ultrasound.

Study	Imaging	Pre-treatment	Post-treatment		Correlation with		
<u>!</u>	modality		< 2 wk	2-12 wk	3-6 M	> 6 M	histopathology
Gianfelice et al. (2003) [12]	MRI 1.5T Signa	-	-	-	-	-	-
Gianfelice et al. (2003) [41]	MRI 1.5T Genesis	Hypo-intense tumour.	-	1 M: little or no change (92%).	-	-	-
Gianfelice et al. (2003) [40]	MRI 1.5T Signa	-	-	-	-	-	Correlation ISI, MDF, PEI and % residual tumour.
Wu et al. (2003)[19]	MRI 1.0T Impact	Tumour enhancement.	7-10 D: No enhancement tumour and 1.5- 2.0 cm margin.	-	-	-	-
Wu et al. (2005)[44]	MRI 1.0T Impact	-	-	3 M: 8.2% ± 6.1 reduction.	6 M: 26.7% ±12.2 reduction.	Reduction 12M: 45.2%±22.1 (21pt), 24M: 72.3%±32.1 (17pt), 36M: 80.3%±38.2 (17pt), 48M: 87.3%±42.3 (16pt), 60M: 90.4%±49.1 (5pt).	-
Zippel et al. (2005) [42]	MRI (unknown)	-	-	-	-	-	-
Khiat et al. (2006)[43]	MRI 1.5T Signa	In all patients strong enhancement.	-				Correlation ISI, PEI and % residual tumour.
Furusawa et al. (2006) [17]	MRI 1.5T (unknown)	-	-	-	-	-	-
Kim et al. (2010)[21]	MRI 1.5T Signa	Internal enhancements: inhomogeneous (3), internal septal (1), rim (1) and homogeneous (1).	-	2 wk: Iso-intense signal, no change in tumour size or SI. Thin rim (50%), nodular (33%) and both (17%). Heterogeneous signals on T2w.	4-6M: signal change on T2w.	11-24M: 46% decrease in tumour size (3pt). 11- 30M: no change in thin rim enhancement of index tumour.	-

Table 2: Imaging findings and outcomes of included studies.

* MRI = magnetic resonance imaging, CR = complete response, PR = partial response, NR = no response, SI = signal intensity, D = days, wk = weeks, M = months, ISI = increase signal intensity, MDF = maximum difference function and PEI = positive enhancement integral.

Table 3: Histopathology	outcomes of	f included	studies.
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Study	Type of	Time of	Type of	Complete histopathological response (%)			
	specimen collected	specimen collection	staining	< 1 M	1-3 M	3-6 M	>6 M
Gianfelice et al. (2003)[12]	Resection	Unknown	H&E	-	-	-	-
Gianfelice et al. (2003) [41]	Biopsy	6 M	Unknown	-	-	Complete necrosis (58%)	2nd treatment: total CR (79%)
Gianfelice et al. (2003)[40]	Resection	3-21 D	H&E	CR (24%), RD <10% (53%) and RD 30-75% (24%)	-	-	-
Wu et al. (2003)[19]	Resection	1-2 wk	H&E	CR (100%) tumour and margin of 1.80±0.58 cm	-	-	-
Wu et al. (2005)[44]	Biopsy	2 wk, 3/6/12 M	H&E	CR (100%) tumour and adjacent margin	Partial fibrosis (100%, 18 pt)	Complete fibrosis (100%, 14 pt.)	Complete fibrosis (100%, 14 pt.)
Zippel et al. (2005) [42]	Resection	7-10 D	Unknown	CR (20%), microscopic foci (20%), 10% RD (30%) and 10-30% RD (30%)	-	-	-
Khiat et al. (2006) [43]	Resection	3-21 D	Unknown	CR (31%), RD <10% (42%), RD 20-90% (27%)	-	-	-
Furusawa et al. (2006)[17]	Resection	5-23 D	H&E	CR (54%), <10% RD (36%) 10-15% RD (10%)	-	-	-
Kim et al. (2010)[21]	Resection + biopsy	3-20 M	Unknown	-	Viable tumour (50%)	No viable tumour (67%)	No viable tumour (67%, 4 pt)

*CR = complete response, RD = residual disease, D = days, wk = weeks, M = months and H&E = haematoxylin and eosin.

Quality assessment

Seven items of the STROBE [39] statement were used for quality assessment of the included cohort studies (*table 4a*). One study was excluded due to a quality score lower than four. All studies included specified study objectives and all but one had clear inclusion criteria and used standardized imaging. A standardized technique was used in six studies and five reported standardized histopathology. Patient follow-up was undertaken until surgery in six studies and normal follow-up performed in two studies. In three studies, patients withdrew during the course of the treatment. The overall STROBE score ranged from four to six (mean 5.3, SD 0.7). For the single RCT [19], the Cochrane checklist [38] was used (*table 4b*). The study was randomized, contained complete outcome data (no short-term outcome data missing), was free of selective reporting and other biases but did not contain a power analysis or blinding (of patients, participants or results).

Study	Study objectives	Inclusion criteria clear	Standardized technique	Standardized histopathology	Standardized imaging	Patient follow- up	Withdrawals from study
Gianfelice et al. (2003)[12]	Y	Y	Ν	Y	Y	N*	Ν
Gianfelice et al. (2003)[41]	Y	Y	Ν	U	Y	N*	Ν
Gianfelice et al. (2003)[40]	Y	Y	Y	Y	Y	N*	Ν
Wu et al. (2005)[44]	Y	Y	Y	Y	Y	Y	Y
Zippel et al. (2005) [42]	Y	Y	Y	U	Y	N*	N
Furusawa et al. (2006)[17]	Y	Y	Y	Y	Y	N*	Y
Khiat et al. (2006) [43]	Y	Y	Y	Y	Y	N*	Ν
Kim et al. (2010)[21]	Y	Y	Y	N	Y	Y	Y

Table 4: Study quality assessment (a) of cohort studies according to the STROBE statement, (b) included RCT assessed according to the "Risk Bias Tool" in the Cochrane Handbook. a)

*Follow-up: Studies in which the lesion was resected, follow-up was performed until surgery.

Study	Adequate sequence generation	Power analysis	Concealed allocation	Blinding	Incomplete data addresses	Free of other bias	Free selective reporting
Wu et al. (2003)[19]	Y	Ν	U	Ν	Ν	Y	Y

Clinical outcomes

Assessment of response to HIFU using imaging:

All studies performed pre- and post-treatment imaging. In two studies [19, 44] (26.9%, 45/167 patients) US colour Doppler was performed prior to the treatment and in one study post-treatment to determine perfusion of the tumour. In one study [44] a SPECT scan was made in 3.6% of all patients (6/167 patients) pre- and post-treatment. Of all patients, 77.8% (130/167 patients) underwent a pre- and post-treatment MRI. [12, 17, 19, 21, 40-44].

Different imaging modalities have been used to determine the response of HIFU (*table 2*). All nine studies [12, 17, 19, 21, 40-44] used MRI pre- and post-treatment; however, different MRI systems and sequences were used. Six studies [12, 17, 21, 40, 41, 43] used the 1.5T Signa excite (GE medical), two studies [19, 44] used the 1.0T Impact (Siemens) and in one study [42] the MRI system was not mentioned. The MRI protocol of seven studies [12, 19, 21, 40-42, 44] consisted of spin-echo T1 weighted sequences and fast spin-echo T2 weighted sequences with fat suppression. Seven studies [12, 17, 19, 21, 40, 43, 44] used dynamic contrast enhanced sequences with Gadolinium (Magnevist, Berlex Itd, Germany) contrast enhancement. Of these three studies [12, 40, 43] also used fast-spoiled gradient-echo sequences with fat suppression.

Another study [12] used T1 weighted spin-echo sequences with fat suppression, captured within a dynamic contrast-enhanced protocol.

Of the 77.8% (130/167) who underwent post-treatment MRI, in one study (12 patients) [12], the results were not reported. In four studies (80 patients), [17, 40, 42, 43] general descriptive findings were reported without quantitative findings. Contrast-enhancement was seen on pre-treatment scans and no enhancement was seen post-treatment. In four studies [19, 21, 41, 44], 31 patients (81.6%, 50-100%) showed an absence of enhancement at the index tumour and a thin rim of enhancement at the periphery. In seven patients (18.4%, 0-50%) nodular enhancement was seen at the periphery of the tumour, consistent with residual disease.

Two studies [21, 44] recorded reduction in tumour size after HIFU treatment on MRI. After six months, a reduction of $26.7 \pm 6.1\%$ was reported and after 11-24 months, a reduction of 46% and 57% (12 months $45.2 \pm 22.1\%$ and 24 months $72.3 \pm 32.1\%$) was observed. MRI performed within the first two weeks after treatment showed transient oedema surrounding the target volume [19]. Any change in tumour size as a result of oedema, was not documented.

MRI was performed immediately after HIFU treatment in three studies [17, 42, 43], within the first two weeks in eight studies [12, 17, 19, 21, 40-43] and at an unknown subsequent time in one study [44]. MRI immediately after HIFU treatment showed decreased enhancement, not sufficient enough to determine response to treatment.

Two studies [41, 43] showed a good correlation between the increase in signal intensity (ISI, r = 0.90 and r = 0.75, respectively), maximum difference function (MDF, r = 0.80), positive enhancement integral (PEI, r = 0.86 and r = 0.78, respectively) and the percentage of residual tumour. In one of these studies [43] a stronger correlation was seen (r = 0.93 (ISI) and r = 0.96 (PEI)) when only MRIs seven days or longer post-treatment were included.

In one study [12] with 24 patients, 95% (18/19 patients) of patients who were considered to have successful treatment based on biopsy results demonstrated a lack of enhancement on MRI. 60% (3/5 patients) of patients who were considered to have had a failed treatment because residual tumour was found on biopsy, demonstrated persistent enhancement on MRI after two HIFU sessions.

Histopathological correlations:

Histopathology was discussed in all nine studies (*table 3*). [12, 17, 19, 21, 40-44] Six studies (68.9%, 115/167 patients) [12, 17, 19, 40, 42, 43] obtained histopathology results by surgical removal of the tumour by either lumpectomy or mastectomy. In two studies (27.5%, 46/167 patients) [41, 44], CNBs were obtained and in the last study (3.6%, 6/167 patients) [21], a combination of CNB and surgical removal was used. To get a clear view of the percentage of complete ablation and residual tumour, the histopathology results are divided into three groups: complete ablation, less than 10% residual tumour, residual tumour between 10 - 90%. Our primary outcome is complete treatment. The threshold of 10% residual tumour was used previously in four studies [12, 17, 42, 43]

Complete ablation or no residual tumour was found in 46.2% (55/119, range 17-100%) of all patients, who underwent surgical excision after HIFU treatment. The weighted summary proportion analysis showed an estimated proportion of 30% (95% CI: 0.2 - 0.4) of patients having no residual tumour after HIFU treatment. The weighted proportions are illustrated in a forest plot (*figure* 2). The I2-statistic of 47.2% confirms the suspected heterogeneity among studies. One study was excluded from the analysis due to 100% ablation.



Figure 2: Random effect analysis demonstrating an estimated 30% (95% CI: 0.2-0.4) of patients with no residual tumour after HIFU treatment.

One study recorded complete necrosis of the tumours in all patients [19]. In five out of seven studies [17, 19, 40, 42, 43], patients underwent surgical resection 1 - 3 weeks post-treatment and in one study [21] the patients underwent surgical resection 3 - 11 months post-treatment. The last study [12] did not discuss the time of surgical removal of the breast specimens. Four studies [12, 17, 19, 40] used haematoxylin and eosin (H&E) for histopathological staining, the other three studies [21, 42, 43] did not report the type of staining.

Residual tumour of less than 10% was found in 29.4% (35/119 patients, range 0 - 53%) [12, 17, 40, 42, 43]. These histopathology results were obtained within the first three weeks after HIFU treatment. Residual tumour between 10-90% was found in 22.7% (27/119 patients, range 0 - 60%) of all patients [12, 17, 40, 42, 43]. Surgical resection of the tumour was performed between one to three weeks after surgery and in one study no time of resection was mentioned. In one study [21], the amount of patients with complete ablation were described but the percentage of residual tumour in the other patients (1.7%, 2/119 patients, range 0 - 33%) was not mentioned. These patients underwent surgery 3 - 11 months post-treatment.

In the three studies [21, 41, 44] using CNBs to determine the amount of residual tumour, 90.0% (43/48 patients, range 79 - 100%) of patients showed no residual tumour. Residual tumour was found in the other five patients, however, no quantitative statements were made. In the first study [44], the CNBs were performed after two weeks, in the second after 6 - 7 months [41] and in the last study [21] after 1 - 20 months. In one study [44], H&E was used for histopathological staining, the other two studies [21, 41, 44] did not report the type of staining used.

One study [44] reported that after three months partial fibrosis, was seen in the CNBs of all patients (18 patients) and after six and 12 months complete fibrosis was visible in all patients (14 patients).

In two studies [12, 17], the percentage of tumour located in the treatment area was determined. The whole tumour was located in the treatment area in 82.5% (33/40 patients, range 58 - 93%) of patients, in 10.0% (4/40, range 7 - 17%) between 90 - 100% of the tumour was located in the treatment area and in 7.5% (3/40, range 0 - 25%) less than 70% of the tumour was located in the treatment area.

One study [19] measured the ablated margin around the tumour, which was 1.8 ± 0.6 cm.

Post-treatment complications:

Complications were described in seven studies (table 1, figure 3). [12, 17, 19, 21, 41, 42, 44]



Figure 3: Short-term complications after HIFU treatment.

Pain was reported in 40.1% (67/167) of patients and was slight in 6.0% (4/67 patients), moderate in 32.8% (22/67 patients), mild in 56.7% (38/67 patients) and unknown in 4.5% (3/67 patients). Skin burns occurred in 4.2% (seven patients; one superficial, five second and one third-degree burn). Oedema around the tumour was noted in three studies and occurred in at

least 16.8% (28/167 patients). In one study [19] oedema was noted, but the number of patients was not reported. The oedema disappeared within two weeks of the treatment. Pectoralis major injuries were reported in one study and occurred in all six patients (3.6%, 6/167 patients). Other complications were claustrophobia (0.6%; 1/167 patients), redness of the skin (0.6%; 1/167 patients) and tenderness of the breast (0.6%; 1/167 patients).

Recurrence:

Recurrence of the tumour was found in two patients (1.2% of total patients, 7.1% of those with follow-up (2/28 patients)) in one study [44]. Both underwent modified radical mastectomy followed by chemotherapy, however one patient died 44 months post-treatment due to metastatic disease.

Cosmesis:

Two studies [42, 44] performed an cosmetic analysis (*figure 4*) after HIFU treatment. Good to excellent cosmetic results were achieved in 92.6% (25/27 patients) and an acceptable result was achieved in 7.4% (2/27 patients). None of the patients were reported to have poor or unacceptable cosmetic results.



Figure 4: Cosmetic outcome of HIFU treatment.

2.4 Discussion

The studies included in this systematic review demonstrate that HIFU has been shown in small series to successfully induce coagulative necrosis in breast tumours. Histopathology showed no residual tumour in 46% of all patients (55/119 patients, range 17 - 100%). Residual tumour of less than 10% was found in 29% (35/119 patients, range 0 - 53%), residual tumour between 10 - 90% in 23% (20/119 patients, range 0 - 33%) and no percentages of residual tumour were mentioned in 2% (2/119 patients, range 0 - 33%) [12, 17, 40, 42, 43]. Post-treatment MRI images showed an absence of contrast enhancement and a thin rim of enhancement at the periphery in 82% of patients (31/38 patients, range 50 - 100%) indicative of coagulative necrosis. [19, 21, 41, 44] MRI of the breast after HIFU treatment [40, 43] showed that there was a positive correlation between the percentage of residual tumour. The most common complications during and post-treatment were pain (40%), skin burns (4%), oedema (>17%) and pectoralis major injury (4%). Recurrence was reported in two patients in which an increase in tumour size was seen after an initial reduction in US size.

This is the first systematic review that describes the clinical efficacy of HIFU in the treatment of breast tumours and therefore, no comparisons with the outcomes of other systematic reviews could be made. Comparing the imaging results, studies, perhaps surprisingly show that USguided HIFU treatment [19, 21, 44] appears to give better results than MRI-guided studies [12, 17, 40-43]. Although only three studies [19, 21, 44] (two research groups) have performed HIFU under US guidance, two of these [19, 44] had an efficacy of 100% and the third [21] had an efficacy of 67%, although the latter included only six patients. More studies are needed to get an idea of the efficacy of US-guided HIFU. It is likely that patients are selected for US treatment and patient selection may be responsible for this observation. A positive correlation was found between the increase of signal intensity and the percentage of residual tumour tissue. [40, 43] Before HIFU treatment, a strong enhancement of the whole tumour was observed in dynamic contrast-enhanced MRI. Post-treatment, no enhancement was seen when the tumour was completely necrosed. When residual tumour was left behind, MRI scans showed a thin rim of enhancement. However, some benign processes such as oedema, fibrosis, necrosis and inflammation can mimic malignant contrast and therefore the time interval between treatment and imaging procedure, as well as the shape of the enhancement curves, must be taken into account. Malignant tissues after HIFU continue to show an irregular border, a rapid enhancement and an early distinct washout phase. [43]

A failure to achieve complete ablation of the tumour may be related to accuracy of targeting the treatment area or to failure of the technique itself. Clearly if the target tumour is not fully located in the treatment field, this can be due to problems in the imaging process or movement of the patient during the treatment. It is not clear on review whether in many of the studies, the whole tumour was actually located in the treatment field and also if the treated area was completely necrosed and therefore what part any difficulties in targeting may play in failure of ablation. This data was reported in only two of nine studies [12, 17]. All other studies only recorded the percentage of cases where complete ablation of the tumour was achieved. It is therefore not evident if this is a failure of the HIFU treatment or of locating the tumour. It is possible that with improved targeting of the tumour, the efficacy of HIFU treatment could potentially be higher than described in these studies. The percentage of residual tumour after HIFU treatment varies within the studies. Studies [46, 47] have proven that even though ablated tissue is easily identified (the yellow-white or sallow, mat coagulation necrosis showed a clear boundary with surrounding tissue), tumour cells which show normal cellular structure (tumour cells outline was remained and no significant changes in nucleus) after staining with H&E were found to be not viable in NADH staining and electronic microscopy (organelles and nucleus were damaged or had disappeared). Therefore, the percentage of residual tumour might be lower than found in the studies. In order to treat patients with HIFU, the histopathology of the tumours must be established prior to treatment for a definitive diagnosis. Surgical resection after HIFU treatment may not provide definitive diagnostic or prognostic factors for the determination of adjuvant systemic therapies. Several prognostic factors (e.g. presence or absence of lympho-vascular invasion) will not be assessable reliably on the limited sampling of a CNB sample. This may potentially limit the value of this technique in the malignant setting until improvements in imaging allow for collection of comparable prognostic factors to histopathology. When only limited examination with small biopsies are performed, clearly it is not possible to conclude whether complete or incomplete ablation has been achieved within either days or months after HIFU treatment. It is possible that sampling after longer periods of time may demonstrate more extensive fibrosis or even additional necrosis related to isolation of any residual tumour from its blood supply.

The extra margin of normal tissue ablated around the target tumour in the included studies varies from 0.5 to 1.5 - 2.0 cm. In two studies [19, 44], the margin was between 1.5 - 2.0 cm, and these are the only two studies having complete necrosis of the tumour in 100% of cases. Both were based on US-guided HIFU treatment. The only other US-guided study [21] applied a margin of 1.0 cm and achieved complete ablation in 66.7% of cases. In the MRI-guided studies [12, 17, 40-43], the margins treated around the tumour were between 0 - 1.0 cm and complete ablation was obtained in fewer cases. This wider treated margin of surrounding tissue could explain the high percentages of complete ablation achieved in the US-guided studies. The width of tissue surrounding the tumour that should be included in the treatment field is not clear from this review of the literature and may potentially not be identical for MRI and US-guided techniques; further research is required. The histopathology and imaging results after HIFU treatment were directly compared in three studies. [19, 41, 44] Two studies with a complete ablation of 100% histologically also showed a complete ablation on MRI. However, in one other study [41] the MRI gave one false positive and two false negatives results when compared to histopathology as the gold-standard. This suggests that MRI is an accurate predictor of complete ablation following HIFU treatment.

The treatment times of HIFU are a major disadvantage of the technique. However, only five studies [12, 17, 19, 21, 44] have reported treatment times, these range between 78 and 171 minutes for a lesion of 1.3 - 3.4 cm. It is imperative to make this treatment a viable alternative not only to patients clinically unfit for surgery that treatment times must be reduced.

The most common complications are local pain, skin burns, oedema and pectoralis major injuries. Pain could be controlled with local anaesthesia or analgesics. Pain can also be avoided by ensuring a distance of at least 0.5 cm from the tumour to the skin and 1.0 cm from the chest wall. If the distance is shorter, decassed local anaesthesia can be added to increase the distance, although this is likely to be absorbed during treatment due to pressure from the device on the skin. In most cases with skin burns, a simple cause could be found such as insufficient tissue between the skin and the lesion or insufficient amount of cooling time in-between pulses. If sufficient distance from the skin and chest wall is maintained during treatment and there is ample time between pulses to cool the skin (or the skin is cooled during the treatment), skin burns should be avoided. Oedema resolved within two weeks post-treatment and pectoralis major injury resolved within six months. To prevent pectoralis major injury a minimum distance of 1.0 cm should be used. Compared to the possible complications of breast surgery (infection, bleeding, incomplete wound healing) these are relatively mild complications. The most significant concern regarding HIFU treatment of malignant tumours is inadequate treatment of the cancer. Recurrence was described in only two patients. [44] However, follow-up was only reported in two studies [21, 44] so this number is likely to be higher overall in all of the HIFU treated breast cancer patients.

The cosmetic result after HIFU treatment was good to excellent in 93% (25/27) of patients asked and acceptable in 7% (2/27). [42, 44] Due to the fact that in six studies HIFU was followed by surgical resection, the cosmetic outcome of HIFU could not be assessed. Lesion resorption is a long process and can take up to six months after HIFU treatment. [41, 44] This could prove to be a challenge during follow-up and may also have a psychological impact on patients believing that they might still have a lesion or even recurrence. [19] Therefore, it is important to inform patients that lesions may remain palpable for a long period and that this does not constitute recurrence. Furthermore, HIFU treatment might also prove challenging for interpretation of future breast imaging, if radiologists are not made aware of the HIFU treatment.

All cohort studies reviewed were performed in different ways, varied in outcome measures and consistency of reporting of results, and results could therefore not, be directly compared with each other in an any quantitative analysis. There are significant variations in, for example, the times of further imaging and subsequent biopsies as well as the lesions included in the studies, the mode of the HIFU treatment, different MRI devices and MRI sequences. Heterogeneity was also found in the width of the surrounding tissue treated, the ablation dose and the frequency of the treatment. Furthermore, inclusion criteria differed in the distance between the lesion and the skin, chest wall and the nipple. Finally, the median amount of patients per study was 16.7, and two studies [21, 42] have a number of patients of ten or fewer. Strict standardization within the setting of RCT's is needed to compare HIFU with breast surgery and to compare MRI-guided HIFU with US-guided HIFU and, in particular, large prospective studies are needed.

Compared with breast surgery, HIFU offers several potential advantages, including reduced recovery time and hospital stay, decreased complication risks and the ability to perform the treatment under local anaesthesia in an outpatient setting [48, 49]; all these factors could lead to a significant cost reduction, but require formal assessment. Furthermore, compared to the use of VAM for therapeutic interventions, HIFU has the advantage of not requiring any puncture of the skin, preventing the risk of infection and haematoma development. HIFU has the added benefit over VAM, that visualization of the tumour does not become increasingly obscured during the treatment due to excessive bleeding. [50] Studies [12, 17, 19, 21, 40-42, 44, 49] still use adjuvant therapy after HIFU treatment to acquire successful removal of the tumour. HIFU could potentially achieve complete removal of the tumour without the need of adjuvant therapy, like radiotherapy, if the ideal margin that needs to be included in the treatment is known.

Compared with other ablative techniques like RFA, cryo-ablation, laser ablation and microwave ablation, HIFU again has the advantage of not requiring any skin incision for instrument insertion. Furthermore, the focal point is fixed in the other techniques and in the case of HIFU, the focal point is flexible in terms of size and shape of the treatment zone. [12] The next chapter compares HIFU with these other ablative techniques in the treatment of breast tumours to evaluate which technique shows the best potential.

3. Systematic Review: Minimal Invasive Ablative Techniques in the Treatment of Breast Tumours

3.1 Materials and Methods

Study selection

A systematic review of the literature was performed using PubMed/Medline library database to identify all studies published up to January 2014 that evaluated the role of ablative techniques for the treatment of breast tumours. The MeSH terms used were ablative techniques, ablative interventions, ablative therapy, thermal ablation, high intensity focused ultrasound, radiofrequency ablation, laser ablation, cryo-ablation and microwave ablation in combination with breast. Except for reports in the English language and human subjects, there were no further restrictions. All obtained abstracts, studies and citations were reviewed. The related articles function was used to broaden the search, and all abstracts, studies and citations obtained were reviewed. References of the articles acquired were also searched by hand. The last search was conducted on February 18th, 2014.

Inclusion criteria

Studies were considered eligible for the systematic review if they addressed the following: (1) studies performed on human subjects with breast tumours, (2) studies using a non-surgical ablative technique including RFA, HIFU ablation, cryo-ablation, laser ablation or microwave ablation as a treatment for breast tumours, (3) studies objectively recorded response to treatment using imaging and histopathology, (4) studies objectively recorded treatment times, complication and/or recurrence rates (5) studies with 20 patients or over included.

Exclusion criteria

Studies that failed to fulfil the inclusion criteria or studies in which the outcomes of interest were not reported or if it was not possible to analyse these from the published reports, were excluded. Conference articles, letters, editorials and case reports were excluded. Studies using laser ablation as a scalpel were also excluded. In the case of studies with overlapping study populations, the most recent study with histopathological outcomes was included. When full text was not available, the study was also excluded.

Data extraction

Each study was initially evaluated for either inclusion or exclusion. The data extracted from the included studies were: first author, year of publication, study design, type of ablative technique, number of included patients, number of included tumours, tumour type (benign/malignant), tumour size, type of imaging guidance, total treatment time, resection (yes/no), treatment margin used, follow-up period, imaging outcome, histopathology staining, outcome of histopathological staining, complications, re-treatment rate and recurrence rate. One reviewer, (M.P) extracted data for all selected studies and a second reviewer (M.A) verified the accuracy of the extracted data. In case of a disagreement the senior author (M.D) made the final decision.

Risk of bias in individual studies

The "Risk of bias" tool presented in the Cochrane Handbook [51] was used to determine the suitability of RCTs selected for inclusion in the quantitative analysis. The study quality of cohort studies was assessed according to the recommendations of the STROBE statement. [52] Seven items of the STROBE statement were considered relevant for quality evaluation. Studies with a score of less than four were excluded. Two reviewers (M.P and M.A) performed the assessment independently. In case of disagreement, a consensual decision was made.

Statistical analysis

All extracted data were tabulated and presented as means and percentages. Numerators and denominators were provided to address outcomes of included studies. For continuous variables the mean (SD), median and range should also be extracted and reported.

3.2 Results

Selected studies

A total of 1532 articles published up to January 2014 were identified from the literature search (*figure 5*). Two articles were identified by searching the references of selected articles. After reviewing the abstracts, 1446 articles were excluded and 88 articles underwent full text examination. A total of 11 articles matched the selection criteria of which six [27, 44, 53-56] were feasibility studies, two [57, 58] were pilot studies, one [26] was a phase I study and one [19] was a RCT. One article [11] included four sub-studies: one phase I, one phase II and two randomized studies in which microwave ablation was compared to breast (conserving) surgery. Only one sub-study (phase II) matched all selection criteria and was included into the systematic review.



Figure 5: Results of systematic search of the literature.

Study characteristics

In total, 11 studies with 377 patients with 383 tumours were included in the systematic review. The included breast tumour types were: invasive ductal carcinoma (80.7%, 309/383 tumours), DCIS (7.8%, 30/383 tumours), invasive lobular carcinoma (3.7%, 14/383 tumours), colloid carcinoma (1.0%, 4/383 tumours), invasive mucinous adenocarcinoma (0.3%, 1/383 tumours), tubular carcinoma (0.3%, 1/383 tumours), medullary (0.3%, 1/383 tumours) and unknown breast carcinoma (6.1%, 23/383 tumours). The characteristics of the studies are summarized in *table* 5. Four studies treated patients with RFA [54-56, 58], two studies used HIFU ablation [19, 44] and two used cryo-ablation. [26, 57] Laser [27] and microwave ablation [11] were both used in one study. One study [53] compared cryo-ablation with RFA. All studies treated patients with malignant tumours and used US as the mode of guided imaging. After ablation, resection of the tumour was performed in nine studies [11, 26, 27, 44, 53, 55-58] and follow-up with biopsies was performed in two studies. [19, 54] All studies described the imaging and histopathological ablation outcomes and the total treatment time; these results are described per ablation technique in *tables 5a-f.*

Table 5: Study characteristics and	outcomes for (a) RFA	, (b) HIFU, (c) cryo-ablation, ((d) laser abla	ation, (e) microwave	ablation and (f) the
single retrospective study.						
(a)						

Author	Patients	Age	Size (cm)	Treatment time (min)	Follow- up	Histopathology	Imaging	Complications and cosmesis
Manenti et al. (2009) [55]	34	53 ± 5 (49-62)	1.9 ± 0.6 (1.7-2.0)	27 ± 3.7 (25-35)	-	H&E (4 wk): No RD 94%. NADH (4 wk): No RD 97%.	MRI (3.0T Achieva Philips) 1 wk: 91% no enhancement, 4 wk: 97% no enhancement.	Skin burn & hyper- pigmentation (1). Cosmesis: excellent (28), good (5), poor (1).
Ohtani et al. (2011) [58]	41	59 (38-92)	1.3 (0.5-1.8, MRI)	9 (6-15)	-	General: No RD 88%. H&E: No RD 12.5% NADH (1-2 M): No RD 100% (12/12)	MRI (3.0T Signa GE medical) 1-2 M: 25/26 (96%) no enhancement	Skin burn (1).
Oura et al. (2007) [56]	52	55 (37-83)	1.3 (0.5-2.0)	12 (5-25)	Every 2-3 M	Cytology (3-4 wk): 42% no RD.	MRI (1.5T Siemens Magnetom) 1-2M: 100% no RD, US 2-3 M: 42% no RD, 100% no vascular flow.	Skin burn (1). Cosmesis: excellent (43), good (6), fair (3).
Yamamoto et al. (2011) [54]	29 (30)	55.9 (38-78)	1.3 (0.5-1.9)	11.4 (6-20)	17 (2-41) M	H&E (3-4 wk): 10%, NADH (3-4 wk): 92%	MRI 3-4 wk: no hyper- vascularity in ablated zone 100%, mean size 3.8x3.3cm.	Third-degree burn (3), overreaction (1). Cosmesis: excellent (28), unknown (1).

* D = days, wk = weeks, M = months, RD = residual disease, H&E = haematoxylin and eosin, NADH = nicotinamide adenine dinucleotide, MRI = magnetic resonance imaging and US = ultrasound.

(b)

Author	Patients	Age	Size (cm)	Treatment time (hr)	Follow- up	Histopathology	Imaging	Complications and cosmesis
Wu et al. (2003) [19]	23	46.5 ± 1.7	3.1 ± 0.79 (2.0- 4.7)	78 (45-210)	-	H&E (1-2 wk): No RD 100% Margin 1.8 ± 0.58 (1.5-2.2) cm	MRI (1.0T Impact) 7-10 days: 100% (3/3) no enhancement, extra margin of 1.5-2.0 cm.	Skin burn (1), mild local pain (1), oedema
Wu et al. (2005) [44]	22 (23)	48.6 (36-68)	3.4 (2.0-4.8)	132 (60-180)	54.8 (36-72) M	Biopsy results (2 wk, 3, 6, 12 M): H&E: no RD 100%	US (Q-2000): 86% absence blood flow, volume decrease: 6M (n=17) 26.7 ± 12.2%, 12M (n=17) 45.2 ± 22.1%, 60M (n=5) 90.4 ± 4.91%. MRI (1.0T Impact): 100% (5/5) no enhancement.	Mild local pain (14), oedema. Cosmesis: good - excellent (16), acceptable (1).

* D=days, wk=weeks, M=months, RD=residual disease, H&E=haematoxylin and eosin, MRI = magnetic resonance imaging and US = ultrasound. (c)

Author	Patients	Age	Size (cm)	Treatment time (min)	Histopathology	Imaging	Complications and cosmesis
Sabel et al. (2004) [26]	29	Median 52.5 (34-77)	1.2 ± 0.5 (0.6-2.0)	10.3 (10-12)	H&E (14D): 85% no RD, 4x DCIS in normal tissue	US: during treatment: Size ice ball: 4.8x3.4x3.3 cm ³ (high freezing cycle 8-10 min), 3.8x2.7x2.7 cm ³ (6 min).	-
Tafra et al. (2003) [57]	24	Mean 61 (41-78)	1.2 ± 0.4 (0.7-2.0)	15.8 ± 7.6 (median 14)	Unspecified: 83% negative margins, diameter 1.4 ± 0.5 cm (+14%)	US during treatment: Ice ball: 3.9 ± 0.3 ($3.2-4.4$) cm (high freeze cycle 8 min) Margin: 0.8 ± 0.2 ($0.5-1.1$) cm	Small seroma (2)

* D = days, wk = weeks, M = months, RD = residual disease, H&E = haematoxylin and eosin and US = ultrasound.

(d)

Author	Patients	Age	Size (cm)	Treatment time (min)	Histopathology	Imaging	Complications and cosmesis
Mumtaz et al.	20 (24)	57 (34-79)	2.0 (0.4-3.3)	8.3	H&E (5D): 65% no RD, 25% beyond margin, 10% missed.	MRI 4hr, 24/48 hr: 65% centric, 25% eccentric,	Severe pain (2)
(1996) [27]					NADH (5D): 100% (2/2), Size: 1.0 (0.5-1.5) cm (-50%)	size 1.0 (0.7-1.8)cm (-50%).	

*hr = hours, D = days, wk = weeks, M = months, RD = residual disease, H&E = haematoxylin and eosin, NADH = nicotinamide adenine dinucleotide and MRI = magnetic resonance imaging.

(e)

Author	Patients	Age	Size (cm)	Treatment time (min)	Histopathology	Imaging	Complications and cosmesis
Dooley et al. (2010) [11]	25	57.2 (no range)	1.76 (0.7-2.8)	159-206 (8), 108-148 (9), 82.8-97.2 (2)	H&E (17D): 68% necrotic tumour in relation to necrotic and viable tumour, 8% no RD.	US, prior to resection: size 1.84 (0.7-3.8) cm (+5%), PR* (16%), SD (52%), PD (32%)	Mild pain (9), erythema (9), oedema (5), first-degree burn (2), third-degree burn (1), severe pain (1).

* CR = complete response, PR = partial response, decrease tumour volume ≥ 50%, SD = stable disease, decrease <50% and PD = progressive disease, increase tumour volume >25%, D = days, RD = residual disease, H&E = haematoxylin and eosin and US = ultrasound. (f)

Author	Patients	Age	Size (cm)	Treatment time (min)	Histopathology	Imaging	Complications and cosmesis (pre- vs. post-treatment)
Manenti et al. (2013) [53]	40 (RFA)	73 ± 5 (64-82)	-	15 ± 3.7 (12-23)	NADH (30-45D): 93% no RD	MRI (unknown) 88% (1 wk) and 93% (4 wk) no enhancement. RFA area: 2.7 ± 0.1 x 4.2 ± 0.1cm	Cosmesis: excellent 23 vs. 34, good: 10 vs. 3, acceptable: 5 vs. 1 and poor: 1 vs. 2.
	40 (cryo)	73 ± 5 (64-82)	-	25	NADH (30-45D): 95% no RD	MRI (unknown) 90% (1 wk) and 95% (4 wk) no enhancement. Size ice ball: $1.6 \pm 0.1 \times 3.1 \pm 0.1 \text{ cm}$	Cosmesis: excellent: 26 vs. 37, good: 8 vs. 2, acceptable: 7 vs. 1 and poor: 0 vs. 0.

* D = days, wk = weeks, M = months, RD = residual disease, NADH = nicotinamide adenine dinucleotide and MRI = magnetic resonance imaging.
Quality assessment

Seven items of the STROBE statement [52] were used for quality assessment of the included cohort studies (*table 6a*). One study was excluded due to a quality score lower than four. All studies included specified study objectives and all but one had clear inclusion criteria. A standardized technique was used in eight studies and seven studies reported standardized histopathology. Standard imaging was performed in all studies but patient follow-up after resection of the tumour was undertaken in only two studies. In two studies, patients withdrew from the study due to pain or other reasons which were not reported. The overall STROBE score ranged from four to seven (mean 5.5 (SD 0.9)).

For the single RCT, the Cochrane checklist [51] was used (table 6b). The study was randomized, contained complete outcome data (no short-term outcome data missing), was free of selective reporting and other biases but did not contain a power analysis or blinding (of patients, participants or results).

(u)							
Study	Study objectives	Clear inclusion criteria	Standardized technique	Standardized histopathology	Standardized imaging	Patient follow -up	Withdrawals from study
Dooley et al. (2010) [11]	Y	Y	Y	Y	Y	N	N*
Manenti et al. (2009) [55]	Y	Y	Y	Y	Y	N	Ν
Manenti et al. (2013) [53]	Y	Y	Y	Y	Y	N	Ν
Mumtaz et al. (1996) [27]	Y	Y	Y	Y	Y	N	Y*
Ohtani et al. (2011) [58]	Y	Y	Y	Y	Y	N	Y*
Oura et al. (2007) [56]	Y	Y	N	U	Y	Y	Ν
Sabel et al. (2004) [26]	Y	Y	Ν	Y	Y	Ν	N
Tafra et al. (2003) [57]	Y	Y	N	U	Y	N	N
Yamamoto et al. (2009) [54]	Y	Y	Y	Y	Y	Y	N
Wu et al. (2005) [44]	Y	Y	Y	Y	Y	Y	Y

Table 6: Quality assessment (a) Study quality assessment of cohort studies and (b) methological characteristics of included RCT.

*Included patients that did not tolerate the treatment. Study quality was assessed according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

⁽b)

Study	Adequate sequence generation	Power analysis	Concealed allocation	Blinding	Incomplete data addresses	Free of other bias	Free selective reporting
Wu et al. (2003) [19]	Y	Ν	U	Ν	N	Y	Y

*Study quality was assessed according to the "Risk Bias Tool" in the Cochrane Handbook.

Outcomes

Total treatment time

In the single study which used laser ablation [27] the standard treatment time in all cases was 8.3 minutes for a tumour of approximately 2.0 cm. In RFA [53-56, 58] patients were treated in between 9 and 27 minutes, dependent on the size of the tumour. Tumours of approximately 1.4 \pm 0.3 cm were ablated in about 14.9 \pm 8.2 minutes. For cryo-ablation [26, 53, 57], the treatment time was similar as RFA, approximately 13.1 \pm 3.9 minutes for an average size of 1.2 cm. In HIFU [19, 44], the treatment times were relatively longer, approximately 105.0 \pm 38.0 minutes for a tumour of approximately 3.3 \pm 0.2 cm and in the study using microwave ablation [11] the duration of the treatment varied between 90 and 180 minutes for tumours of about 1.8 cm. The treatment times are shown in *table 7*.

	Mean treatment time	Mean tumour size
RFA	14.9 ± 8.2 minutes	1.4 ± 0.3 cm
HIFU	105.0 ± 38.0 minutes	3.3 ± 0.2 cm
Cryo	13.1 ± 3.9 minutes	1.2 ± 0 cm
Laser	8.3 minutes	2.0 cm
Microwave	90 - 180 minutes	1.8 cm

Table 7: Mean treatment times of ablative techniques

Baseline imaging and assessment of response to treatment

In RFA, before treatment all studies performed a mammogram, US and MRI. [54-56, 58] Furthermore, in one study a positron emission tomography computed tomography (PET - CT) was performed [58] and in another study a contrast enhanced multi-detected computed tomography (MDCT) was performed pre-treatment. [54] In HIFU, both studies performed an US, colour Doppler (Q-2000 Siemens, Erlanger, Germany), chest radiograph, bone scan and MRI. [19, 44] In cryo-ablation, both studies [26, 57] performed a US and one study [57] performed an MRI pre-treatment. In the laser ablation study [27], MRI's were performed and in the microwave ablation study [11], US scans were performed pre-treatment. In the study [53] comparing RFA with cryo-ablation, US and MRI's were performed.

Post-treatment all RFA studies [54-56, 58] performed an MRI; one study [56] also performed an US post-treatment. In HIFU, studies [19, 44] performed MRI post-treatment and one study [44] performed US, colour Doppler and single positron emission computed tomography (SPECT, Technetium-99m, Dicom, Siemens Erlanger, Germany). In the cryo-ablation studies [26, 57], both performed US scans during the treatment to control the ice ball size. Post-treatment, no imaging was reported to be performed. In the laser ablation study [27], all patients received an MRI and in the microwave ablation study [11], the patients received an US post-treatment. In the study [53] combining RFA with cryo-ablation all patients received a post-treatment MRI.

In RFA, three studies [53, 55, 58] used a 3.0T MRI device (Achieva, Philips healthcare, The Netherlands; Signa, GE medical systems, Japan), one study [56] used a 1.5T MRI (Magnetom, Siemens, Erlanger, Germany) and in one study [54], the MRI device was not reported. In HIFU

the single study [19] using MRI used the 1.0T Impact device (Siemens, Erlanger, Germany) and in cryo-ablation [53] the 3.0T Achieva (Philips healthcare, The Netherlands) was used and in another study [57] the type of MRI device was not reported. In laser ablation [27], a 1.0T Magnetom (Siemens, Erlanger, Germany) was used.

MRI results were described in six studies [19, 44, 53-55, 58] using three ablation techniques (table 8). In RFA, MRI in two studies [55, 58] showed an absence of contrast enhancement in 96.7% (58/60 patients) of all patients 1 - 2 months post-treatment compared to pre-treatment scans, indicating complete necrosis although histopathological compete response was only seen in 92.0% (69/75 patients, not all patients received an MRI). In one patient residual peripheral enhancement was visible after 1 - 2 months due to intolerable pain during the treatment and in one patient peripheral enhancement was seen after four weeks. Another RFA study [54] reported that no hyper-vascularity of the ablated tumour was found in all patients. For HIFU [19, 44] an absence of enhancement and a thin rim of enhancement indicative of coagulation necrosis was visible post-treatment in all patients (8/8 patients). In the study comparing cryo-ablation and RFA [53], MRI showed no enhancement after RFA in 92.5% (37/40 patients) after four weeks. In 7.5% (3/40 patients), the enhancement increased on the four-week scan suggesting residual tumour, which was confirmed by histology. For cryo-ablation, no enhancement was seen in 95.0% (38/40 patients) after four weeks. In 5.0% (2/40 patients), increased enhancement was seen suggesting residual tumour, which was again confirmed by histology.

	MRI: Absence enhancement	US: Disappearance	Timeframe imaging*		
RFA	96.7% (58/60)	42.3% (22/52)	1-2 M (MRI), 2-3 M (US)		
HIFU	100% (8/8)	86.4% (19/22)	Unknown		
Article	rticle comparing RFA and cryo-ablation:				
RFA	92.5% (37/40)	-	4 wk		
Cryo	95.0% (38/40)	-	4 wk		

Table 8: Post-treatment imaging results seen on MRI and US.

Measurements of tumour / ablation / margin were performed with MRI or US in eight studies [11, 19, 26, 27, 44, 53, 54, 57] using all five ablation techniques. With RFA [53, 54], the ablation sizes were 3.8 cm x 3.3 cm after 3 - 4 weeks and 2.7 cm x 4.2 cm after 1 - 4 weeks on MRI. With HIFU [19, 44], the measured margin of normal tissue around the ablated area was 1.5 - 2.0 cm on MRI scans. On US, a decrease in volume of $26.7 \pm 12.2\%$ was measured after six months, $45.2 \pm 22.1\%$ after 12 months and $90.4 \pm 4.9\%$ after 60 months. With cryo-ablation [26, 53, 57], the ice ball reached a maximum size of 3.8 cm x 2.7 cm x 2.7 cm after a high freezing cycle of six minutes (US), 3.9 cm after eight minutes (US), 4.8 cm x 3.4 cm x 3.3 cm after 8 - 10 minutes (US) and 1.6 cm x 3.1 cm after 10 minutes (MRI). In one study, a margin of 0.8 ± 0.2 cm around the ablated tumour was ablated during the treatment and in the laser ablation study [27] the ablated diameter measured 1.0 (range 0.7 - 1.8) cm on MRI scans after 24 - 48 hours which corresponds to an average decrease of 50%. With microwave ablation [11], a tumour increase of 5% was observed (1.84 cm, range 0.7 - 3.8 cm) on US scans after 17 days.

Two studies [44, 56] reported US results, in RFA 42.3% (22/52 patients) of all scans demonstrated a tumour disappearance after 2 - 3 months indicated by an absence of vascular flow. In HIFU, 86.4% (19/22 patients) of all colour Doppler scans showed an absence of blood flow indicating cell death. In the remaining 13.6% (3/22 patients), the scan was not sufficient sensitive enough to detect the destruction of blood flow vessels. SPECT was used in one study [44], five patients had positive lesions pre-treatment and the uptake disappeared post-treatment indicating an absence of viable tumour cells, one patient had a negative lesion both pre- and post-treatment.

Histopathology

Histopathological outcomes for studies were assessed. To determine the grade of the tumour and the receptor status pre-treatment, seven studies [11, 19, 26, 27, 44, 54, 57] performed CNBs in all patients, in one study [58] VAB or CNBs were performed and in two studies [53, 55] VABs were performed. Only one study [56] did not perform any biopsy previously to the ablation treatment.

In nine studies [11, 26, 27, 44, 53, 55-58], all patients underwent surgical excision by either BCS or mastectomy post-treatment. Two studies [19, 54] performed CNBs or VAB (number of cores not reported) to determine if there was residual tumour left. In the three RFA studies [55, 56, 58], resection of the tumour was performed immediately after treatment in 7.1% (9/127 patients), after 3 - 4 weeks in 67.7% (86/127 patients) and after 1 - 2 months in 25.2% (32/127 patients). In the HIFU study [44], resection was performed after 7 - 14 days and in cryo-ablation [26, 57], 24 patients underwent resection immediately post-treatment and 27 patients underwent resection after 7 - 30 days. In the study using laser ablation [27], resection was performed after five days and in the study using microwave ablation [11], 17 days post-treatment. In the study [53], combining RFA and crvo-ablation resection was undertaken after 30 - 45 days. H&E findings were reported in five studies (two RFA [55, 58], one HIFU [44], one cryo-ablation [26] and one laser ablation [27]). Nicotinamide adenine dinucleotide (NADH) was used in four studies (two RFA [55, 58], one laser ablation [27] and the combined study [53]) and staining was unknown in three studies (one RFA [56], one cryo-ablation [57] and one microwave ablation [11]). For the studies using biopsies, these were obtained 3 - 4 weeks after RFA [54] and two weeks, three, six and 12 months after HIFU treatment [19]. Both studies used H&E staining and one study [19] used NADH staining. One HIFU study [19] also used Victoria bleu and Ponceau's staining. A transient area intermediate between necrosis and normal tissue was not reported in any of the studies. Independent pathological review was not undertaken in any of the studies.

H&E staining was used in four ablation techniques (*table 9*) following resection of the tumour and no residual tumour was found in 94.1% (32/34 patients) after RFA [55, 58], 100% (23/23 patients) after HIFU treatment [44], 85.1% (23/27 patients) after cryo-ablation [26] and 65.0% (13/20 patients) after laser ablation [27]. In a RFA study [58] it was stated that H&E could not demonstrate complete tumour cell death when resection was performed immediately after the treatment. In the study using cryo-ablation [26], in 14.8% (4/27 patients) DCIS was found within normal tissue surrounding the ablated region. In the laser ablation study [27], 25.0% (5/20

patients) had extensive tumour beyond the margin and in 10.0% (2/20 patients) the tumour was completely missed. One study [58] using RFA did not report the H&E staining results.

	H&E complete ablation	NADH complete ablation	Timeframe histology*
RFA	94.1% (32/34)	95.3% (82/86)	R: 0 or 1-2M, CNB: 3-4 wk
	89.7% (26/29,CNB)		(H&E) and R: 4wk (NADH)
HIFU	100% (23/23)	-	R: 1-2 wk
	100% (22/22,CNB)		CNB: 2 wk, 3,6,12M
Cryo	85.1% (23/27)	95.0% (38/40)	R: 0 or 1-4 wk (H&E)
			and 4-6 wk (NADH)
Laser	65.0% (13/30)	100% (2/2)	R:1 wk

 Table 9: Post-treatment H&E and NADH staining findings after surgical excision or core needle biopsy.

*CNB = core needle biopsy, H&E = haematoxylin and eosin, NADH = nicotinamide adenine dinucleotide, R = surgical excision, wk = week and M = month.

In the two studies, using biopsies post-treatment H&E staining found no residual tumour in 89.7% (26/29 patients) and degenerative change in 10.3% (3/29 patients) after RFA. Post HIFU treatment, no residual tumour was found in all patients (22/22 patients) [19].

NADH was used in three techniques: RFA, cryo- and laser ablation. In three studies, RFA [53, 55, 58] showed no viable cells in 95.3% (82/86 patients) of all patients after approximately four weeks. One study [58] stated that NADH staining was indispensible to evaluate cell death. For laser ablation [27], the result was 100% (2/2 patients) after five days and for cryo-ablation there was complete ablation in 95.0% (38/40 patients) after 30-45 days.

Four studies described general histopathology results (no staining was mentioned), two RFA studies [56, 58] showed 62.4% (58/93 patients) of complete tumour ablation after approximately four weeks. The cryo-ablation study [57] showed that 83.3% (20/24 patients) had negative margins. One microwave ablation study [11] showed that 68.0% (17/25 patients) of all necrotic and viable tumour areas were necrotic and complete ablation was found in 8.0% (2/25 patients) after 17 days.

Three studies looked at the shrinkage of the tumour after ablation. A HIFU study [44], showed a margin of 1.8 ± 0.6 cm after 1-2 weeks. A cryo-ablation study [57] showed an increase of 14% immediately after the treatment, however an extra margin of 0.8 cm was not taken into account and excluding this margin showed a decrease of 50% compared to the tumour size pre-treatment. In a laser ablation study [27], imaging and histopathology measurements showed a decrease of 50% in tumour size.

Three studies [27, 53, 55] looked at the correlation between MRI *versus* the histopathological determined ablation volumes and found good to large correlations (r = 0.8 (Spearman), r = 0.9 (unknown) and r = 0.9 (Pearson's)).

Treatment margins

The ablative studies used different treatment margins; only two studies looked at the margin post-treatment. In a HIFU study [19], the aim was to ablate an extra margin of normal tissue between 1.5 - 2.0 cm surrounding the tumour itself. Post-treatment histopathology showed a margin of 1.8 ± 0.6 cm (range 1.5 - 2.2 cm). In a cryo-ablation study [57], the plan was to ablate a margin of 0.5 cm of normal tissue during the first half of the study and 0.5 - 1.0 cm of normal tissue during the second half of the study and post-treatment US showed a margin of 0.8 ± 0.2 cm. No post-treatment margins were reported in the other nine studies.

Post-treatment complications

Nine studies recorded one or more complications during or post-treatment (*figure 6*). Most common complications were pain at the site of ablated tissue and skin burns. Other complications described were erythema (9/25 patients, 36.0%, microwave ablation), oedema (5/25 patients, 20.0%, microwave ablation), small seroma (2/24 patients, 8.3%, cryo-ablation), overreaction (1/29 patients, 3.4%, RFA) and hyper-pigmentation (1/34 patients, 2.9%, RFA). Both HIFU studies reported an unknown number of patients with oedema.





Mild local pain was described in 26.7% (24/90 patients) with two techniques, 15 patients developed pain during HIFU-treatment [19, 44] and nine patients after microwave ablation [11]. Severe pain was described in 3.3% (3/90 patients), two after laser ablation [27] and one after

microwave ablation [11]. Skin burns occurred in 4.9% (10/204 patients), six developed during RFA [54-56, 58], three during microwave ablation treatment [11] and one during HIFU [19]. Third-degree burns occurred three times in RFA and once in microwave ablation. Other skin burns were first-degree burns.

Recurrence was reported in two HIFU patients [44] after 18 and 22 months. Other studies did not look at recurrence since the studies were treat and resect studies.

Cosmetic outcome:

Cosmesis was discussed in five studies [44, 53-56] with three techniques (*figure 7*). For RFA, cosmesis was excellent in 85.8% (133/155 patients), good in 9.0% (14/155 patients), fair in 2.6% (4/155 patients), poor in 1.9% (3/155 patients) and unknown in 0.6% (1/155 patients). With cryo-ablation the cosmesis was excellent in 92.5% (37/40 patients), good in 5.0% (2/40 patients) and fair in 2.5% (1/40 patients) and in HIFU cosmesis was excellent in 94.1% (16/17 patients) and acceptable in 5.9% (1/17 patients).



Figure 7: Cosmesis after HIFU, cryo and RFA ablation.

3.4 Discussion

Although the trials conducted to date document some potential of minimal invasive techniques none of these trials included an adequate sample size calculation. The only comparative trial also did not included an adequate sample size calculation. The studies included in this systematic review demonstrate that ablative techniques are currently being evaluated in small, often uncontrolled studies that are unlikely to change clinical practice or provide the basis for phase III trials.

Some specific comparisons are feasible based on the published evidence. In terms of treatment time RFA, laser and cryo-ablation are the most time-effective ablative techniques. Post-treatment MRI and US indicate that RFA and HIFU are potentially the most effective ablative techniques. When comparing the outcomes in terms of cell death, H&E showed the best results for HIFU (23/23 no residual tumour) and RFA (32/34 no residual tumour) and NADH showed the best outcomes with both RFA (82/86 no residual tumour), cryo- (38/40 no residual tumour) and laser ablation (2/2 no residual tumour). However, the short amount of patients with NADH

staining in laser ablation makes this technique less promising than RFA. No conclusion could be made from the general histopathology results. Skin burns occurred in ten patients, of which most occurred after RFA, local pain was described in 27 patients and pain was most often described in HIFU. Analysing the histopathology and imaging results, treatment times and complications, RFA and HIFU show the best potential to minimal invasively treat breast tumours.

Post-treatment MRI indicate that HIFU (23/23 no residual tumour) and RFA (32/34 no residual tumour) have the highest percentages of complete ablation. The time of the MRI is also important in this case, when there is a longer period between the treatment and the MRI, there is more time for tissue that has been isolated to die. As a result, the enhancement will decrease and the efficacy will increase. This could explain the difference between the laser ablation study [27] and the other studies [19, 44, 53-56, 58] because in laser ablation an MRI was performed four and 24 or 48 hours post-treatment. In all other studies, MRI's were made after a few weeks. US scans showed an absence of blood flow when complete ablation was accomplished. Analysing the results of these two ablation studies [44, 56], it is noticed that the results were lower than determined in MRI or histopathology results. This could insinuate that US is not an accurate image modality to visualize tumour necrosis. However, only two studies described the number of patients with complete ablation. Other studies measured different parameters such as the size of the lesion or the ice ball, and these results could not be compared because they are dependent on the cryo-probe settings and the freezing cycle times.

In general, very often there is a mismatch between imaging and histopathology results. It is therefore important for the future of any image guided technique that the tumour must be completely visible with the used imaging technique. This factor could potentially explain the reason for residual tumour to be left behind. Comparing the H&E staining, this was performed after surgical excision or after biopsies were taken from the tumour. However, resection or biopsies were not always performed at the same time post-treatment. When the specimen is resected a longer period after the treatment, there is a higher chance of complete ablation. Tissue that was not ablated by the treatment but has been isolated is still viable shortly after the treatment. After a few weeks, this tissue has died due to a shortage of blood supply and as a result, the percentage of complete ablation increases. In the four RFA studies [53-55, 58], biopsies or resection was performed after 3 - 4 weeks. In the HIFU studies [19, 44], biopsies were taken at two weeks, three, six and twelve months or resection was performed after 1 - 2 weeks. For cryo-ablation [26, 53], resection was performed after 14 days (6 - 300 days) and for laser ablation [27], resection was performed after five days (1 - 15 days). Comparing the H&E staining results and the period between treatment and staining, HIFU [19, 44] is most likely to be the most effective treatment. Furthermore, biopsy results cannot give complete reassurance of complete ablation since not the whole tumour is excised. Therefore, these results need to be separated for patients, which underwent surgical excision and biopsies post-treatment.

Limitations exist in the comprehensive recording of reported histopathological outcomes. NADH staining was performed in three types of ablation techniques, RFA, cryo- and laser ablation [27, 53-55, 58]. All techniques showed complete ablation in almost all patients. Remarkable is that H&E results showed a lower amount of patients with no viable tumour compared to NADH

staining. One study [58] stated that H&E could not demonstrate complete tumour cell death when resection was performed immediately post-treatment and that NADH staining was indispensible to evaluate cell death when comparing imaging results with H&E and NADH staining results, this is likely to be the case. The most reliable way to determine cell death (especially right after resection) seems to be NADH staining. No statements about the most effective ablation technique could be made because only five studies in three techniques [27, 53-55, 58] performed NADH staining. Several studies [11, 56, 57] do not describe the staining technique they used in their pathological examination and therefore the results cannot be compared to other studies. In addition, in the study by Ohtani et al. [58] both H&E and NADH staining was used. The combined complete ablation results for H&E and NADH staining were mentioned, however it was not possible to re-calculate this result from the individual NADH and H&E results. Histopathology results described the number of patients with complete ablation. For more accurate results, the percentage of tumour in the ablated zone and the percentage of ablation of the treated zone must be described. In the included studies, the number of patients in which the ablation technique was not complete was known, but the percentage of residual tumour in these patients was unknown in most studies. In order to calculate the effectiveness of the studies, the percentage of ablation in all patients must be known and not just the number of complete ablations.

Not all ablative techniques use an extra margin of normal tissue to make sure the whole tumour was ablated, only three of the included studies described the used margin, which was between 0.5 - 1.0 cm in the case of cryo-ablation [57] and in both HIFU studies [19, 44] a margin of 1.5 - 2.0 cm was used. For RFA, laser ablation and microwave ablation no margins were described, however a certain margin is expected in these techniques due to the irregular shape of breast tumours. Without a margin the risk of not treating the whole lesion at once is large because not all tumour tissue might always be visible on imaging. In the case of HIFU, the margin of 1.5 - 2.0 cm could be the reason that the efficacy was 100%, in the case of such large margins, the tumour is most likely to be within the ablated area, even if imaging is not showing all the correct tumour margins.

The treatment times show that RFA, laser and cryo-ablation have the shortest treatment times. The treatment time of the laser ablation study [27] was constant (8.3 minutes) and was not dependent on the size of the tumour. Compared to other ablative techniques this is the fastest treatment in larger lesions, however in smaller lesions, cryo-ablation [26, 57] and RFA [54-56, 58] had shorter treatment times $(13.1 \pm 3.9 \text{ versus } 14.9 \pm 8.2 \text{ minutes})$. Comparing the size of the lesions treated with cryo-ablation and RFA (1.2 versus 1.4 cm) it is concluded that cryo-ablation might be a slightly faster technique. The treatment times in the microwave ablation [11] and HIFU [19, 44] studies were considerably longer (90-180 and 105 ± 38 minutes). Even though the lesions treated with HIFU were almost double the size (mean 3.3 cm) of the lesion treated with cryo-ablation and RFA, the treatment time was still considerably longer. In the case of microwave ablation, the treatment time can be decreased by increasing the temperature during the treatment. [11]

Analysing the complications, RFA and cryo-ablations seem to be the safest techniques, having the fewest complications. However, complications like oedema and erythema might not have

been reported by the patients or might not have been considered as a complication in all studies. Furthermore, not all studies might have given their patients questionnaires describing the level of pain during and after treatment. Skin burns were the most serious complications described in these studies, explanations for the skin burns were not given in most studies, however in two patients the skin burn was caused due to lesions located close to the skin or the treatment was performed right after biopsies were taken. Pain was the second most serious complication, but in most cases the pain could be resolved with local anaesthesia or analgesics. Based on the number and severity of complications cryo-ablation, laser ablation and HIFU were considered the safest techniques. Recurrences were found in two HIFU patients [44], however only three studies had follow-up, therefore this number could be higher.

In this study, only one RCT [19] and one retrospective analysis [53] were included and therefore no meta-analysis could be performed. The single RCT compares HIFU with breast (conserving) surgery and the retrospective analysis compares RFA with cryo-ablation. More RCTs or retrospective analysis comparing ablative techniques are needed to get a better look at the differences between the techniques. All cohort studies were not randomized and the heterogeneity is considerable. For example, in one study using cryo-ablation the resection was performed during the same treatment and not post-treatment as in the other studies. The device was put on a low freezing setting while the surgeon resects the ice ball. It is therefore important to standardize some of the treatment protocols to get a better comparison of the studies using the same technique and between studies using different techniques. These differences make it hard to compare the included studies.

Compared to breast surgery these techniques have the advantage of intra-operative imaging to get more control of the actions taken during the treatment and to keep the tumour visible during the whole procedure. Other advantages are lower complication rates, minimal invasiveness and therefore a shorter hospital stay and a shorter recovery. These advantages potentially result in a decrease in costs compared to breast surgery. Adjuvant therapies were performed in the same way as with normal breast cancer patients. Ideally, in the future, it would be possible to use one of the ablative techniques without the need of further adjuvant therapy such as radiotherapy.

In the next chapter one of these promising ablative techniques: HIFU, is discussed in more detail.

4. High Intensity Focused Ultrasound

4.1 The History of HIFU

The first use of focused ultrasound (FUS) was in 1942, however, the first report in humans was not until 1960. [59] Fifty patients with Parkinson were treated and although resolution of symptoms was claimed, this technique was not further used for the treatment of Parkinson, probably due to the development of the drug L-dopa. [60]

Later, FUS was proposed for the use in ophthalmology to demonstrate cataract formation when targeting the lens of the eye. HIFU has also been successful in the treatment of intra-ocular pressure, glaucoma, intra-ocular tumours, retinal detachment, vitreous haemorrhage and sealing traumatic capsular tears, nevertheless, with the development of laser surgery more success and a broader application within the ophthalmology department was obtained due to the decreased complexity of this technology. HIFU is therefore no longer used in ophthalmology. [60]

In the 1970s, US at lower intensities was used for the treatment of tumours. [61, 62] The goal was to induce hyperthermia in the entire tumour volume and maintain this temperature for about an hour. Unfortunately, there was a lack of uniform heating and maintenance of the entire tumour volume due to a lack of feedback control of the delivered acoustic power to the tumour. [62]

The first clinical application of HIFU was the use of extracorporeal shockwave lithotripsy as a method for treating kidney stones. [62] HIFU was re-discovered in 1990, due to the development of modern technology and advanced imaging methods and the realization that HIFU can produce instant cell death to the focused areas of tissue. [62]

Currently, HIFU has been successfully used in the treatment of both benign and malignant tumours in the liver, breast, kidney, uterine, prostate and pancreas. HIFU has also been used in the treatment of osteosarcomas and as a pain relief in bone metastasis. [60]

4.2 The HIFU Technique

US wave propagation

In US, a wave moves from the transducer through different layers of the dermis towards the destined treatment site within the target organ. A part of the energy carried by the sound wave is reflected every time the wave reaches an tissue interface, while the rest of the energy passes through the tissue layer. The transmission coefficient of each tissue depends on the difference in acoustic impedance which is dependent on (1) the density of the tissue layer; (2) the speed of sound between the two tissue layers and (3) the thickness of the tissue layers. [60, 63] When there is little difference between the acoustic properties, the transmission coefficient is close to unity. For the transmission of US energy from the transducer to the body tissue it is important to minimize the effect of reflection at tissue interfaces. Water-like media are therefore optimal since the acoustic properties and therefore there is much more reflection at these tissue interfaces. [60]

When US propagates through soft tissue layers, the induced pressure fluctuations lead to shearing motion of tissue at a microscopic level. This results in frictional heating, the primary mechanism for US induced hyperthermia. [60] In an inhomogeneous media, the incident wave is scattered in all directions due to the small regions with different acoustic properties from their surroundings. This causes a loss in acoustic intensity in the direction of sound propagation. The loss in incident acoustic energy is characterized by the attenuation coefficient, which is the sum of the scattering and the absorption coefficient. [60]

The attenuation coefficient is related to the US frequency, in most tissues therefore US is ideal for the use of non-invasive therapy. However, it causes some challenges in optimising HIFU induced hyperthermia. Unlike other ablative modalities the attenuation of sound through water-like media at US frequencies is considerable low so that adequate amounts of energy can be delivered to the required depths in tissue during clinical treatments. By increasing the US excitation frequency, both absorption and attenuation coefficients are increased, resulting in a higher heat deposition of surrounding tissues and a lower penetration depth. Therefore, the optimal treatment frequency is application dependent, and a compromise is needed between the desired penetration depth and heating rate. [60, 64] Fatty tissues such as the breast have relative high attenuation coefficients while brain and most abdominal organs have low attenuation coefficients. Most tissue attenuation coefficients apart from fat, increase with increasing temperature and during HIFU treatment, this creates significant challenges in HIFU treatment planning. [60, 65]

Non-linear wave propagation and cavitation

In most studies, HIFU transducers with frequencies between 0.5 - 8 MHz are used. If the resulting sound wave propagates linearly through soft tissue, the heating rates are dependent on the incident US intensity and the local absorption coefficient. Any non-linear mechanisms that gives rise to higher frequency components in the sound field will also yield enhancing heating as implied by the frequency dependency of the absorption coefficient. Two mechanisms will be explained: non-linear wave propagation (*figure 8b*) and cavitation (*figure 8c and d*). [60]

When a single frequency sound wave with a large amplitude propagates through a non-linear medium, the waveform becomes gradually shocked, resulting in energy leakage from the fundamental frequency into its higher harmonics. The extend of leakage is dependent on the (1) amplitude of the incident wave, (2) non-linearity of the medium and (3) distance that the wave needs to travel. The non-linearity for most soft tissues is close to water, but for fat the non-linearity is almost doubled. In HIFU, non-linear effects become more significant when there is an increased treatment depth, or if a region of high intensity happens to be coincident with a layer of fatty tissue. In HIFU, at the intensities used, non-linear propagation is a significant contributor to the heating observed. [60, 62]

The peak rarefaction pressure of an US wave with a large amplitude may be sufficiently large for small cavities to form, which contain some of the gas originally dissolved in the surrounding medium as well as vapour. Cavities could also arise from the thermal effects alone. Relatively large vapour bubbles are formed when the tissue temperature reaches the boiling point. The behaviour of these cavities under the influence of a sound field is known as acoustic cavitation.

There are two types of cavitations, stable and inertial. [62] The type of observed cavitation activity is dependent on the bubble size compared to the linear resonance size, the insonation frequency and on the relative contribution of vapour and gas pressure to the total pressure inside the bubble. [60]

Stable cavitation (*figure 8c*) describes the repeated oscillations of cavities whose size is normally close to, or greater than the linear resonance size for the insonation frequency. This can result in period-doubling oscillations of the cavity wall. Inertial cavitation (*figure 8d*) describes the explosive growth of cavities with a initial size of about one third of resonant size, and its subsequent intense collapse under the effect of the inertia of the surrounding fluid. Inertial collapse generally occurs over a single or small number of acoustic cycles and results in broadband noise emissions. [60, 62]



Figure 8: Microbubble oscillations (a) linear oscillation, (b) non-linear oscillation, (c) stable cavitation and (d) inertial cavitation. Figure from Stride *et al.* (2009) [66]

Cavitation exists of two main mechanisms which contribute to the significance of cavitation for enhanced heating: if the pressure amplitude is sufficiently large for stable or inertial cavitational activity to exist in a tissue volume, strong scattering of the incident wave by these multiple bubbles will result in acoustic energy being trapped within the cavity. This results in enhanced heating due to viscous absorption of the trapped excess energy. In inertial cavitation the violent bubbles collapse as a result of a redistribution of energy received by the bubble at the fundamental frequency into broadband noise emissions. The frequency dependency of the absorbed coefficient means that higher frequency emissions are more absorbed than attenuated leading to enhanced heat disposition in the immediate surroundings of the inertially cavitating bubble. [60]

In HIFU, cavitation will lead to a region with increased effective absorption and attenuation coefficients. Furthermore, the bubbles will result in acoustic impedance changes of the tissue volume, yielding larger reflection coefficients at the boundaries of the cavitational region. The relative increase of any coefficient in any given medium will depend on the range of bubble sizes, type of cavitation activity and the density of the bubbles. [60]

In HIFU treatment, both inertial cavitation and non-linear propagation are most likely to play a significant role. At a certain pressure amplitude, the relative contribution of these two mechanisms are dependent on the cavitation threshold, tissue temperature, distance over which US propagates and the non-linearity of the tissues in the US path. [60]

4.3 Treatment Devices

Effective HIFU treatment is dependent on the completion of three different stages. At first an assessment of the tumour visibility with diagnostic US is required. This involves making sure that there is a suitable acoustic window through which the treatment can be delivered, that the target boundaries can be clearly identified and that no sensitive normal tissue structures lie in the beam path. The target volume must be accurately identified and spatially localised.

The second stage is to determine the suitable US exposure to achieve ablation. In US-guided treatment this is determined by adjusting the focal peak intensity and the exposure time until a hyper echoic mark (HEM) is visible at the target. In MRI-guided treatment these two are varied until the required temperature is reached. During treatment delivery, an indicator of tissue change is required. This enables assessment of treatment progress and provides feedback which allows adjustment of exposure parameters in real-time. In US-guided treatment the indicator is the HEM on the image and in MRI-guided treatment this is the temperature rise determined by spin-lattice relaxation time (T1), proton resonance frequency, proton diffusion related sequences. [60, 67]

The third stage is the assessment of the extend of tissue ablation post-treatment. US and contrast enhanced MRI allow visualization of the vasculature. Successful HIFU treatment leads to the occlusion of blood vessels in the target volume and thus a reduced contrast uptake post-treatment. [60, 68]

4.4 Treatment delivery

Focussing of the US beam

In HIFU, a US beam needs to be able to destroy a focused region of tissue within a short time with minimal effects to surrounding tissues. Focusing of the US beam may be achieved in a number of ways but the simplest method is the use of a single element spherical shell of piezo-electric material capable of delivering high power. Quartz used to be the material of choice

however, most recently piezo-ceramics and piezo-composite materials are used. The single element gives no flexibility as to focal length, this may be achieved combining the transducer with lenses of different specifications. [60]

US transducers

Transducers for clinical use can be divided in three categories: (1) extra-corporeal, (2) transrectal and (3) endoscopic transducers. Extra-corporeal transducers are currently used for targeting organs which are readily accessible through an acoustic window on the skin such as the breasts; trans-rectal transducers are used for the prostate and endoscopic transducers are developed for biliary duct and oesophageal tumours. [60]

The acoustic energy can be delivered in different ways. For small volumes, short single pulses can be generated with the transducer held stationary. This results in a well-defined lesion with dimensions determined by the focal region of the transducer. If a larger volume needs to be treated, the transducer can be moved in discrete steps and give pulses at each position, where the distance between the pulses will determine whether lesions are overlapping or separate. This is dependent on the necessity to achieve confluent regions of cell killing. Another option would be to move the active transducer in pre-determined trajectories, when the correct combination of transducer velocity and US energy are used, confluent volumes of cell damage can be obtained. [60]

Extra-corporeal transducers are generally integrated with either MRI or US in order to plan treatment, detect movement during treatment and monitor response in real-time. MRI has the advantage of excellent anatomical resolution, high sensitivity for lesion detection and temperature mapping. With MR thermometry, the thermal dose can be calculated and the regions in which the thermal dose has achieved cytotoxic levels can be represented. Temperature imaging with MRI is challenging due to the high amount of fat and lack of reliability of water proton phase shift based measurements within fat. [69]

US offers real-time visualization of the targeted volume thereby detecting movements made by the patient, guidance of the energy deposition within the treated area (hyper-echogenic cross visible during pulse application) and rapid real-time assessment of the volume of coagulative necrosis during treatment (HEM visible on screen). [19, 20] The thermally ablative region is not visible until gas bubbles have been induced. However, it is not yet clear if the HEM is created by acoustic cavitation or thermal exsolution of tissue gas. [60] US is nonetheless an excellent modality for the guidance of HIFU treatment.

In December 2013 we started a feasibility trial using circumferential US guided HIFU in the treatment of breast FAD. The methods and results of this trial will be discussed in the next chapter.

5. High Intensity Focused Ultrasound in the Treatment of Breast Fibroadenomata: the HIFU-F trial

5.1 Materials and Methods

Patients

A prospective trial was set up for a total of 50 patients recruited at Guy's and St. Thomas' hospital in London, with an interim proof of principle analysis which has been undertaken at 20 patients. All patients eligible for the study underwent triple assessment and went through a multi disciplinary meeting (MDM) either at Guy's or another hospital in the United Kingdom. Patients were identified in three ways: (1) at the MDM, where all patients were discussed in which CNB or FNAC was performed, (2) patients scheduled for surgical excision of a FAD and (3) patients visiting the breast clinic requesting for surgical excision. All patients were approached in the breast clinic or by telephone, asking if they would be interested in receiving a patient information sheet (PIS) regarding the HIFU-F trial, which described the procedure in detail and mentioned the advantages, disadvantages and potential complications of the treatment. If the patient was interested, a second telephone call was made to determine if the patient would like to participate in the study and to answer any questions. If the patient agreed to participate in the trial, the patient was scheduled for an appointment in the breast clinic, if requested by the patient, or directly for HIFU treatment.

Two patient screening logs were updated: (1) for the MDM, to determine how many patients had a FAD biopsied and how many of them were eligible for the HIFU-F trial and (2) for patients which accepted or declined the HIFU-study after being approached by one of the investigators.

Informed written consent was obtained for the HIFU-F trial on the treatment day or at a prior hospital visit.

Inclusion and exclusion criteria

The inclusion criteria for the HIFU-F trial were as follows: adult patients (age \geq 18 years) visiting the one-stop breast clinic with a symptomatic FAD, either a palpable lesion or pain developing from this lesion; visible on US, graded either U2: benign or U3: indeterminate. Patients of 25 years or older underwent a FNAC or CNB to confirm the final diagnosis of a FAD (graded C2/B2).

Exclusion criteria were FAD with atypia or suspicion of phyllodes on biopsy (graded B3/C3 or greater), pregnant or lactating patients, patients with a history of laser or radiation therapy to the ipsilateral breast and patients with breast implants. There were no restrictions in size, margin to the skin or chest wall and pectoralis major muscle. If patients had multiple FAD in one breast only the largest symptomatic FAD was selected for treatment, other FAD could be treated in another HIFU session, if requested by the patient. In the case of a malignant lesion in the ipsilateral or contralateral breast, the treatment of the malignant lesion had priority and the patient was excluded from the study.

The primary outcome measure was the change in size of the FAD as recorded on US. Secondary outcome measures were complications of the treatment, patient recorded outcome measures, mean treatment time and cost-effectiveness of the treatment.

HIFU treatment

Patients enrolled in this trial were treated using the US-guided Echopulse device (Theraclion Ltd, Malakoff, France, *figure 9*). The device contained a cooling and coupling device to cool the skin and prevent burning. The breast lesions were ablated under real-time US guidance using a 7.5 - 12 MHz diagnostic US transducer (Theraclion Ltd, Malakoff, France). Therapeutic US energy was produced by a 5.6 ± 0.1 cm diameter 3.0 MHz imaging transducer with a central hole of 1.1 ± 0.1 cm for the coaxial imaging transducer. The transducer ablates a tissue volume of approximately 0.9 cm in length and 0.2 cm in width. The minimum distance between the skin and the top of the lesion must be 0.5 cm. Furthermore, the minimum distance between the posterior edge and the skin is 1.4 cm and the maximum is 2.6 cm (*figure 10*).



Figure 9: Echopulse device (I) and VTU unit (r). Figure by Theraclion Ltd (Malakoff, France).

A visualisation and treatment unit (VTU) model is connected to the Echopulse, the main functions of the VTU are imaging of the target tissue, HIFU power delivery and measuring the cooling liquid temperature and pressure within the membrane covering the transducer. The skin is cooled during the treatment, cooling liquid is regulated at the membrane and flows from the device towards the probe.



Figure 10: Focal point depth range: (I) minimum and (r) maximum depths of the Echopulse.

All patients were treated as a day-case procedure in main theatre/day surgery or the clinical research facility at Guy's hospital under subcutaneous degassed local anaesthesia (1.0% Lidocaïne with adrenaline and 0.25 - 0.5% Bupivacaïne, ratio 1:1) injected under US guidance. The first four patients also received topical anaesthesia (Emla crème) on the skin prior to treatment. Applying Emla crème was not continued due to insufficient time for the crème to work. Dependent on the position of the FAD and the size of the breast, the patient was placed supine or laterally and an immobilisation system and/or micro-foam were used to immobilize the breast.

After a handheld US scan, to determine the visibility and dimensions of the FAD, the probe was placed on top of the FAD. The treatment was guided using US; the lesion and the skin were outlined in the radial and anti-radial view by the surgeon using the system software (*figure 11*). For every radial slice, treatment pulses were visualised and the skin and FAD outlines were adjusted when needed. A safety margin of 0.5 cm in which no treatment is possible will be projected on the screen. Procedure started with a single pulse in the centre of the FAD to determine the right energy level, if the right energy level had been obtained a HEM was visible right after administering of the pulse and/or during application of the pulse a hyper-echoic cross was visible. After the right energy level was determined, treatment was able to begin. In the HIFU-F study, only the circumference was ablated: two rings around the FAD were treated and the centre of the FAD was deselected.



Figure 11: Treatment planning of a fibroadenoma with the Echopulse device.

The Echopulse device included a laser pointed at the treated breast which detects any movement made during the treatment. If repositioning was needed it could be performed at any time during the treatment. Each pulse was followed by a resting period, the length of both periods were determined by the applied power, the frequency used for the treatment, the depth of the tissue that needs to be ablated and if pulses have been applied to any area between the probe and the target. After the final pulse, a screenshot was made, showing the progress of the treatment and a post-treatment handheld US scan was performed to determine if there were any direct changes in the tissue. The patients skin was observed for any direct skin changes and patients were discharged half an hour after treatment following hospital protocol.

Treatment times

Treatment times at start (first pulse administered) and end (last pulse administered) of the treatment were written down. The actual treatment time for the amount of pulses was compared to the treatment time to complete whole lesion ablation. Whole lesion ablation treatment time was determined by comparing the whole amount of pulses with the amount of pulses treated during the HIFU treatment using *formula 1*.

$$Whole \ lesion \ time = \frac{Nr \ of \ pulses \ whole \ lesion \ * \ circumferential \ time}{Nr \ of \ pulses \ circumferential \ lesion} \ (1)$$

Follow-up

Follow-up appointments at two weeks, three, six and twelve months were booked for the patient to visit the breast clinic or the clinical research facility. At these visits, the treatment site and lesion were examined and the patient was asked about the level of pain they have experienced during and after the treatment. Short-term complications were assessed during the breast clinic visits. A handheld US scan was performed at each follow-up appointment to determine the three dimensions of the FAD and the degree of swelling.

The degree of decrease of the FAD was determined by calculating the volumes using *formula 2* with A, B and C being the longest diameter of the FAD in length, width and height.

$$V = \frac{4}{3} * \pi * \left(\frac{1}{2}A\right) * \left(\frac{1}{2}B\right) * \left(\frac{1}{2}C\right)$$
 [70] (2)

Patients who have not experienced at least a 50% reduction in size of their FAD at six months follow-up will be offered the opportunity to undergo an additional HIFU treatment – as per standard protocol.

Case report forms

Paper and digital case report forms (CRFs) were developed at the beginning of the trial. CRF's contained relevant pre-treatment patient details, treatment details and follow-up details. CRF's were submitted before every treatment, after every treatment and after every follow-up visit.

Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2007. A two-sample T- test using equal or unequal variances was used to determine the significance of the reduction in treatment time and the reductions in FAD volumes. The variances were determined using a two-sample F-test.

5.2 Results

Study characteristics

Research ethics approval was obtained on August 28th, 2013 and the Echopulse device arrived in the hospital on October 11th, 2013. A site initiation meeting was scheduled on November 15th, 2013 to introduce the HIFU-F trial to the consultants at Guy's hospital and to get a demonstration on how the device works on a liver phantom. Local research and development delayed the start of the trial and eventually approved the trial on December, 12th 2013.

Recruitment

A total of 322 patients with CNB or FNAC proven FAD were discussed at the MDM between December 12th, 2013 and November 20st, 2014 (*figure 12*). Of these, 153 patients (47.5%) met the inclusion criteria and 113 patients (73.9%) were contacted.

In the period between December 2013 and May 2014, only recruitment of patients with painful FAD and/or a size considered suitable for surgical excision was possible, this resulted in the loss of 27 out of 68 eligible patients (39.7%) in that period. Five patients (7.4%) were missed and 36 patients (52.9%) were contacted by the team. An investigators meeting took place on May 15th, 2014 with the recruitment results above presented. After a long discussion the attending consultants and researchers agreed to approach more suitable patients and not only the patients suitable for surgery.

Between June and November 2014, 85 patients were eligible and 77 patients (90.6%) were contacted by the team, three patients (3.5%) were missed and five patients (5.9%) were not contacted due to a disagreement during the recruitment at the MDM.



Figure 12: Patient recruitment per month at the multi disciplinary meetings.

Of the 113 contacted patients 25 patients (22.1%) were recruited for participation of the HIFU trial. From 50 patients (44.2%) the responses are still awaiting. These patients are currently being contacted by the HIFU team. Reasons for declining participation include: no reason given in 17 patients (15.0%), preferred leaving the FAD alone in seven patients (6.2%) and preferred surgical excision in 14 patients (12.4%).

Study and patient characteristics

An overview of the study and patient characteristics were given in *table 10*. From December 2013 till November 12th 2014, 25 patients with symptomatic palpable FAD underwent circumferential HIFU treatment and an interim analysis was performed.

Table	10:	Study	and	patient	characteristics.
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n	25		
Age (mean (SD, range))	30.2 (7.6, 18	8 - 45) years	
Pain	9/25 patient	S	
Lesion volume (mean (SD, range))	9.2 cm ³ (16.	2, 0.4 - 69.4	cm ³)
Cytological (FNAC) / histological (CNB)	CNB: 21	FNAC: 2	None: 2
confirmation FAD			
Nr of circumferential rings completed	None: 1	One: 10	Two: 14

Nine out of 25 patients (36%) requested intervention for the treatment of pain/discomfort related to the lesion. Patients had a mean age of 30.2 years (SD 7.6, range 18 - 45 years). All patients gave written consent to participate in the trial after the procedure was fully explained. The lesions had an average size of 2.9 cm (SD 1.8 cm, range 1.0 - 10.0 cm) and a mean volume of 9.2 cm³ (SD 16.2 cm³, range 0.4 - 69.4 cm³). FAD were located 0.4 cm (SD 0.3 cm, range 0.2 - 1.5 cm) from the skin and 0.5 cm (SD 0.3 cm, range 0.02 - 1.3 cm) from the pectoralis major muscle. The measurements were taken from the last US scan performed previously to HIFU treatment and are susceptible to different pressures of the US probe on the breast given by radiologists and inter-observer variability of the different radiologist. When not all three dimensions were determined, one radiographer was asked to determine the other dimensions to keep the inter-observer variability to a minimum. Subcutaneous local anaesthesia was injected between the skin and the FAD and between pectoralis major and the FAD to increase the distance to a minimum of 0.5 cm. Furthermore, in cases in which the lesion was close to the skin or pectoralis major the treatment was focused to the bottom half or the top half of the lesion creating a sufficient distance between the FAD and the skin / pectoralis major.

Sixteen lesions were located in the right and nine in the left breast. Six lesions were located at 12 o'clock, three at two o'clock, two at three, four, five, eight, nine, ten and eleven o'clock and the other lesions were located at one and six o'clock. In 22 patients, the centre of the FAD was selected, in one patient, the bottom was selected (FAD was close to the skin), in another patient, the top was selected (FAD was close to chest wall) and in the last patient only the left side of the FAD was treated due to the large size of the FAD.

Two circumferential rings were successfully treated in 14 patients (*figure 13*); one circumferential ring was completed in ten patients, in which six patients almost completed two rings apart from one or two pulses due to unreachable areas with the device (closer than 0.5 cm from the skin or pectoralis mayor or out of the range of the device (see red areas in *figure 13*) or due to pain during the treatment. In one patient, no completion of a circumferential ring was obtained due to RSI pain in her arm, this patient was therefore not able to lie still for the aimed 45 - 60 minutes. This patient decided to have her lesion excised three months after HIFU treatment due to an absence of decrease in the FAD.



Figure 13: Treatment planning in (I) whole lesion ablation and (r) HIFU-F trial: two circumferential rings were treated by deselecting the centre of the lesion.

During the excision of the FAD in this patient, it was noticed that the FAD had broken down in smaller pieces (*figure 14*), however the complete lesion was surgically excised. The tissue felt

necrotic and it was noticed that the complete lesion was larger than initially measured on US. Pathological results showed a FAD with no atypia and no necrosis, however sclerosis of the tissue was found.



Figure 14: Macroscopic image of excised fragments of a FAD treated with HIFU.

Mean energy per lesion was 135.4 Joule (SD 19.0, range 96.1 - 158.9 Joule, n=19) and mean power per lesion was 33.6 Watt (SD 4.7, range 24.1 - 39.9 Watt, n=19). Both were determined in 19 treatments due to an error of the device while producing the report in one patient and for five patients the reports were not transferred to us.

Treatment times

Average treatment time for approximately 64.8 pulses (SD 22.2, range 23.5 - 105.0 pulses) was 38.5 minutes (SD 12.0, range 18.0 - 66.0 minutes). Comparing the amount of pulses with the total amount of pulses to ablate the whole lesion, the complete ablation treatment time was determined to be approximately 72.3 minutes (SD 44.2, range 23.2 - 185.7 minutes). Circumferential ablation reduced treatment time by an average of 36.4% (SD 18.9%, range 5.0 - 76.8%). A two-sample T-test assuming unequal variances showed a significant reduction in treatment time (P = 0.0001, two-tailed).

Pain symptoms

During treatment 22/24 patients (91.7%) were found to have some degree of painful and/or burning sensation (*figure 15*). Minimal pain (maximum pain scored 0 - 4 out of 10) was found in seven patients (39.2%), intermediate pain (maximum pain scored 5 - 7 out of 10) was found in three patients (12.5%) and severe pain (maximum pain scored 8 - 10 out of 10) was found in 14 patients (58.3%) (*figure 15*). In one patient the maximum level of pain during the treatment was unknown, this data will be obtained at the two-week follow-up. Mean maximum pain level during treatment was 6.8 (SD 3.0, range 0 - 10, n = 24). By rescheduling the treatment or moving to another part of the treatment planning, the treatment was continued with agreement of the patient. It was found that patients sometimes felt a 'pulling' sensation when the treatment was close to the pectoralis major. In three patients (12%) pain was radiating to the arm, this was the case when lesions were located in the upper outer quarter of the breast. A burning sensation around the skin was felt when the applied treatment pulse was closer to the skin.

Right after treatment the maximum pain was minimal in 19 patients (76.0%), intermediate in two patients (8.0%) and severe in none of the patients. In four patients (16.0%) the amount of pain right after the treatment was unknown. Mean maximum pain right after treatment was 1.5 (SD 1.8, SD 0 - 6, n = 21).



Figure 15: Pain scale during and right after HIFU treatment.

Follow-up in 21 patients (n = 22) at approximately 17.3 days (SD 5.7 days, range 5 - 30 days) showed 5/8 patients (62.5%) with a decrease in pain compared to pre-treatment, of which in two patients (25.0%) the pain had resolved. In three patients (37.5%) the pain stayed the same and an additional two patients (25.0%) developed post-treatment pain. In the other 10 patients no pain occurred post-treatment (*table 11*).

Follow-up in 12 patients (n = 16) at approximately 106.7 days (SD 18.5 days, range 78 - 156 days) showed 7/8 patients (87.5%) with a decrease in pre-treatment pain of which in six patients (75.0%) the pain had resolved, one patient (12.5%) had a decrease in pain and one patient (12.5%) continued to have persistent pain. One patient which developed post-treatment pain had a resolution of the pain after three months.

Follow-up approximately 181 days (SD 11.7 days, range 155 - 197 days, n = 10) showed resolved pain in 5/6 patients (83.3%) and one patient in which the developed post-treatment pain had resolved. One patient kept having pain, which was probably not related to the FAD but due to hormonal change prior to her menstrual period.

	Two weeks	Three months	Six months
	(n = 22)	(n = 16)	(n = 10)
Pain (n=8)			
- Pain the same	3	1	1
- Reduction in pain	5	7	5
- Resolution of pain	2	6	5
No pain (n=12)			
- No pain (n=14)	12	7	2
- Pain developed	2	0	0
- Resolution of pain	0	1	1

Table 11: Post-treatment pain at two weeks, three and six months.

Complications:

Short-term complications (*table 12, figure 16*) found at two weeks (n=22) were ecchymosis (n = 8), erythema (n = 6), hypo-pigmentation of the skin (n = 1), dimpling of the skin (n = 1), numbness of the skin (n = 1) and a superficial first-degree skin burn (n = 1). The erythema and ecchymosis completely resolved within the first month post-treatment and the superficial first-degree skin burn resolved completely after one month without the need of intervention. All other single case short-term complications resolved within the first month. The patient with the first-degree skin burn opted for a second HIFU treatment for a FAD in the contralateral breast one month after her first HIFU treatment.



Figure 16: Short term complications (a + b) two images of ecchymosis at two weeks, (c) hyperpigmentation at three months and (d) first-degree skin burn at two weeks post-treatment.

A complication found at three months (n = 16) was slight hyper-pigmentation of the skin in five patients and mild hyper-pigmentation in one patient, this patient showed hypo-pigmentation at

two weeks and dimpling of the skin right after HIFU treatment. In most cases of hyperpigmentation the patients did not notice the slight changes in skin pigmentation.

At six months (n = 10) hyper-pigmentation of the skin was found in two patients.

	Two weeks	Three months	Six months
	(n = 22)	(n = 16)	(n = 10)
Erythema	6	0	0
Ecchymosis	8	0	0
Hypo-pigmentation	1	0	0
Hyper-pigmentation	0	6	2
First-degree skin burn	1	0	0
Dimpling of skin	1	0	0
Numbness of skin	1	0	0

 Table 12: Short-term complications at two weeks, three and six months post-treatment.

Patients felt the FAD changing in appearance; the FAD became flatter a therefore less visible and palpable to the patient and the physician. In some patients with larger FAD, the lesion was previously visible when looking at the breast or through the clothes. These patients noticed that the FAD was not visible anymore post-treatment.

Patients were overall not bothered by the remaining lesion, they were well informed about the fact that they would be able to feel the FAD for a couple of months post-treatment and they are aware that the lesion is completely benign.

Volume measurements by US

US scans at two weeks (n = 22) post-treatment showed hyper-echogenicity and oedema at the circumference of the lesion in some patients. Overall, the FAD had a mean volume of 5.7 cm³ (SD 8.2 cm³, range 0.3 - 37.8 cm³) which resulted in a mean decrease in size of 14.3% (SD 24.9%, range \uparrow 50.0% - \downarrow 62.7%) (*table 13, figure 17*). In the cases of an increase, the follow-up scan was performed shortly after HIFU treatment and swelling of the tissue due to erythema could explain the slight increase in size. A two-sample T-test, assuming equal variances showed no significant reduction in size after two weeks (P = 0.70, two-tailed).



Figure 17: US images of first patient (l) pre-treatment (2.1 x 0.9 x 2.0 cm) (m) two week follow-up (1.9 x 0.9 x 1.3 cm) and (r) three months follow-up (1.6 x 0.7 x 1.2 cm).

At three months (n = 16) the US volume of the FAD was 5.4 cm³ (SD 6.9, range 0.1 - 28.2 cm³). This resulted in an average decrease in size of 41.7% (SD 28.6%, range \uparrow 23.1% - \downarrow 77.8%). A two-sample T-test, assuming unequal variances showed no significant reduction in size after three months (P = 0.32, two-tailed).

	Mean volume (SD, range)	Mean decrease (SD, range)
Pre-treatment	9.2 cm ³ (16.2, 0.4 - 69.4 cm ³)	-
Two weeks	5.7 cm ³ (8.2,	↓14.3%
(n = 22)	0.3 - 37.8 cm ³)	(24.9%, ↑50.0% - ↓62.7%)
Three months	5.4 cm ³ (6.9,	↓41.7%
(n = 16)	0.1 - 28.2 cm ³)	(28.6%, ↑23.1% - 77.8%)
Six months	4.6 cm ³ (8.0,	↓59.6%
(n = 10)	0.5 - 26.5 cm ³)	(15.2%, ↓39.0% - ↓80.5%)

At six months (n = 10) the mean volume was 4.6 cm³ (SD 8.0 cm³, range 0.5 - 26.5 cm³) a decrease in size of 59.6% (SD 15.2%, range \downarrow 39.0 - 80.5%). No significant difference was found due to the limited amount of patients with six months follow-up (P = 0.35, two tailed), but the results are in accordance to our goal of obtaining a reduction of 50% after six months.

The residual volume of the FAD (in %) followed over time for all patients can be found in *figure 18.*



Figure 18: Residual FAD volume (in %) per patient followed over time.

5.4 Discussion

Circumferential HIFU ablation shows a significant decrease in treatment time compared to whole lesion ablation, this decrease is dependent on the size of the lesion and patient movement during the treatment. Follow-up at two weeks showed a reduction in pain in 5/8 (62.5%) patients with a resolution of pain in two. At three months a reduction of pain was seen in 7/8 patients (87.5%) with a resolution of six of them. Short-term complications at two weeks were ecchymosis (n=8), erythema (n=6), hypo-pigmentation (n=1), dimpling of the skin (n=1), numbness (n=1) and a superficial first-degree skin burn (n=1). At three months only slight hyper-pigmentation (n=6) was seen, all previously described local complications had resolved. Two weeks post-treatment, a mean decrease in volume of 14% was visible on US scans and after three months a further decrease in volume of 42% reduction was seen.

Only 25 patients were recruited during the last ten months due to multiple aspects. Only patients with symptomatic palpable FAD which requested for intervention or would be suitable for surgical excision were suitable for recruitment, even though the approval from the ethics committee stated otherwise. This decreased the potential amount of patients eligible for the study. Not all consultants were equally co-operating with the trial and some did not inform the investigators about suitable patients. Another aspect which slowed down the process was finding a suitable treatment room. At first patients were treated at main theatre or day surgery. Patients had to be scheduled first on the list because the Echopulse device needed to be moved to the operating theatre and the device had to be set up. However, this was not approved because local anaesthetic cases are normally scheduled last on the list. If HIFU cases would be scheduled last on the list, it would not be possible to finish the list on time and therefore another location to treat patients had to be found. The radiology department was too small and too busy to treat patients and the breast clinic did not had the right facilities for HIFU treatments. Almost two months, in which patients could have been treated, were lost before all approvals to treat patients at the clinical research facility at Guy's hospital were obtained. Once treatment of patients started at this facility, the whole treatment process became much smoother. In the end, it was decided to move all follow-up appointments to this facility as well to be able to see the patients quicker and to keep the patients out of the breast clinic.

During the planning stage of the treatment it was noticed that the pre-treatment imaging probe was of poor quality and after administration of local anaesthesia and placement of the treatment probe on the lesion the quality dropped even more. This was due to two aspects, first the quality of the treatment probe was indeed lower than the imaging probe and second the local anaesthesia seemed to make the images blurry. As a result, in some patients it was hard to distinguish the actual lesion from the surrounding tissue. This was more common in patients with intermediate lesions of <1.5 cm. Another factor limiting the view during treatment was oedema which developed at the beginning of the treatment, this factor played a bigger role in patients with larger FAD which would need longer treatment.

Administration of local anaesthesia needs to be optimised. Throughout the trial it was observed that administration of local anaesthesia has improved, however patients were still able to feel some degree of pain. Every painful pulse stopped the treatment due to patient movement

caused by the pain or treatment planning which needed to be adjusted. Optimizing the administration of local anaesthesia will improve the reduction in treatment time as well. Sedation or general anaesthesia would not be an option in our trial because the patient will need more time to recover and it is our aim to have the patient in the hospital as short as possible. Furthermore, patients could potentially develop complications from the general anaesthesia. Applying Emla crème would be an option since there is more time between consent of the patient and the HIFU treatment since treatment at the clinical research facility started, Emla crème now has more time to work. Giving the patient oral analgesics pre-treatment would be another option to decrease the inter-treatment pain. During follow-up it was addressed by several patients that they would prefer another HIFU treatment over surgical excision. However, some patients would not like to have another treatment due to the pain experienced during treatment.

It was noticed during treatment that the lesion stayed in the same location but the skin kept moving down compared to the skin drawn during planning. As a response repositioning was needed to get the skin on the same location, otherwise the treatment pulse would be too close (< 0.5 cm) to the actual skin. The lowering of the skin might be caused by the local anaesthesia dissolving to the nearby tissue under the pressure of the treatment probe. Software could be developed which recognises the skin automatically before each pulse and adjusts this and the safety margin before administering the next pulse. Another solution would be a program which recognises that the previously drawn skin does not correspond to the real skin anymore and stops the treatment in order for the surgeon to re-draw the skin. With the previously drawn skin in the planning stage, the HIFU device must be able to recognise the changes in pixel values in the scan as the skin overlying the breast tissue.

During treatment, a laser pointer detected every movement made due to actual movement of the patient, insufficient immobilisation of the breast and/or breathing of the patient. These movements unnecessarily slowed down the treatment. Therefore the breast of the patient needs to be immobilised better. With every movement, treatment planning is moved as well which makes the treatment less accurate. In some cases, the laser pointer had to be moved to a different area of the body which moves less frequently to prevent the laser pointer from blocking the treatment continuously. However, in case of breast movements these would not be detected anymore and the treatment would be less precise.

During some treatments the probe needed to be repositioned quite a few times due to aspects mentioned before. As a result, some of the planned pulses were not available anymore, as it was impossible for the probe to reach these areas. They appeared red on the screen and it was not possible to treat at these locations. In two patients this prevented the team from completing the goal of two complete circumferential rings. In some cases, these locations were retrieved by repositioning again but this lengthens the treatment time. The probe head should get a wider range in which it can move to treat breast lesions, especially in larger lesions this would be necessary.

Time per pulse is dependent on the power used for the pulse, depth of the FAD, frequency of the treatment transducer and if there are any previous pulses performed in the plane of the US

beam. This influences time per pulse because treated tissue has a different refractive index which results in more scattering and therefore a higher power is needed to get enough US waves to reach the targeted tissue. It is advised that when multiple treatments are applied to the same breast the lesions located the deepest will be treated first before treating the top lesions.

In two patients, it was impossible to treat the circumference of the whole lesion because the whole lesion was too large to fit on the Echopulse screen. At pre-assessment it was explained to the patient that it might not be possible to treat the whole FAD and that there are low expectations of a considerate shrinkage of the FAD post-treatment, but the patients wanted to continue with the HIFU treatment. In the first patient, the inner part of the FAD was treated. It was expected that the inner part of the FAD will shrink due to the treatment and hopefully the whole FAD will shrink as a result as well. At three months, a reduction in volume of 35.9% was seen on US, which is a promising result in her case. It still might be needed to give this patient a second treatment in order to completely treat the FAD and get an even bigger decrease in volume, but six months results will be determined first before making any decisions. In the second patient only one side of the FAD was treated. If the treatment makes a significant decrease in size a second treatment of the other side could complete the HIFU treatment. Looking back, it might have been better to treat the bottom part of the FAD first and then the top. However, the maximum treatment depth of 2.6 cm from the skin makes it complicated to treat the bottom of these large FAD. Due to the safety margin around the skin and the small margin between the skin and the FAD in patients with large FAD, treating the top layer is also complicated. Therefore the best treatment would be to treat the left side of the FAD first and the right side in a second treatment session. The response of the first treatment can be assessed in these patients and after six months the best treatment option can be selected (either HIFU or surgical excision).

In another patient it was not possible to complete a single circumferential ring around the FAD due to RSI pain in her arm. It was previously mentioned to the patient that she must be able to lie comfortably for 45 - 60 minutes with her arm behind her head and she was happy to do this. During the treatment, the team became aware that this was not possible and that the RSI prevented her from completing her treatment. As a result of the incomplete treatment, no decrease in size was visible after three months and the patient opted for surgery.

Even though a decrease in treatment time was seen (from pulse one till the last pulse) of approximately 36.4%, the treatment time could be decreased even more by improving the treatment in a few aspects. The immobilisation of the patient needs to be optimised as mentioned before. With every breath the patient takes, the breast and the FAD move along which makes the treatment less accurate and it takes longer to treat at the right location because repositioning is needed. The amount of errors the Echopulse device produces can be decreased. In a few patients, there were a lot of technical hardware / software errors regarding movement of the probe head, elevated temperatures of the cooling liquid in the probe head and/or due to movement of the patient. These errors resulted in a longer treatment time than initially expected, and in some cases, handheld probe repositioning or shutting down of the device was needed to solve the errors. This takes a lot of unnecessary time and needs to be

improved. A technician of the company was available during every treatment to give support in the event of technical errors.

At two weeks, no significant decrease in size of the FAD was seen on US, this was not expected due to local swelling of the treatment site and time the body needs to remove the necrotic tissue. However, an average decrease of approximately 14.3% was found. This could be because it was complicated to get the maximum diameter on the screen and freeze it at the right moment. Furthermore, inter-observer variability between radiologists could also make a difference in US measurements. At this moment, 16 patients have been followed up at three months and ten at six months and the average decrease in volume was 41.7% and 59.6% respectively. No significant difference was found due to the limited amount of patients. At six months a decrease in volume of about 50% was expected, this expectation was obtained from previous studies using whole lesion ablation. Hynynen *et al* [31], treated eleven FAD and found a similar decrease in volume of 32% (1.9 to 1.3 cm³) measured on T2-weighted MRI after six months. Kovatcheva *et al* [71] found a decrease of 60.7 \pm 16.0% (12 patients) after six months with US.

Analysing the decrease in volume of the FAD shows that one patient is not responding to the HIFU treatment. At two weeks the FAD increased in size for 10% and at three months the FAD had increased in size for 23%. Two complete circumferential rings were treated in this patient, but the treatment was not effective enough. This could be due to movement during the treatment as a response to pain or movement of the patient in general. The six month follow-up will confirm if the FAD is not responding to the treatment or if the FAD is responding and the increase in volume was due to local oedema.

In general, six patients developed hyper-pigmentation of the skin. Some patients had not noticed the hyper-pigmentation themselves. It was expected that the hyper-pigmentation would be caused by a short distance between the skin and the lesion. However, analysing these distances it appeared there is no correlation. Patients with darker skin were also expected to have a higher chance in developing hyper-pigmentation, however in our case three patients were Caucasian, one was Asian and two had a black background. Topical crèmes to fade the skin pigmentation like hydroquinone could resolve the hyper-pigmentation.

One patient developed a superficial first-degree skin burn during the treatment; this could have been caused by the micro-foam used for immobilisation of the breast. The micro-foam might have folded the skin during the treatment causing the US waves to pass three layers of skin. Because of the different refractive index of the skin compared to the breast tissue, energy was reflected and absorbed at the skin and as a result, temperature rises and a skin burn developed. There could also have been some air in between the layers which also causes the temperature to rise at the skin because of the different refractive indexes, and cause a skin burn. No treatment was required for the first-degree burn and the burn completely resolved one month post-treatment. When placing the treatment probe on the skin, care is needed to make sure the probe is not placed on the site of injection. Slight air bubbles might be left at this site and when located in the US beam this might cause cavitation and in the worst case cause a skin burn.

Some patients reported feeling pain or shocks of the HIFU pulses going through their arm, in these patients the lump was located close to the pectoralis muscle and when pulses were given close to the muscle, the muscle itself was warmed up as well resulting in a painful feeling or shock going to the arm. An solution would be to add a function to draw the pectoralis major muscle and / or chest wall as well during the treatment planning. A safety margin similar to the skin can be added to protect the muscle form potential damage.

At the beginning of the trial the investigators had a different definition of circumferential ablation. Our goal was to treat the whole circumference of the FAD. With the Echopulse it was only possible to ablate a single 'donut' shape. To treat multiple layers the whole planning process needed to be performed again and this would almost double treatment time. In this case, it is important to treat the bottom layer first before treating the top layer. However, it was determined by the team that the donut shape would be sufficient enough for the trial and therefore only single layers of circumferential treatments were applied.

Patients and clinicians asked about the potential risk of damage to the breast ducts and this influencing lactation or breast feeding on the long-term. During HIFU treatment, the ducts are not located within the treatment area, the surrounding tissue is not damaged and therefore lactation and/or breastfeeding will not be influenced by this treatment. Questions were also raised about if certain types of FAD are more suitable for HIFU treatment than others. Unfortunately, the specific type of FAD are not mentioned in the histology and cytology reports and therefore it was not possible to look at these outcomes, future work could look at this aspect and see if there is a correlation between the decrease in size and the type of FAD.

Comparing circumferential HIFU treatment with whole lesion treatment shows that there is a reduction is time, without a loss in pain relief, FAD reduction or an increase in short-term complications. When compared to VAM or surgical excision the procedure takes the same amount of time, however the patient can be discharged almost straight after. Furthermore, the absence of possible general anaesthetic and operational complications and the absence of a scar are further advantages of HIFU treatment. However, HIFU is not eligible for all patients with FAD, in this trial, it was experienced that patients with a FAD of about 1 - 3 cm are ideal candidates for HIFU.

6. Conclusions

Minimally invasive ablative techniques have been shown to successfully induce coagulative necrosis in breast tumours with minimal complication rates and reliable follow-up imaging. Complete ablation has not been consistently reported on histopathology and no imaging modality has been able to confidently predict the percentage area of complete ablation. Response to treatment can be monitored with either US or MRI, however none of the trials compared both modalities. Consistent tumour and margin necrosis, which represent oncological safety combined with reliable follow-up imaging are required before these minimally invasive ablative can be evaluated within large, prospective clinical trials. Adequately powered and prospectively conducted clinical trials are needed to validate the efficacy of ablative techniques comparing outcome to surgery.

Circumferential HIFU ablation of FAD is feasible with a significant reduction in treatment time. At two weeks a slight decrease in volume of the FAD is visible and minor to mild short-term complications are found. At three months post-treatment a resolution of pain symptoms is seen, minor short-term complications are found and on US a potential significant reduction in volume is visible. Six months follow-up showed a further decrease in volume on US and minor complications. Further patient follow-up is required to establish the effect of circumferential HIFU treatment.

7. Future Work

The following aspects need to be taken in consideration before (circumferential) HIFU treatment can be used as a standard treatment of care.

Patients need to be informed about what they can expect during and after HIFU treatment. At this moment not enough data is obtained to draw any hard conclusions about the degree of reduction the patient can expect post-treatment at three, six and 12 months and what the patient will experience during and in the first weeks post-treatment. It is advised to complete the recruitment and 12 month follow-up of all 50 patients. In this way, HIFU treatment can also be optimized in terms of immobilisation, administration of local anaesthesia and occurrence of errors during the treatment. From the follow-up data the expected decrease in FAD volume can be determined. It would also be possible to inform patients about a potentially increased risk of developing specific short-term complications like hypo- or hyper-pigmentation.

Another way of obtaining more information regarding the treatment is to collect data of multiple centres which are using the same device and to compare this data retrospectively. The patient group and therefore the collectable data would be much larger and more can be said in terms of decrease in size, short-term complication rate and reduction of pain symptoms. However, other centres have used whole lesion ablation and not circumferential ablation which will make it harder to compare the data. Another way is to compare these results with each other in a case-control study. In this way, it is possible to determine the decrease in time and the difference in reduction of the FAD of circumferential treatment compared to whole lesion ablation.

Long-term follow-up could be useful to determine the impact of the treatment on the tissue in the long-term. It would be interesting to see if the slight hyper-pigmentation visible at three months will resolve completely and/or if the breast tissue will restore itself and if the FAD will resolve completely.

Reduction in FAD volume after HIFU treatment needs to be compared to a control group of patients with FAD which have been discharged from the breast clinic and not received any treatment. Patients need to be matched for age and FAD size. Matching for the time of FAD appearance would make the comparison more precise but an even larger population is needed for this. The comparison can be made by getting the control patients to undergo an US scan to determine the natural behaviour of a FAD within a period of six months. Guy's hospital is currently awaiting ethics and research and development approval on the amendment of the HIFU-F trial including this control study. The new protocol is translated into an article which can be found in Appendix II.

Ideally a study comparing HIFU with surgical excision would be performed, however there are no outcome measures which can be compared, due to the fact that in surgical excision the lesion is completely removed and after HIFU no histopathology is obtained. It would be possible to compare patient recorded outcome measurements like cosmetic outcome and overall satisfaction after HIFU treatment or surgical excision. Patient recorded outcome measures and user acceptability by both surgeons and radiologists need to be obtained from the HIFU trial as well. This could be obtained by getting the patients to submit a questionnaire regarding the trial. User acceptability can only be obtained by including other sites using the same device, currently there are at least twelve sites where the Echopulse or a MRI guided HIFU device is used for the treatment of FAD in commercial treatment or clinical trials.

Improvement in the reduction in time can be obtained if it would be possible to visualise the vessels going to the FAD during treatment, for example with colour Doppler. In this case, pulses should only be applied at these areas to obtain isolation of the FAD from its blood supply and this would severely reduce the treatment time.

The Echopulse device might need some improvements to optimize the treatment. The possibility to apply multiple pulses at the same time would make it possible to treat patients quicker. This function might only be allowed in larger lesions and at sites not close to the border of the FAD or close to the skin or pectoralis major. The treatment images can get a higher quality by using a treatment transducer with a higher gain. At the moment it is sometimes very hard to distinguish the FAD from the surroundings which complicates the treatment procedure. The treatment planning can be improved by adding a function to draw the pectoralis major and / or chest wall and add a safety margin similar to the skin. This would protect the pectoralis muscle and chest wall from potential damage during the treatment. For the first pulse the right power needs to be obtained in which a HEM is visible. A function could be added which recognizes the HEM by itself as an increase in signal intensity at the target volume. A similar function could recognise the skin as well and give an error or adjust the drawn skin when the skin is not corresponding to the skin drawn during treatment planning. The possibility to select the next treatment pulse would be a great outcome. At the moment the surgeon needs to deselect all planned pulses before he can select the next pulse. A deselect all button or the possibility to select the next pulse right away would decrease the treatment time. Finally the device should be adjusted in a way that it performs less errors. These have been very time consuming during the previous treatments.

During this trial, it is found that this new technique is less suitable for patients with a large FAD because the lesion is most likely located close to the skin and/or to the chest wall and as a result the patient will have more pain during the treatment. Another disadvantage of treating large lesions is that it takes more time to treat, it could become more uncomfortable for the patient if she needs to lie still for more than an hour. It is therefore advised to treat patients with a FAD of about 1 - 3 cm. Smaller lesions are most often not felt by the patient and not suitable for treatment because only a few pulses are needed to treat the whole lesion and therefore, circumferential treatment is not possible in these cases. In FAD of these sizes it might be better to wait and see if the FAD increases or decreases in size.

A cost-analysis is needed to determine if HIFU treatment is more cost-effective compared to surgical excision and how many treatments need to be performed to be able to reimburse the purchase of the Echopulse. It would also determine if purchasing the device is less costly compared to leasing the device or shipping the device up and down to France for every treatment.
A comparative trial using US *versus* MRI-guided HIFU would be an option. At the Royal Marsden in London, MRI-guided HIFU is used for pain relief due to bone metastases. If this device would be suitable to treat breast lesions as well, it might be possible to treat a few patients and compare these results with US-guided HIFU. The advantage of MRI-guided HIFU is that it can measure the temperature during the treatment and therefore the pulse can be stopped at a certain temperature. In some applications this is important because if the tissue temperature reaches boiling point, cavitation will occur and this might destroy nearby tissues as well. Also when ablating blood vessels, higher temperatures might cause boiling of the tissue and as a result the vessel will not be coagulated but due to the bubbles arising a bleeding will be developed.

Before US-guided HIFU can be considered for use in breast cancer there are a few aspects that need to be considered. This circumferential treatment or any other HIFU method needs to prove a significant decrease in size and at a certain moment in time no residual tumour tissue can be left behind in all patients. Therefore, the treatment at this moment is not consistent enough for use in malignant lesions. Furthermore, lesions must be well-defined on US, if the lesion is not well defined the chance of not treating the whole lesion is considerable. Therefore on histology, the patient should not have any evidence of DCIS, it is reported that DCIS is not clearly visible on US and the risk of not treating the whole extend of the tumour would increase. The ideal treatment margin for HIFU treatment needs to be determined, would the same margin as in surgical excision be sufficient enough or is an extra margin needed to reassure complete ablation. The disadvantage of treating an extra margin is that not all lesions will be suitable for HIFU treatment due to the safety distances needed between the skin and the lesion and the pectoralis major muscle and the lesion. The device also has a limiting depth of 2.3 cm at which it can treat. As a result, only small breast cancers are eligible for treatment. Histopathological staining must be able to accurately measure the amount of cell death post-treatment. It is advice to use NADH or H&E staining if histopathological tissue is not obtained shortly after HIFU treatment. Currently, skin toxicity occurs in some patients after HIFU treatment. This skin toxicity must be explained and decreased in occurrence before HIFU can be applied to breast cancer. HIFU should be used together with adjuvant therapies like radiotherapy, chemotherapy or endocrine therapy and therefore a protocol needs to be developed in which all required information to determine additional adjuvant therapy are obtained prior to HIFU treatment. Grading of the tumour and receptors of the lesions must be determined pre-treatment and a treatment planning including the adjuvant therapies must be made.

HIFU ablation is a promising technique however, sufficient amounts of work needs to be performed before (circumferential) HIFU treatment can be used as a standard treatment of care.

8. List of publications and presentations

Publications:

Abstracts:

- Focused Ultrasound Symposium, Maryland, USA: 'High Intensity Focused ultrasound in the treatment of breast fibroadenomata: The HIFU-F trial.'

Book chapters:

- 'Clinical application of ultrasound-guided focused ultrasound ablation for breast tumours', Therapeutic Ultrasound, CRC press Taylor & Francis group. (Appendix III)

Presentations:

National:

- Oral presentation Ultrasound Symposium, Enschede, The Netherlands: 'High Intensity Focused Ultrasound (HIFU) in the treatment of breast lesions: a systematic review'.

International:

- Oral presentation ABS London Regional Breast Symposium, Guy's and St. Thomas's Hospital, London: 'A systematic review: High Intensity Focused Ultrasound (HIFU) in the treatment of breast lesions'.

- Oral presentation Focused Ultrasound Symposium, Maryland, USA: 'High Intensity Focused ultrasound in the treatment of breast fibroadenomata: The HIFU-F trial.'

- Poster presentation Focused Ultrasound Symposium, Maryland, USA: 'High Intensity Focused ultrasound in the treatment of breast fibroadenomata: The HIFU-F trial.'

- Poster presentation, BASO/ESSO, Liverpool, UK: 'High Intensity Focused Ultrasound (HIFU) ablation in the treatment of breast tumours: A systematic review'.

- Poster presentation, BASO/ESSO, Liverpool, UK: 'Minimal invasive ablative techniques in the treatment of breast tumours: A systematic review.'

Awards:

- Young Investigators Award, Focused Ultrasound Symposium 2014, awarded for the abstract: 'High Intensity Focused ultrasound in the treatment of breast fibroadenomata: The HIFU-F trial.'

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Appendix I. Ablative Techniques in the Treatment of Breast Fibroadenomata

Introduction

Breast fibroadenomata (FAD) are benign breast lesions which can develop at any age but most often during the second and third decades of a woman's life. Ten percent of all women have FAD and about 50% of all breast biopsies are FAD. Studies showed that within five years, up to 59% of FAD will show a decrease in size or complete resolution. [1] FAD transformation into a malignant lesion is exceptionally rare (0.002 - 0.0125%) and women with FAD have a 1.3 - 2.1 increased risk of breast cancer compared to the general population. [2-4]

Patients present themselves in the breast clinic with a palpable lesion detected during self- or medical examination. FAD are generally solitary, smooth, non-tender, mobile masses of about 1-3 cm. [2, 4] FAD are diagnosed by triple assessment which entails physical examination, imaging by ultrasound and/or mammogram and a fine needle aspiration or core needle biopsy to get conformation of the diagnosis. [2, 4] Overall diagnostic efficacy of triple assessment is approximately 70-80% and in 95% an accurate differentiation is provided between a benign and a malignant lesion. [2, 4] Management of non-palpable FAD is reassurance with or without follow-up. For palpable lesions, there are three treatment options: reassurance (with or without follow-up), vacuum assisted mammotomy (VAM), which is not licensed for the treatment of FAD, or surgical excision. [2] Intervention is offered to patients with large FAD, fast growing lesions or to patients requesting for removal. [4] In other cases, removal might involve unnecessary excisions of benign lesions and unbecoming cosmesis. [2]

Ablative techniques offer the opportunity to treat the FAD without creating scarring or poor cosmesis. Advantages of minimal invasive ablative techniques are also the ability to image the progress during the treatment. We reviewed the current evidence of non-invasive ablative techniques in the treatment of breast FAD including high intensity focused ultrasound (HIFU) ablation; cryo-ablation and laser ablation.

High intensity focused ultrasound

HIFU is a non-invasive ablation technique. [19] During HIFU, an US beam generated by a piezoelectric US transducer propagates through tissue as a high-frequency pressure wave. [20] The beam is focused onto the targeted tissue and the energy from the beam elevates the temperature up to 60-95^oC within a few seconds. This is done without causing damage to direct adjacent tissues, allowing a focused ablation leading to protein denaturation and coagulative necrosis. [20, 21]

Hynynen *et al.* [31], evaluated the feasibility of HIFU guided by magnetic resonance imaging (MRI) for the non-invasive treatment of 11 FAD in nine patients (median age 29 years, range 19-38 years). Patients were included if they were 18 years or older, with a FAD confirmed by histology located 1.5 cm or more from the skin and rib cage. Pregnant or lactating women, FAD with calcifications on mammography and patients with standard MRI contraindications were

excluded. The lesions had a volume of 1.9 cm³ (0.7-6.5 cm³) and ablation was performed using a power between 28-50 W. Follow-up with clinical examination and MR imaging was performed at two and ten days and six months and thereafter (median six months, range 1.5-4 years). A decrease in volume of 50-90% was defined as a partial response. A decrease above 90% was categorised as complete response and a decrease between 10-49% as minor response. T1weighted images showed that six patients had a complete response, two patients had a partial response, one had a minor response and two had no response to treatment. T2-weighted images showed a median volume decrease from 1.9 to 1.3 cm³ at six months post-treatment. Pain was recorded as slight in four patients, moderate in two and severe in one patient. Tenderness was common up to 10 days post- treatment and oedema was visible up to two days.

Tempany *et al.* [72], evaluated the safety and efficacy of HIFU treatment using the InSightec device. The goal was to obtain a decrease in size on physical examination of 50% or greater and 65% or greater on MRI. Between January 2003 and October 2005 102 patients were enrolled. Inclusion criteria were female patients of 18 years or older with a histological confirmed single FAD, visible on non-contrast MRI with a FAD of 0.5 cm or larger. Exclusion criteria were FAD over 3.5 cm on MRI, FAD with a distance to the skin of less than 0.5 cm or to ribs less than 1 cm, patients with micro-calcifications within the FAD, patients with intolerance to MRI contrast agents, hemolytic anemia, unstable cardiac status, cardiac pacemaker, a ASA score >2, severe cerebrovascular disease, patients which will not fit in the MRI, patients on anti-coagulation therapy, patients with a history of breast cancer, laser or radiation therapy to the target breast and patients with a history of chemotherapy. The follow-up period was not mentioned.

Kovatcheva *et al.* [32], included 27 patients to demonstrate the efficacy of HIFU in the treatment of breast FAD. The trial started on March 2011 and finished in January 2014. The primary outcome measure was the reduction in volume of breast FAD on US. Patients included were 18 years or older with a single FAD confirmed by triple assessment and a size between 1-3 cm at its largest dimension. Exclusion criteria were pregnant or lactating patients, patients with a BIRADS score of higher than two on mammography or with micro-calcification within the FAD. Patients with a history of breast cancer, laser or radiation therapy, patients with breast implants or breast cysts, FAD which are not clearly visible on US and patients participating in other trials using drugs or devices. Follow-up was performed at one, three, six, nine and 12 months.

Boulanger *et al.* [33], designed a multicentre, open uncontrolled trial for the observation of histological changes in FAD following HIFU. The study included 24 patients between October 2011 and February 2014. The included patients will receive HIFU treatment and six months post-treatment the need for surgery was evaluated. The primary outcome measure was the HIFU induced tissue necrosis assessed by histology of excised gland or reduction of FAD volume. Secondary outcome measures were volume reduction on US, pain scoring during HIFU, evaluated by the visual analog scale and adverse effects. Inclusion criteria were patients of 18 years or older, with FAD diagnosed by triple assessment and a size superior at 1 cm at its largest dimension. Exclusion criteria were pregnant or lactating patients, FAD with micro-calcifications on mammogram, patients with a history of laser or radiation therapy, patients with breast implants, FAD not clearly visible on US and patients participating in other trials using

drugs or devices. Patients are followed-up at two, four six and 12 months or until surgery if they opt for surgical excision.

In the mono-centre, open-label uncontrolled study by Hahn *et al.* [34], 27 patients were recruited between December 2013 and January 2016 to evaluate the efficacy of HIFU in the treatment of FAD. The secondary outcome measure was the tolerability of HIFU. Patients were included if they were 18 years or older with at least one diagnosed FAD based on triple assessment and a longest diameter of 2.5 cm. Patients were excluded if they were pregnant or lactating, had a history of ipsilateral breast cancer or radiotherapy to the targeted breast within five years of inclusion, patients with breast implants, patients with FAD previously treated by interstitial laser therapy or cryo-ablation within one year of inclusion, patients with FAD which are not clearly visible on US and patients participating in other trials using drugs or devices within three months of inclusion. Patients were followed-up for 12 months.

Douek *et al.* [73], performed a feasibility study to assess the treatment of FAD with a circumferential signification HIFU treatment. The trial started in January 2014, will recruit 50 patients and the estimated completion date is October 2016. The primary outcome measure is the change in size of FAD as recorded on US imaging. Secondary outcome measures are complications, patient recorded outcome measures, mean treatment time and cost-effectiveness of the HIFU treatment compared to surgical excision of FAD. Patients eligible for this trial are patients \geq 18 years of age with FAD diagnosed according to local hospital protocol visible on US (graded U2 benign or U3 indeterminate) and for patients \geq 25 years of age, confirmation is required by either cytology (C2) or histology (B2). The definitive diagnosis of FAD must be confirmed by the breast MDM. Exclusion criteria are FAD with atypia or suspicion of phyllodes (graded B3/C3 or greater), pregnant or lactating women, women with breast implants and women with a history of laser or radiation therapy to the targeted breast.

Benin *et al.* [35], designed a FDA approved feasibility study to determine the safety and efficacy of the Echopulse device (Theraclion Ltd, Malakoff, France). The trial started in April 2014 and the estimated completion date is May 2016. The aim is to include 20 patients. Primary outcome measures are the change in volume of the FAD measured by US, size of FAD on physical examination, patient recorded outcomes by measuring the patient rated pain and the patient response to satisfaction questionnaires. The secondary outcome measure is the incidence of adverse events. Patients are included if they are 18 years or older, with a palpable histological confirmed FAD with a size of 1 cm or greater and a volume between 2-10 cc. Exclusion criteria were pregnancy or nursing patients, patients with breast implants, patients with a cyst in the FAD and patients participating in another trial involving an investigational drug, device or biologic. Patients are followed-up at three, six and 12 months.

Cryo-ablation

Cryo-ablation uses freezing instead of heating in the treatment of breast tumours. It is accomplished by inserting a cryo-probe under US guidance into the target tissue. The freezing process involves two phases: freezing and thawing. Four mechanisms destroy the tumour cells: direct damage by (1) intracellular ice formation and (2) osmotic dehydration, indirect damage

due to (3) ischemia and (4) immunologic response. The treatment has good precision and control because the formation of an ice ball can be clearly visualized with US. [25, 26]

Klein *et al.* [74], performed a study to determine whether the ice-sense cryo-ablation system is safe and effective in the treatment of benign breast tumours such a FAD. The primary outcome was the engulfment of the ice-ball as seen on US imaging. The secondary outcome measure was the adverse events. 54 patients were enrolled between April 2009 and January 2013. The patients were 18 years or older, with core biopsy proven FAD, visible on US and a size between 0.5-3.0 cm. Exclusion criteria were pregnant women, superficial FAD, patients with a history of breast cancer, patients which had major surgery in the last three months, patients with a terminal illness or a life expectancy of less than two years, patients carrying contagious diseases such as TBC, HEP or HIV, and patients participating in other trials using drugs or devices. Follow-up was performed for 12 months.

Hahn *et al.* [25], performed a prospective multicentre trial to evaluate cryo-ablation under US guidance in the office setting for patients with FAD. Histological confirmed FAD with a maximum dimension of 3 cm were included. 23 patients were treated and all attended follow-up at one week, three, six and 12 months. The ice-ball engulfed the treated FAD in 91.3% and a sharp reduction in volume was observed at six months. Four minor adverse events occurred. In 96% of cases, patients and physicians rated the cosmetic results as excellent or good.

Kaufman et al. [75], performed a prospective non-randomised multicentre trial on 63 patients (mean age 34 (range 13-66 years)) with 78 biopsy proven US-visible benign breast lesions (mean size 2.0±0.8 cm (range 0.7-4.2 cm)) of which 85% were FAD. They were treated with the Visica treatment (Sanarus Medical, Pleasanton, California) between June 2000 and August 2002. Exclusion criteria were, invisible tumours on US, patients with a history of ipsilateral breast cancer, patients with any other suspicious lesions, or refusal to undergo post-treatment medical photography. The system uses a freeze-thaw-freeze technique. Follow-up was performed at one and six weeks for examination and three, six and 12 months for US. Palpable lesions that became non-palpable had a volume of 1.8 cm³ and the lesions that remained palpable were 3.8 cm³. Median residual tumour at three months was 54.9%, 32.2% at six months and 11.7% at 12 months. 82% of lesions were palpable pre-treatment and posttreatment 27% were palpable. Overall cosmesis was good to excellent in almost all patients and patient satisfaction at 12 months was good to excellent in 92% and unsatisfactory in five patients (8%) due to remaining palpability. Complications were mild ecchymosis, oedema, tape blisters (n=9 at 6 weeks and n=2 at 12 months), skin de-pigmentation (n=6), keloid at probe entry (n=2), breast abscess (n=1) and pain (n=3).

A multicentre trial was set up by Edwards *et al.* [76] using the Visica cryo-ablation system (Sanarus Medical, Pleasanton, California). Two freeze-thaw cycles were used. Follow-up was performed at six and 12 months. 53 sites treated 310 FAD with a mean diameter of 1.8 cm. Pre-treatment, 77% of FAD were palpable. Complications were infection (2%), ecchymosis, hematoma (amount comparable to surgical excision), tape blisters (5%) and de-pigmentation (1%). 92% of patients were satisfied with the procedure and 91% would recommend it to a

friend. A palpable lump was found in 50% at three months and in 33% at six months. Average residual lesion volume was 49% at six and 3% at 12 months.

Littrup *et al.* [77] performed a study to assess freezing protocols, imaging and clinical outcomes of percutaneously US guided cryo-therapy for breast FAD. 42 FAD were diagnosed in 29 patients. The US guided Visica treatment system was used with a freeze-thaw-freeze cycle. Follow-up was performed at one and six weeks, three, six and 12 months. Patient age was 26.6 years (13-50 years) with a volume of 4.2 ± 4.7 cm. At three months it became harder to visualise the FAD from its surroundings and at six months four FAD showed fragmentation. At 12 months five FAD could no longer be identified and the FAD showed a reduction of 73% to 0.7 ± 0.8 cm (P<0.001). All patients were happy with the cosmesis and in three patients scarring at the insertion site lead to hypo-pigmentation which resolved at 6-12 months. One patient had a keloid and at the end of the trial two patients had surgical excision.

Caleffi *et al.* [78] used the Visica system to carry out interstitial US-guided cryo-ablation of 124 benign breast tumours in 102 patients. 42 were treated between December 1999 and August 2000 with a Double HI-Freeze technique and 82 breast tumours were treated with a tailored Freeze technique between July 200 and August 2002. Patients were eligible if the lesion was confirmed by FNAC or CNB and was visible on US. Any evidence of DCIS or pre-malignancy excluded the patients. Further exclusion criteria were lesions suggestive of malignancy, history of ipsilateral breast cancer, or aversion of post-treatment medical photography. All patients were followed-up for 12 months and the mean age was 38 years. The Double HI-Freeze group had a mean age of 45 years, a mean size of 1.4 ± 0.6 cm and a mean treatment time of 16.1 minutes, the Tailored freeze group had a mean age of 34 years a mean size of 2.1 ± 0.8 cm and a mean time of 14.7 minutes. With the double freeze group 14 lumps were palpable pre-treatment and at one year post-treatment 24/36 lesions were palpable. With the tailored freeze group there was a reduction in volume of 91% at 12 months. No serious adverse events occurred apart from ecchymosis, discomfort, oedema visible, tape blisters (n=6) and keloid (n=2). Patient satisfaction was excellent in 92% of all patients.

Laser ablation

In laser ablation, tumours are destroyed using direct heating with low-power laser light energy delivered via thin optical fibers. [27] Upon absorption in the tissue, heat is produced, inducing lethal thermal injury. [18] The size and shape of thermal lesions are difficult to predict, however owing to biologic variability, fiber tip charring and changing optical and thermal properties of the tissue during interstitial laser photocoagulation. [27]

DeLay *et al.* [79], performed a observational study to monitor long term safety and effectiveness of the Novilase device. 500 patients will be recruited between December 2008 and December 2014. Patients are included when 18 years or older with a FAD confirmed by CNB, tumors detected either by physical exam or imaging, not exceeding 2 cm in diameter and measure at least 0.5 cm away from the skin and chest wall. Exclusion criteria are pregnant or lactating women, tumours suggestive of phyllodes or atypia, patients with equivocal pathology report and FAD with stromal solidarity.

Basu et al. [80], included 27 patients in a uncontrolled prospective study to evaluate the effects of interstitial laser hyperthermia in FAD of the breast. Inclusion criteria were patients with FAD confirmed by FNAC and with lumps up to 2 cm on physical examination. Exclusion criteria were patients of 35 or older, pregnant women and lumps present for less than one year. For the procedure the Lasermatic (model 5050-23, Combolaser, Helsinki, Finland) was used with ND:YIG bare quartz fibers of 600µ in diameter. Two watts of laser energy were delivered in continuous wave mode for 300 sec. Follow-up was performed at two, four and eight weeks. At eight weeks, ten patients with residual lumps of more than one cm in diameter underwent excision biopsy for histopathology examination. The mean age was 21.8 years (14-35 years) with a mean duration of the lump of 16.4 months. All patients experienced a warmth sensation locally during the procedure. Immediately post-treatment US showed a hyper-echoic zone with a narrow rim of hypo-echogenicity (0.3-0.5 cm). Blanching of the skin at the needle insertion site was seen in eight patients. These patients showed epithelial breakdown and hyper-pigmentation during follow-up. At two weeks all lumps were tender and less mobile and US showed a decrease in size and a narrower hypo-echoic rim. At four weeks US showed a heterogeneous echo pattern. At eight weeks further reduction was seen. Histopathology in ten patients showed fibrotic tissue and tissue than was adhered to the surrounding tissue. A statistically decease in size was found clinically (60-70% reduction, mean form 2.6±0.79 to 1.25±0.6 cm) and on US (40-50% reduction, from 2.17±1.03 to 0.68±0.39 cm).

Lai *et al.* [81], evaluated the feasibility of laser ablation as a minimal invasive technique for treating FAD. All patients had palpable FAD confirmed by triple assessment and were informed of the conventional management options. One to four 19 G needles were inserted under US guidance and connected with the semiconductor diode laser (Diomed 25, Diomed Ltd, Cambridge, UK). A power of 2.5 W was used for 500 seconds. Patients were seen at 2-4 weeks for a check up and at three, six and 12 months for an US. 24 patients with 29 FAD were treated with a median age of 26 (18-42 years) and lesions of 2.5 cm (1.4-3.5 cm). 28 lesions showed some reduction in size and six patients had surgical excision post-treatment. The median reduction in size was 38% at three months, 60% at six months and 100% at 12 months. Lumps were no longer palpable and no FAD increased in size. In 11/17 lesions which showed strong enhancement on pre-MRI, showed zones of non-enhancement post-treatment. Most patients and resolved within one week. Three patients showed a small skin burn around the needle insertion point, and in one patient there was persistent oily discharge for three weeks.

Discussion

The use of minimally invasive techniques like laser, cryo- and HIFU ablation enables the patient to undergo treatment without general anaesthesia, scarring and risks of complications possible with the currently used techniques. A disadvantage of these techniques is that the FAD is not immediately removed but will slowly decrease in size in the months following treatment. Patients must therefore be well informed to prevent them from developing anxiety towards the lump.

Objectively only the cryo- and laser ablation techniques can be compared since for HIFU only one trial has currently published results and two other trials have only presented their

preliminary results. For cryo-ablation five out of six studies have published and in laser ablation two out of three studies reported their results.

Looking at the efficacy of the treatment, in HIFU [31] a decrease of 32% was seen after six months. In cryo-ablation [75-78] there was a mean decrease of 40.6% at six months and 87.3% at 12 months. For laser ablation [81] the decrease was 60% at six months and 100% at 12 months.

Complications like oedema, pain, tenderness and bruising are common in all techniques, tape blisters, hematoma and keloids occur in cryo-ablation and the tape blisters result in depigmentation of the skin near the needle insertion point. In laser ablation, skin burns were more common, however these could occur in HIFU as well.

In general no distinct difference can be given between the three techniques, efficacy and complications are similar in all techniques. Furthermore, more results from large trials are needed to give more conclusive outcomes.

Conclusions

Minimally invasive ablative techniques like HIFU, cryo- and laser ablation, are promising in the treatment of breast FAD. More published outcomes are needed to objectively compare these techniques.

Appendix II. High Intensity Focused Ultrasound for the Treatment of Fibroadenomata (HIFU-F) Study.

Background

Fibroadenomata (FAD) are the most common benign breast lesions in woman. FAD can occur at any age but are more common between the age of 20 - 30 years. FAD occur in about 10% of all woman and account for about 50% of performed breast biopsies. [1] Studies revealed that up to 59% of FAD will show regression or complete resolution within five years. [1] Malignant transformation within FAD is considered exceptionally rare (0.002 - 0.0125%) and there is a 1.3 - 2.1 increased risk of breast cancer in women with FAD compared to the general population. [3] FAD are encapsulated from their surrounding tissues and can be considered as an aberration of normal development rather than a true neoplasm. On histology, FAD consist of combined proliferation of epithelial and fibroblastic tissue elements which are slowly growing and oestrogen (ER) dependent. [4, 5]

The diagnosis of a FAD can be made by triple assessment. The first step is physical examination. In 50 - 67% of cases the lesion identified as a FAD is actually a FAD due to the similar characteristics with other benign diseases. [2, 4] Therefore, more accurate diagnostic methods are required to get the correct diagnosis. The second step is imaging. Ultrasound (US) is the main diagnostic imaging method used for the diagnosis of FAD. [2] However, not all FAD have the same characteristics and not all FAD are visible on US images. [2, 4] A fine needle aspiration cytology (FNAC) or core needle biopsy (CNB) is the third step and can be used to get final confirmation of the diagnosis. In one study, it was found that only 82% of CNB proven FAD could be visualized with US. [2] The overall diagnostic efficacy of this triple assessment is approximately 70 - 80% but an accurate differentiation between a benign and a malignant lesion is provided in 95%. [2, 4]

The management of non-palpable FAD is reassurance with or without follow-up. For palpable FAD, there are currently three main treatment options available: reassurance (with or without follow-up), vacuum assisted mammotomy (VAM), which is not officially licensed for the treatment of FAD only to obtain the diagnosis of a lesion, or surgical excision. In the case of reassurance, it is advised for patients up to 35 years to use a follow-up protocol in which the patient comes back every six months to determine if the lesion has changed in size. [2] Intervention is normally offered to patients with large FAD, rapidly growing lesions or to patients requesting for removal of the lesion due to anxiety or discomfort. [4] In other cases, surgical removal might involve unnecessary excisions and unbecoming cosmesis. [2, 36] A new technique in the treatment of FAD is high intensity focused ultrasound (HIFU) ablation. This a non-invasive ablative technique in which the FAD is treated with focused consecutive repeated US pulses, while surrounding tissues are not damaged.

The basis of HIFU therapy is a HIFU pulse of several seconds period generated by a piezoelectric US transducer. The US field is insonated via a coupling media overlaying the tissue to the targeted area. Due to the high local concentration of acoustic energy in the focal spot, the tissue in a small volume is heated rapidly and a sharp circumscribed lesion caused by thermal coagulation will be induced. [37] HIFU has been clinically applied to the treatment of

invasive breast cancer and demonstrated on pathological assessment coagulative necrosis, regression of tumour size and loss of proliferative activity with a minimal side effect profile. [12, 17, 19, 31, 42, 44, 82, 83] Pathological examination with Victoria blue and ponceau's histochemical staining has been used to assess tumour vascular wall destruction, and immunohistochemical staining for proliferative markers using biotin-streptavidin-peroxidase. [19] Cell viability has been determined by staining for active dehydrogenase using 2, 3, 5-triphenyltetrazolium chloride (TTC). [83]

The current limitation of HIFU is the prolonged treatment times of the procedure. Since a single pulse generates a rather small tissue lesion, a lot of these consecutive repeated pulses have to be applied with an adequate idle time in between to prevent overheating until a large tumour is ablated. [37] We propose to overcome the prolonged treatment times by applying HIFU pulses to the circumferential surface area of lesions rather than their whole volume. We propose to perform this upon a cohort of patients with benign breast tumours in the form of FAD using the US guided Echopulse [™] (Theraclion, Malakoff, France) HIFU system, which is CE marked for the treatment of breast FAD.

Design

Patients

We will undertake treatment on 50 patients with confirmed FAD via triple assessment and agreement on multi disciplinary meetings (MDM). Patients are identified in three ways: (1) at the MDM, where all patients were discussed in which CNB or FNAC was performed, (2) patients scheduled for surgical excision of a FAD and (3) patients visiting the breast clinic requesting for surgical excision. All patients are approached in the breast clinic or by a telephone call asking if they are interested in receiving a patient information sheet (PIS) regarding the HIFU-F trial, which described the procedure in detail and mentioned the advantages, disadvantages and potential complications of the treatment. If the patient is interested, a second telephone call is made to determine if the patient would like to participate in the study and to answer any questions. If the patient agrees to participate in the trial, the patient is scheduled for an appointment in the breast clinic, if requested by the patient, or directly for HIFU treatment. Patients who are willing to participate in the study will provide informed written consent on the day of elective surgery or at a prior hospital visit (during pre-assessment). Details of all patients approached about the trial will be recorded on the patient-screening log and kept in the Investigator Site File (ISF).

Inclusion and exclusion criteria

Patients eligible for this trial are patients \geq 18 years of age with FAD diagnosed according to local hospital protocol visible on US (graded U2 benign or U3 indeterminate) and for patients \geq 25 years of age, confirmation is required by either cytology (C2) or histology (B2). The definitive diagnosis of FAD must be confirmed by the breast MDM.

Exclusion criteria are FAD with atypia or suspicion of phyllodes (graded B3/C3 or greater), pregnant or lactating women, women with breast implants and women with a history of laser or radiation therapy to the targeted breast.

HIFU treatment group

All patients in the HIFU treatment group will attend the HIFU treatment using the US-guided Echopulse device (Theraclion, Ltd, Malakoff, France) as a day-case procedure to be performed in the Clinical Research Facility (CRF) at Guy's Hospital. The breast lesions are ablated under real-time US guidance using a 7.5 - 12 MHz diagnostic US transducer (Theraclion, Malakoff, France). Therapeutic US energy is produced by a 5.6 \pm 0.1 cm diameter 3.0 MHz imaging transducer with a central hole of 1.1 \pm 0.1 cm for the coaxial imaging transducer. The transducer ablates a tissue volume of approximately 0.9 cm in length and 0.2 cm in width.

Patients will be placed in a supine position and degassed local and/or topical anaesthetic (Emla cream [™]) will be administered subcutaneously under US guidance. The breast will be immobilised with a immobilisation system. A coupling media in the form of a gel pad attached to the device will be lowered onto the treatment site. The Echopulse [™] will be used to image the lesion in two perpendicular dimensions and set to deliver HIFU to the circumferential surface area of the lesion by deselecting the centre of the lesion. A laser pointer detects any movement made during the patient and pauses the treatment if there is a lot of movement. Treatment times at start (first pulse administered) and end (last pulse administered) of the treatment are written down. The actual treatment time for the amount of pulses is compared to the treatment time to complete whole lesion ablation. Once the treatment is completed the patient will be observed in the discharge suite for a period of one hour prior to discharge subject to satisfactorily complying with local day case discharge protocols. All patients will be reviewed post-operatively at two weeks, three, six and 12 months with a repeat US scan at each appointment.

Patients who have not experienced at least a 50% reduction in size of their FAD at six months follow-up will be offered the opportunity to undergo an additional HIFU treatment – as per the standard protocol.

Control group

Another 50 patients with core biopsy confirmed symptomatic palpable FAD who have been discharged from the breast clinic since the HIFU-F trial started (December 12, 2013) will be recruited. These patients will be contacted six months after their discharge and will be offered an US scan in order to determine the decrease or increase in size of the FAD.

This cohort will be selected in order to identify the natural course of a FAD over a period of six months from diagnosis. Analysis will be performed to compare the difference in size between the HIFU-treatment group *versus* control group initially when 20 control patients have been recruited.

Outcome measures

The primary outcome measure is the change in size of FAD as recorded on US imaging. Secondary outcome measures are complications, patient recorded outcome measures, mean treatment time and cost-effectiveness of the HIFU treatment compared to surgical excision of FAD.

All patients will be asked prior to receiving HIFU treatment and again at six and 12 months posttreatment to submit sections of the Breast-Q Breast Conserving Therapy Modules to obtain patient recorded outcome measures."

A two-sample T-test will be performed to determine if there is a significant difference in FAD volume reduction between the study group and the control group. To determine if the variances are equal or unequal we will perform a two-sample F-test.

Discussion

At our unit over 600 FAD were identified on US imaging in 2012 of which six underwent VAM and 60 formal surgical excision. Difficulties could be encountered on recruitment of patients from the MDM. Clinicians from different surgical and radiological specialties could be hesitant to allow recruitment and treatment of patients with HIFU who have symptomatic FAD, but would not generally be offered intervention. Patients offered surgical excision are usually patients with atypical FAD, lesions with a suspicion of phyllodes tumour, fast growing and/or very large FAD. Most of these patients are not eligible for HIFU treatment. Large lesions are not an exclusion criteria, but HIFU treatment would take significantly longer and multiple treatments might be needed to treat the whole FAD.

In general, FNAC and CNB could be performed in all patients, however in woman < 25 years no FNAC or CNB is performed when both US and physical examination reveals a solid lesion with typical benign characteristics. This is due to the low incidence of breast cancer in woman of this age group. [6, 7]

The primary outcome measure is the decrease in size of the FAD as visible on US. US as an imaging modality was chosen because it is more accessible to patients and staff than for example CT or MRI. Furthermore, the FAD must be visible on US to be eligible for HIFU, it should therefore be possible to visualise and measure the FAD post-treatment as well. Disadvantages of US as an imaging modality are the intra-observer variability between the measurements performed by the different radiologists. This is solved by asking only one radiologist to perform the measurements if any dimensions are missing. The US screen must be freezed at just the right moment to visualise the maximum diameters. With CT or MRI this would be much easier to determine. The ideal imaging modality would be accessible and accurately measure the response of the treatment in two aspects: the change in FAD size in three dimensions and the changes in the tissue surrounding the FAD.

In this protocol the diameters of the FAD are measured in three dimensions to calculate the volume of the FAD. Measuring one diameter is not sufficiently enough since the decrease in size could be different in the different dimensions and with the volume these changes in diameters are all taken into account.

The disadvantage of this trial is that no histology or cytology is obtained post-treatment. Only in patients opting for surgical excisions after HIFU treatment the histology could be obtained. Pathological findings could tell us more accurately about the changes in FAD size and surrounding tissues over time. CNB or FNAC could be performed to obtain this as well however these are often not pleasant for patients and it could prevent them from participating in the HIFU-F trial. The changes can be imaged as well but histology/cytology would give the final diagnosis. It was determined that histology post-treatment in the patients opting for surgical excision would be sufficient enough to get a view of the texture changes post-treatment.

Appendix III. Clinical application of ultrasound-guided focused ultrasound ablation for breast tumours Background

Breast fibroadenomata

Breast fibroadenomata are the most common benign breast lesions, they occur in about 10% of all woman and are the diagnosis in about 50% of all breast biopsies. They develop during the second and third decades of a woman's life. Studies revealed that up to 59% will show a decrease in size or complete resolution within five years. [1] Transformation into a malignant lesion is very rare (0.002 - 0.0125%). [2-4] Compared to the general population, women with breast FAD have a 1.3-2.1 increased risk of developing breast cancer. [2] Patients with FAD usually present themselves with a palpable lesion detected during self- or medical examination.

FAD are normally solitary, non-tender, smooth, mobile masses of about 1.0-3.0 cm. [2, 4] In 20% of cases, FAD are multiple or 4.0 cm or larger (FAD of 5.0 cm or larger occur in 5% of cases). [2] The diagnosis of a FAD is made by triple assessment, in which the first phase is physical examination. In 50 - 67% of cases the lesion identified as a FAD by physical examination is actually a FAD. This is due to similar characteristics with other benign diseases. [2, 4] More accurate diagnostic methods are required to get the final diagnosis. The second phase is imaging, ultrasound (US) is the main diagnostic imaging method used. FAD can be seen as oval smooth solid masses with even low-level internal echoes. [2] In 25% of FAD, features like an irregular border are suggestive of a possible malignant lesion. [2, 4] A fine needle aspiration cytology (FNAC) or core needle biopsy (CNB) is the third phase and can be used to get the final diagnosis. One study reported that only 82% of CNB proven FAD could be visualised with US. [2] The overall diagnostic efficacy of this triple assessment is approximately 70-80% and an accurate differentiation between a benign and a malignant lesion is provided in 95%. [2, 4]

The management of non-palpable lesions is reassurance with or without follow-up. For palpable lesions, there are three main treatment options available: reassurance (with or without follow-up), vacuum assisted mammotomy (VAM), which officially is only licensed to obtain the diagnosis of a lesion but not for the treatment of FAD, or surgical excision. Intervention is in general only offered to patients with large or fast growing FAD or to patients requesting for removal of the lesion due to anxiety or discomfort. Patients with a family history of breast cancer are advised to get interventional treatment as well. [4] In other patients, surgery might involve unnecessary excisions of benign lesions and unbecoming cosmesis. [2]

VAM is performed under US guidance with local anaesthesia. Compared to surgical removal VAM is less invasive with a better cosmetic outcome. Complete removal of the FAD is found in 75-100% of all cases. The disadvantage of VAM is the reduced visibility due to blood, air, local anaesthesia and/or soft tissue oedema. Furthermore, lesions close to the skin <0.5 cm and of a size larger than approximately 3.0 cm are not suitable for treatment. In some lesions close to the pectoralis major and/or skin local anaesthesia can be injected between the lesion and the

skin to increase the distance. Possible complications following VAM are hematoma, skin defect and in rare cases a pneumothorax. [8]

With surgical removal the lesion is usually excised under general anaesthesia. It is the best option in the case of large or multiple FAD or lesions that have the appearance of a phyllodes tumour. [2] The main advantage is the complete removal of the FAD. Disadvantages are the scarring, possible damage to the ducts, cosmetic outcome and chances of anaesthetic and/or operative complications. [2, 4]

High intensity focused ultrasound (HIFU) ablation could be used for the treatment of FAD. The advantaged are the absence of a scar, improved cosmesis and the possibility to treat patients under local anaesthesia in a outpatient setting.

Breast Cancer

Breast cancer is the most common cancer in women in the United Kingdom with just under 50,000 women diagnosed with invasive breast cancer in 2010 and 11,684 deaths in 2011, it is the second most common cause of death from cancer in women. [9] In men, breast cancer diagnosis and death due to breast cancer account for about 1%. [10]

Breast cancers are diagnosed at an increasingly earlier stage due to the wider use of mammographic screening. [10-12] The breast cancer diagnosis is made using triple assessment containing a combination of the following modalities: physical examination, mammography, US, magnetic resonance imaging (MRI) and/or breast biopsy. [10] The American Joint Committee on Cancer (AJCC) has staged breast cancer according to the tumour, regional nodal status and distant metastasis (TNM) classification. [10] Surgery in the form of either breast conservation (BCS) or mastectomy followed by adjuvant therapy constitutes the main stay of treatment for early stage breast cancer. [11, 12] Patients who undergo BCS or mastectomy might also have their sentinel lymph nodes removed under the arm to determine if cancer cells have spread to the lymphatic system. [10] Adjuvant therapy. [10]

BCS has proved to be effective and well accepted by patients diagnosed with breast cancer. However, BCS could be associated with changes in the size and symmetry of the treated breast and this could lead to a reduced patient quality of life. [15] This makes non-surgical techniques without the removal of breast tissue more attractive. Breast conservation is dependent upon clear margins, however, it is not possible to visualize the tumour intra-operatively to aid in determining clear margins. This lack of intra-operative target definition results in higher reexcision rates aimed in an attempt to excise the residual tumour tissue. There is a medical need to develop minimally invasive ablative techniques to further reduce re-operation rates by defining the target and the tumour margins intra-operatively. These techniques have the advantage of not requiring general anaesthesia, a reduced recovery time because the treatment is under local anaesthesia, absence of scarring and consequently possible economic benefits. [17] Non-surgical techniques including HIFU are currently under investigation for local treatment of breast tumours. [18]

Clinical use

Several clinical trials have been executed over the last years using US guided HIFU for the treatment of breast FAD or breast cancer.

Fibroadenoma

More recently, five studies [32-35, 73] have performed or are currently performing US guided HIFU trials on breast FAD. All trials used the Echopulse device (Theraclion, Malakoff, France) which is CE marked and has FDA approval for this application. In general, patients were eligible for participating in the study if they were 18 years or older, with a palpable FAD diagnosed by triple assessment (and a mammogram if the patient is 35 years or older), which must be visible on US and the patient must have given written informed consent. Exclusion criteria were pregnant or lactating women, patient with breast implants, patients with a history of breast cancer, laser or radiation therapy to the ipsilateral breast and patients participating in other trials using drugs or devices.

Kovatcheva *et al.* [32], included 27 patients to demonstrate the efficacy of HIFU in the treatment of breast FAD. Between March 2011 and January 2014, 27 patients were included in a trial to determine the reduction in volume on US after HIFU. The study included patients with a FAD size between 1.0-3.0 cm and excluded patients with breast cysts or micro-calcifications on mammography. Follow-up was performed at one, three, six, nine and 12 months and at the moment the researchers are awaiting long-term follow-up results.

Boulanger *et al.* [33], designed a multicentre, open uncontrolled trial for the observation of histological changes in breast FAD following HIFU. The study included 24 patients with indication for surgical excision between October 2011 and February 2014. The patient will receive HIFU treatment and the need for surgery is evaluated six months post-treatment. Primary outcome measure is the HIFU induced tissue necrosis assessed by histology of excised tissue or reduction of FAD volume. Secondary outcome measures are volume reduction of the FAD on US, pain score during HIFU treatment, evaluated using the visual analog scale, and post-treatment complications. The study only included patients with a FAD of 1.0 cm at its largest dimension and excluded patients with micro-calcifications within the FAD as visible on mammogram. Patients are followed-up at two, four, six and 12 months or until surgery if the patient opts for surgical excision.

In the mono-centre, open-label uncontrolled study by Hahn *et al.* [34], 27 patients are recruited between December 2013 and January 2016 to evaluate the efficacy of HIFU in the treatment of breast FAD. The secondary outcome measure is the tolerability of HIFU. Patients were included if the FAD is 2.5 cm or less in maximum diameter. Patients were excluded if they had interstitial laser therapy or cryo-ablation of a FAD within one year of inclusion. Follow-up was performed for 12 months.

Douek *et al.* [73], performed a feasibility study to assess the treatment of FAD with a circumferential HIFU treatment. Between January 2014 and October 2016, 50 patients will be included. The primary outcome measure is the change in size of FAD volume as recorded on

US imaging. Secondary outcome measures are post-treatment complications, patient recorded outcome measures, mean treatment time and cost-effectiveness of the HIFU treatment compared to surgical excision of FAD. Patients are eligible if the local breast MDM confirms the diagnosis of FAD. FAD with atypia or suspicion of phyllodes (graded B3/C3 or greater) will be excluded from the study. Follow-up is performed at two weeks, three, six and 12 months with physical examination and an US to determine the decrease in FAD volume.

Brenin *et al.* [35], designed a feasibility study to determine the safety and efficacy of the HIFU treatment. 20 patients are aimed to be included between April 2014 and May 2016. Primary outcome measures are the change in volume of the FAD measured by US, size of FAD on physical examination, patient recorded outcomes by measuring the patient rated pain and the patient responses to satisfaction questionnaires. The secondary outcome measure is the incidence of adverse events. Patients are eligible if they have a FAD with a size of 1.0 cm or greater with a volume between 2-10 cc. An additional exclusion criteria are patients with a cyst within the FAD. Patients are followed-up at three, six and 12 months.

Breast cancer

For breast cancer, three trials [19, 21, 44] were conducted between April 1998 and December 2006. All trials used the JC-HIFU therapeutic system (Chongqing HAIFU technology Company, People's Republic of China). In two studies by Wu *et al.*, the therapeutic US beam is produced by a 12.0 cm diameter transducer with a focal length of 9.0 cm and a frequency of 1.6 MHz. In the study by Kim *et al.*, the transducer had a diameter of 15.5 cm, a focal length of 13.5 cm and a therapeutic frequency of 0.8 MHz. Inclusion criteria were patients of 18 years or older with histological proven invasive breast cancer, single palpable tumours with at least 0.5-1.0 cm distance to the skin and chest wall and 2.0 cm from the nipple.

Wu *et al.* [19] included 48 patients in a randomised control trial to investigate the efficacy, safety and feasibility of HIFU ablation. Patient were eligible if the lesion was not greater than 6.0 cm in diameter and boundaries were visible with colour Doppler US imaging. Exclusion criteria were patients with breast implants, patients without stable haemotogenic parameters and patients with a history of active myocardial infarction within the last six months. Patients were randomized to two groups; the control group (n=25) in which modified radical mastectomy was performed, and the HIFU group (n=23), in which HIFU treatment was followed by mastectomy within two weeks. HIFU was performed under IV sedation (n=4) or general anaesthesia (n=19) and a 1.5-2.0 cm of normal tissue was ablated. The target tissue was exposed at acoustic focal peak intensities from 5000-15000 W cm⁻² and a 3.5-5.0 MHz imaging transducer was used. Follow-up was performed previously to surgical excision.

Wu *et al.* [44] included 22 patients between April 1998 and April 2001, in a non-randomized prospective trial to determine patient acceptance, tumour regression, pathological changes, cosmesis, local recurrence and survival post-treatment. Patients were eligible if the lesion was 5.0 cm or smaller, the patient was not suitable or refused modified radical mastectomy or surgical excision, the lesion was visible on US and the patient had a Karnofsky score of 70% or higher. Exclusion criteria were patients with three or more lesions or patients with metastases visible on bone scan or radiograph. Treatment was performed under sedation (n=8) or general

anaesthesia (n=14) and a margin of 1.5-2.0 cm of normal tissue was ablated. The target tissue was exposed at acoustic focal peak intensities from 5000-15000 W cm⁻². Follow-up was performed at two weeks, three, six and 12 months with a physical examination and US guided needle biopsy.

Kim *et al* [21], included six patients with small or intermediate sized (5.0 cm or less) invasive cancer to evaluate MRI features after HIFU ablation between March and December 2006. The lesion boundary need to be visible on US and the patient must not have evidence of distant metastases. In patients with large tumour sizes (5.0 cm or greater) chemotherapy was performed for three cycles to shrink the lesion. The lesions were ablated using a 3.5 MHz diagnostic US transducer and patients were treated under general anaesthesia. Follow-up was performed at two, four and then intervals of 4-6 and 5-8 months with MRI.

Orgera *et al.* [84], published a case report in which US guided HIFU was performed in a nonresectable retroperitoneal lymph node of a women with invasive mixed ductal and lobular carcinoma. The patient underwent quadrantectomy and sentinel lymph node biopsy and received radiotherapy and adjuvant hormonal therapy for two years. A month after the last treatment, US showed a single 3.0 cm hypo-echoic solid mass considered to be a metastatic lymph node which was confirmed by a US guided core biopsy. The patient was not suitable for resection and a month later the lesion had grown by 1.0 cm. The patient was enrolled on a phase I study for HIFU treatment of solid tumours under general anaesthesia using the JC HIFU system with a 0.8 MHz therapeutic probe and a 1.0-8.0 MHz imaging probe. The patient was followed up at five and eight months.

Treatment outcomes

Fibroadenoma

For the treatment of FAD with US guided HIFU two out of five studies have published some results at the Fourth International Focused Ultrasound Symposium. The other trials have not published their results thus far.

Kovatcheva at al. [85], presented the results of 20 symptomatic patients (mean age 29.4 \pm 10.8 years) with 26 FAD treated under conscious sedation in an outpatient setting. A second HIFU session was performed in seven patients due to a reduction of less than 50% or an absolute volume value that exceeded 1.5 ml at six months. This second treatment was performed between month six and nine. At three months the mean volume reduced from 3.00 \pm 2.81 to 1.87 \pm 2.06 ml (p=0.099), 1.36 \pm 1.40ml (p<0.001) at six months and 0.75 \pm 0.66 ml (p<0.001) at 12 months. At 12 months the FAD volume reduction was 73.3 \pm 10.9% (range 47.2-92.6%). Patient which received a second treated had a significantly larger reduction in volume (p<0.05) than patients with one treatment. Subcutaneous oedema or mild skin redness and irritation were observed in seven patients.

Douek et al. [85], presented the results of 13 patients with symptomatic FAD which underwent circumferential HIFU treatment. Seven patients had pre-treatment pain or discomfort due to the FAD. The average treatment time was 36 ± 12 minutes, and circumferential treatment reduced

the treatment time by an average of $44 \pm 21\%$ (p=0.005). Follow-up at two week showed a reduction of pain in five patients with a resolution in two of these. An additional patient developed new pain after two weeks. Short-term complications were erythema in four patients, ecchymosis in four patients, temporarily numbness of the skin in one patient and a first degree skin burn in one patient.

Breast cancer

In the treatment of breast cancer, all three studies have reported the outcomes of the trials.

Wu *et al.*, [19] included 23 patients with a median age 46.5 ± 1.7 years. The tumours were 3.1 ± 0.79 cm (2.0-4.7 cm) in size. The mean treatment time was 78 minutes (range 45-210 minutes). A two-week follow-up was performed to evaluate potential complications of HIFU. Oedema was noticed surrounding the tumour but disappeared within 7-10 days. Fourteen patients experienced mild local pain, warmth and sensation of heaviness in the treated breast and one patient had a minimal skin burn. Macroscopic and histological examination showed complete homogenous coagulative necrosis of the target tissue in all patients, which included a margin of 1.80 ± 0.58 cm. At the margin between treated and untreated tissue there was a rim of congestion present which represented a inflammatory reaction to thermal ablation. Victoria bleu and ponceau's histochemical staining showed that vascular elasticity and collagen fibrin were collapsed and disturbed. MRI showed an absence of enhancement at the periphery. Immunohistological staining post treatment with PCNA, CD44v6 and MMP-9 demonstrated no expression within the tumour cells suggesting a loss of ability to proliferate, invade and metastasize.

Wu *et al.*, published another three articles [83, 86, 87] with results from the same patient population. The first article [86] used immunohistochemical staining, messenger RNA in-situ hybridisation and telomere repeat amplification protocol-enzyme-linked immunosorbent assay techniques to detect tumour expression of proliferation cell nuclear antigen (PCNA), cell adhesion molecule CD44v6, matrix metalloproteinase-9 (MMP-9), erbB2 mRNA and to measure telomerase activity in both groups. In the HIFU group, significant alterations in PCNA, CD44v6, MMP-9 and erbB2 mRNA expression and a decrease in telomerase activity were found. The second article [87] used biotin-streptavidinperoxidase immunohistochemical technology to stain a variety of cellular molecules expressed on breast cancer cells, including tumour antigens and heat-shocking protein 70 (HSP-70). After HIFU ablation, some tumour antigens remained in the tumour debris, which could provide a potential antigen source to stimulate anti-tumour immune response. The third study [83] looked at the therapeutic effects in the treated region by using terminal deoxynucleotidyl transferase-mediated nick end labelling (TUNEL) methods. After HIFU treatment no apoptotic cells were detected in either treated tumour or normal breast tissue.

Wu *et al.*, [44] included 22 patients with a median age of 48.6 years (range 36-68 years). Median tumour size was 3.4 cm (2.0-4.8 cm) and the mean treatment time was 132 minutes (range 60-180 minutes). The median follow-up was 54.8 months. Colour Doppler US imaging was repeated every 3-6 months postoperatively and showed a heterogeneous increase in grey-scale within the treated lesions in 15 patients and an absence of blood flow in 19 patients. On US, the tumour disappeared in eight patients, whilst in 14 patients the tumour decreased in size.

In two women, an increase in tumour size was seen after an initial reduction, due to local recurrence. A decrease in volume of $26.7 \pm 12.2\%$ was measured after six months, $45.2 \pm 22.1\%$ after 12 months and $90.4 \pm 4.9\%$ after 60 months. MRI showed an absence of contrast enhancement in the treated region and a thin peripheral rim of enhancement surrounding the coagulative necrosis indicating an inflammatory reaction to thermal ablation. SPECT results showed a disappearance of the uptake after HIFU treatment. Mild local pain was felt in 14 patients and oedema was noticed immediately after treatment and disappeared within two weeks. Haematoxylin and eosin staining on the CNB at two weeks revealed no viable cells in all patients. Coagulative necrosis of the treated tumour and the margin of normal tissue was seen. At three months partial fibrosis was visible and complete fibrosis was visible at six and 12 months. Two patients. Cosmesis was good to excellent in 16 patients and acceptable in one patient.

Kim *et al* [21], included six patients with a median age of 62.1 years (range 46-68 years). Median tumour size was 2.56 cm (1.2-3.7 cm) and a maximum power of 35W was used. The mean treatment time was 171 minutes (range 80-285 minutes). Complete ablation comprised those patients with no enhancement of the index tumour and thin rim enhancement on subtracted MR imaging. After the first session, three patients had complete ablation (50%) and after a second session performed in two patients, one more patient (17%) had a complete ablation. The other two patients underwent surgery after the first treatment due to oedema and nipple depression. Two patients with complete ablation also underwent surgery and coagulative necrosis was found, fibrosis with foreign body reaction corresponded to the thin rim of enhancement visible on MRI. Injury to the pectoralis major muscle and oedema was found in all patients.

Expert commentary

Several studies have used US guided HIFU in the treatment of breast tumours. However. it is remarkable that the first studies were performed not on patients with benign lesions but on patients with breast cancer. The first study was a treat and resect study, but the following two studies did not excise the treated tissues unless there was a potential of residual or recurrence of breast cancer. However, for both studies, tissue for histopathology was obtained by letting all patients undergo a needle biopsies to determine if there was any residual tumour left.

The first studies which treated breast cancer were performed using the JC-HIFU therapeutic system (Chongqing HAIFU technology Company, People's Republic of China) and the most recent studies, which were all performed on FAD, used the Echopulse device (Theraclion, Malakoff, France). The first device was not used for benign lesions in published trials, but used on breast cancer right away. The second company is aiming on treating breast cancer, but is optimizing the US guided HIFU technique with the FAD trials before starting clinical trials on breast cancer.

After the study by Kim et al, no other studies have been reported using US guided HFU in the treatment of breast cancers. This raises questions, was the treatment not effective enough or is the treatment currently already generally used for the treatment of breast cancer. Currently

there are trials using MR guided HIFU for the treatment of breast cancer, the technique should therefore not be the limitation. The limitation might be the difficulty to visualise breast cancers using US. However, with a strict selection process US guided HIFU trials in the treatment of breast cancer should be possible in the future.

For the trials treating FAD, not much results have been reported thus far. All results previously reported from Kovatcheva and Douek *et al.*, have been published at the Focused Ultrasound Symposium, however these are preliminary results and even though they show promising results, no conclusions can be made from these results. It is expected that within the next year the first results of these trials will be published.

All breast cancer studies, even though they have limited amounts of patients (23, 22 and six), show promising results (23/23, 22/22 and 4/6 complete ablation), however long-term follow-up is needed to determine the recurrence rates. Currently, only one study had a follow-up of five years and showed two recurrences.

The current limitation of HIFU is the treatment time. In the study by Douek *et al.* this drawback is tackled by a circumferential ablation technique. This techniques shows a potential significant reduction in time which would make the treatment more available to patients.

Post-treatment imaging is currently not conclusive enough to determine if the treatment was successful or not. Therefore in the published studies all patients underwent either core biopsies or surgical excision post-treatment. MRI has been used in all studies to determine complete ablation which was recognised by an absence of pre-treatment enhancement and a thin rim of enhancement at the periphery. This thin rim could however also represent residual disease and therefore histopathology was needed to confirm the complete treatment.

Common complications included mild local pain combined with a warmth and heavy sensation at the treatment site, oedema, pectoralis major injury and in a rare case a skin burn. These are common complications for HIFU treatment guided with either US or MRI.

Summary

US guided HIFU studies performed on breast cancer show promising results in terms of complete coagulative necrosis, however for the treatment of breast FAD, no final results have been published and therefore no conclusions can be made. Large prospective clinical trials are needed with reliable follow-up imaging to determine the efficacy of US guided HIFU in the treatment of both FAD and breast cancer.