Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Medicine 2036

Optimizing the magnetic tracer technique for sentinel lymph node detection and tumour localization in breast cancer surgery

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ACTA UNIVERSITATIS
UPSALIENSIS
2024

ISSN 1651-6206
ISBN 978-91-513-2073-1
urn:nbn:se:uu:diva-525195

UPPSALA
UNIVERSITET

Dissertation presented at Uppsala University to be publicly examined in Ingång 21, Västmanlands Sjukhus Västerås, Västerås, Thursday, 16 May 2024 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish. Faculty examiner: Professor Jonas Manjer (Lund Universitet, Institutionen för kliniska vetenskaper i Malmö).

Abstract

Jazrawi, A. 2024. Optimizing the magnetic tracer technique for sentinel lymph node detection and tumour localization in breast cancer surgery. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 2036. 58 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-2073-1.

Breast cancer is the most common form of cancer in women, and the primary treatment modalities are still breast-conserving surgery (BCS) and sentinel lymph node dissection (SLND) in most cases. Superparamagnetic iron oxide nanoparticles (SPIO) are gaining momentum as a tracer for sentinel lymph node detection. The aim of this thesis is to further refine the magnetic method and investigate its postoperative effects.

Paper I: This feasibility study, involving 79 patients, explored the use of SPIO-guided Magnetic resonance imaging (MRI)-lymphography and magnetic-guided axillary ultrasound (MagUS) with core biopsy for sentinel lymph node (SLN) localization and SLN status. MagUS, outperformed baseline axillary ultrasound and successfully traced SLNs in all cases, detecting macro-metastases accurately and missed only one micro-metastasis. The findings suggest that the MagUS technique enables minimally invasive approach in axillary mapping that can meet tailored patient needs and reduce the need for diagnostic surgery.

Paper II: This study aimed to compare skin staining incidence and size between different doses of SPIO and blue dye (BD), evaluating their persistence over time. Among 270 women receiving SPIO, 204 also received BD. At six months, 21.5% had SPIO stains and 25% had BD stains Incidence and size decreased reciprocally, with no significant difference between the tracers regarding skin staining after 24 months.

Paper III: This study compared the magnetic technique using Magseed® for non-palpable breast tumor localization with guidewire localization and SPIO for sentinel lymph node detection. In a prospective analysis of 426 women, reoperation rates, resection ratios, and SLN detection were assessed. No significant differences were found between the techniques in terms of re-excisions, resection ratios, or SLN detection. However, the magnetic technique showed more successful localizations, shorter operation time, and better overall experience among surgeons, radiologists, and theater coordinators, making it a good alternative for BCS.

Paper IV: In this prospective observational study, the impact of postoperative MRI outcome was explored in patients undergoing BCS with a peritumoral SPIO injection for SLN detection. The study affirms SPIO as a safe tracer for SLN detection without compromising MRI interpretation after BCS, ensuring reliable breast cancer recurrence assessment.

Keywords: Breast Cancer, Sentinel Node, Super paramagnetic ironoxide nanoparticles, SPIO, Skin Staining, Magnetic resonance imaging, magnetic seed, guidewire

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ISSN 1651-6206
ISBN 978-91-513-2073-1
URN urn:nbn:se:uu:diva-525195 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-525195)

To Alexis, my lovely daughter, Baba loves you more than life. To my beautiful wife, thank you for always believing in me even when I doubted myself. To my dear mother and father, thank you for guiding me in life.

'*The only limit to our realization of tomorrow will be our doubts of today.*' - *Franklin D. Roosevelt*

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Jazrawi A, Pantiora E, Abdsaleh S, Bacovia DV, Eriksson S, Leonhardt, Wärnberg F, Karakatsanis A. Magnetic-guided axillary ultrasound (MagUS) sentinel lymph node biopsy and mapping in patients with early breast cancer. A phase 2, single-arm prospective clinical trial. *Cancers (Basel)*. 2021;13(17):4285. https://doi.org/10.3390/cancers13174285

II. Jazrawi A, Wärnberg M, Hersi A.-F, Obondo C, Pistioli L, Eriksson S, Karakatsanis A, Wärnberg F. A Comparison of skin staining after sentinel lymph node biopsy in women undergoing breast cancer surgery using blue dye and superparamagnetic iron oxide nanoparticle (SPIO) tracers. *Cancers (Basel)*. 2022;14(23):6017. https://doi.org/10.3390/cancers14236017

III. Pantiora E*, Jazrawi A*, Hersi A-F, Abdsaleh S, Ahlstedt H, Molnar E, Wärnberg F, Eriksson S, Karakatsanis A. Magnetic seed *vs* guidewire breast cancer localization with magnetic lymph node detection: A randomized clinical trial. *JAMA Surg*. 2024; 159(3):239–246. https://doi.org/10.1001/jamasurg.2023.6520.

IV. Jazrawi A, Pantiora E, Abdsaleh S, Wärnberg F, NG Lian C, Zouzos A, Gagliardi T, Karakatsanis A, Eriksson S. Prospective evaluation of imaging artefacts in patients undergoing breast conserving surgery and sentinel lymph node dissection with the magnetic technique. Manuscript.

*Shared first authorship

Contents

Abbreviations

ALND	Axillary lymph node dissection
ARV	Actual resection volume
AUS	Axillary ultrasound
BD	Blue dye
BCS	Breast-conserving surgery
BMI	Body mass index
CIS	Carcinoma in situ
CNB	Core needle biopsy
CT	Computed tomography
DCIS	Ductal carcinoma in situ
FISH	Fluorescence in situ hybridization
HER2	Human epidermal growth factor subtype 2
IBC	Invasive breast cancer
IHC	Immunohistochemical/immunohistochemistry
IQR	Interquartile range
LCIS	Lobular carcinoma in situ
LN	Lymph node
MagUS	Magnetic-guided axillary ultrasound
MRI	Magnetic resonance imaging
MRI-LG	Magnetic resonance imaging lymphography
NAT	Neoadjuvant treatment
OR	Odds ratio
ORV	Optimal resection volume
RI	Radioactive isotope
SLN	Sentinel lymph node
SLND	Sentinel lymph node dissection
SPIO	Superparamagnetic iron oxide nanoparticles
Tc99	Technetium-99 (medical radioisotope)
TNM	Tumour, Node, Metastasis (cancer-staging system)
WHO	World Health Organization

Introduction

Breast cancer is the most common form of cancer among women globally, with 9491 newly diagnosed cases in Sweden in 2022. There is still an increasing trend for newly diagnosed breast cancer both in Sweden and internationally, especially in the developed world (1), which is thought to be caused primarily by improved living conditions.

The first descriptions of breast cancer date back to ancient Egypt, but it is unclear how this form of cancer was treated at that time. The first surgical technique was described at least as early as 548 AD, when a mastectomy was performed during the Byzantine Empire (2–4). Since then, treatment options have progressed with the advent of numerous factors that facilitate surgery, such as anaesthesia and antiseptic drugs.

The primary treatment for breast cancer is currently surgery, which developed from the radical mastectomy described by Halsted and Meyer, involving the removal of the entire breast gland, the pectoral muscle and 30–40 axillary lymph nodes (LNs). Today, surgical treatment for breast cancer mainly comprises breast-conserving surgery (BCS) (5), where the relationship between the tumour and breast size should be such that locally radical surgery can be performed with good cosmetic results. The primary surgical treatment today in Sweden is a combination of BCS and adjuvant radiation therapy, which provides oncological results equal to those of mastectomies (6–8). The introduction of mammography screening also allows for earlier tumour detection, making BCS quite likely. Axillary staging is currently mostly performed using sentinel lymph node dissection (SLND), which is mainly conducted using radioactive isotope (RI) tracers in combination with blue dye (BD), which is considered to be the ‘gold standard’ method for SLND (9, 10). The axilla is the initial site of metastases in most patients with breast cancer and is considered one of the most important prognostic factors, as it determines the need for radiotherapy and neoadjuvant treatments (NATs).

Following the success of mammography screening, tumours are now more often detected at an earlier stage and are thus usually smaller and more often not palpable at the time of detection. This creates difficulties during surgery, as the surgeon cannot feel and palpate the tumour during the procedure, which

makes it more difficult to predict resection margins (11). Therefore, several studies have been conducted in this field to develop methods for localizing non-palpable tumours (12).

Background

Breast cancer ranks as the predominant type of cancer that affects women in both industrialized and developing nations. In recent decades, there has been a consistently global increase in the occurrence of breast cancer. Interestingly, this increase was accompanied by a concurrent decrease in mortality rates (13). This increasing incidence globally is thought to be related to the prevalence of risk factors for breast cancer, such as high body mass index (BMI), increasing population age, physical inactivity, early menarche and late menopause, addition of hormone therapy, fewer pregnancies and reduced breastfeeding. The introduction of screening modalities for breast cancer has also contributed to an increased incidence of diagnosed cases; however, breast cancer screening is more common in industrialized countries (14, 15).

Mortality from breast cancer is decreasing, with a 5-year survival rate of 83%, while the 10-year survival rate is 71% in Sweden (16). These results are primarily because of the introduction of new oncological therapies and additional treatments. Developments in oncological treatments have made great progress in recent decades from essentially only including selective oestrogen receptor modulators and basal cytostatic drugs. A variety of different treatment therapies are now currently available, such as aromatase inhibitors and monoclonal antibodies. In addition, radiotherapy has also been developed to target tumours with greater precision and dosage volumes. These developments in this field have decreased the morbidity that usually follows NATs. Despite the development of these adjuvant treatments, surgery is still the main method for treating and staging breast cancer.

The Halsted radical mastectomy had a profound impact on patients in terms of both its functionality and postoperative complications. The primary technique today is BCS, which is central for breast surgery. This technique was made possible following treatment combinations, including BCS and postoperative radiotherapy of the breast, which have been shown to have comparable results in terms of recurrence rates compared with mastectomy alone (6–8, 17).

Alongside the development of breast surgery, axilla staging developed during the same time frame. Prior to the development of BCS, a complete axillary

lymph node dissection (ALND) was performed routinely, where approximately 10–20 LNs were removed in levels I and II of the axilla during axillary clearance. However, this technique has been shown to be related to a high degree of morbidity with an increased risk of lymphedema (18). Therefore, there was a need to identify another method for properly staging the axilla with less morbidity. During the 1990s, SLND was introduced and validated. The underlying concept for the use of SLND was largely based on the anatomy of lymph drainage from the breast (9, 10, 19). When breast cancer starts to spread, tumour cells will be found in the sentinel lymph node (SLN) because the lymph primarily drains to these nodes. If there are metastases, the SLN will contain tumour cells. If the SLN is healthy and there are no tumour cells, the remaining axilla are considered as being healthy. In this way, SLND becomes both a diagnostic and staging procedure, and it is possible to significantly reduce the postoperative complications that the earlier axillary surgery with ALND entailed, such as lymphedema, chronic pain and wound infections (20, 21). SLND is currently the gold standard method for staging the axilla.

Today, the 'dual technique' is the gold standard for identification and localization of SLNs. The technique is based on combining the RI technetium-99 (Tc99) in combination with BD. This technique is well proven and has been validated with a detection of ≥90% (18, 19, 22). However, the method has some disadvantages, which limit its use. The use of BD has a small risk of producing anaphylactic reactions and is therefore preferred to be given after the induction of anaesthesia when the patient's airway is secured. In addition, BD can cause a skin discoloration that takes a long time to disappear in some patients (23). The largest problem with the dual technique is the strict regulation regarding the handling of the RI and its short half-life of 6 hours. Among other things, the use of RIs requires access to nuclear medical facilities. The radiation itself also entails a certain risk for health-care personnel, while the short half-life causes logistical problems regarding surgery planning. All these factors limit the use of this method, especially in developing countries.

Breast carcinoma in situ

The in situ and invasive phases are two phases in the development of breast cancer. During the in situ phase, the carcinoma is still restricted and has not penetrated the milk duct epithelia. In the invasive phase, however, the cancer has broken through the membrane and infiltrated the breast tissue; therefore, it has the potential to produce metastases.

Traditionally, in situ breast cancer was categorized into ductal breast carcinoma in situ (DCIS) or lobular breast carcinoma in situ (LCIS). DCIS accounts for approximately 10% of all diagnosed breast cancer cases in Sweden (24). DCIS is a precursor to invasive cancer; if left untreated, there is a high risk of transformation to invasive cancer. DCIS is associated with invasive ductal carcinoma, while LCIS is largely associated with invasive lobular carcinoma. Unlike DCIS, the status of LCIS has been re-evaluated and is now seen more as a risk factor for developing breast cancer than the earlier idea that it was an actual precursor. The annual risk to develop breast cancer from LCIS is estimated to be around 2% (25). However, the most common in situ form is DCIS, which has an increased risk of 20%–50% of developing an invasive component if left untreated within a period of 10 years (26). Based on these facts, DCIS is therefore usually treated as a small node-negative breast cancer; however, there are no clear guidelines regarding axilla management for these patients (27, 28).

DCIS is currently divided into three grades based on histopathology. The factors evaluated during DCIS are the number of mitoses, pleomorphism, chromatin and nucleoli appearance. The tumour grading goes from I–III, where I means that the cell most resembles a healthy cell, while grade III shows a low degree of differentiation and thus deviates the most from a normal cell. Regarding DCIS, axillary staging with SLND is advised in mastectomy cases and when there is suspicion of invasiveness based on imaging, clinical examination or biopsy results. In situations involving high-grade DCIS, extensive tumour spread and palpable tumours, a significant number of cases are upgraded to invasive cancer in the final postoperative pathology report. However, due to the risk of arm morbidity persisting even after a SLN biopsy, it is primarily recommended to consider reoperation with a SLN biopsy only after pathology results confirm invasiveness (29).

Histopathological classification and intrinsic biological subtypes

Invasive breast cancer can be classified according to the type of tumour cells based on the World Health Organization (WHO) classification, according to the degree of differentiation (i.e. Elston–Ellis classification) and according to the tumour biology (i.e. endocrine receptors, oncogenes and cell proliferation).

Tumour differentiation (Elston–Ellis classification)

The Elston–Ellis classification system assesses tumour cell morphology microscopically and compares it with that of normal 'healthy' cells to provide what is generally known as the 'grade of differentiation'. The concept of differentiation is common in cancer biology and refers essentially to the question: 'How much or how little do the cancer cells resemble cells from the healthy tissue they originate from?' The Nottingham (i.e. Elston–Ellis) classification is a modification of the previous Bloom–Richardson grading system (34, 35) and assesses three variables: nuclear morphology, tubule formation and mitotic rate, where each variable is scored individually on a scale of 1 to 3 (i.e. 1 = the best and 3 = the worst). These scores are then combined into a cumulative score that correlates with the grade of differentiation, which is then assigned a grade: Grade I (score, 3–5), Grade II (score, 6–7) and Grade III (score, 8–9).

Tumour biology

The further classification of breast tumours and decisions about further therapeutic treatments is very much based on immunohistochemical (IHC) techniques, which are used to identify the endocrine properties that a tumour expresses. The main two hormone receptors being tested for are those for oestrogen and progesterone. Internationally, there is a cut-off limit of 1% to confirm a tumour as being hormone receptor-positive, which means that at least 1% of the tumour cells express these receptors. When defining the tumour as 'hormone receptor-positive' in Sweden, the limit is ≥10% (29).

Another receptor protein that has a role in breast cancer tumour biology is the human epidermal growth factor receptor subtype 2 (HER2), a tyrosine kinase receptor. The cancer cell expresses this receptor on the cell surface and those cells with HER2 overexpression are associated with a more aggressive course and have a poorer prognosis. IHC techniques are used to confirm HER2 status and give a score between 0–3+, where 0–1+ is considered as HER2 negative, 2+ as borderline and 3+ as HER2 positive. If the IHC is considered borderline, a fluorescence in situ hybridization (FISH) or silver in situ hybridization test might be applied to determine whether the tumour cells should be graded as HER2 positive. HER2-positive cancers are currently treated with adjuvant therapy in the form of monoclonal antibodies.

Proliferating cancer cells express an antigen called the KI-67 protein. Anti-KI-67 is a monoclonal antibody directed against this protein, which is expressed as a percentage and used as a marker for assessing tumour proliferation rates.

Intrinsic biological subtypes

Tumours in patients with breast cancer show a great deal of biological heterogeneity, which has in turn led to a need for a classification that accounts for all the different biological factors that influence the choice of treatment and prognosis. The ground-breaking research by Perou et al. defined different profiles based on gene expression and the Cancer Genome Atlas Network further refined research based on their work, which has led to an updated definition of the different subtypes (31, 32). Hence, physicians can now refine and specify the appropriate oncological treatment based on the different subtypes as follows:

- Luminal A – Oestrogen and progesterone receptors positive, HER2-negative and low to intermediate Ki-67.
- Luminal B – Oestrogen and progesterone receptors positive and high Ki-67. Can be HER2-negative or -positive.
- HER2-positive – Oestrogen and progesterone receptors negative and HER2-positive. The prognosis has become better since the introduction of treatment with monoclonal antibodies targeting the HER2 receptor.
- Basal-like or triple-negative – Oestrogen and progesterone receptors negative, HER2-negative, and high expression of Ki-67. This form of breast cancer has the worst prognosis.

Staging

Breast cancer is staged according to the tumour, node, metastasis (TNM) staging system developed by the Union of International Cancer Control. This system addresses three different factors: T relates to the size of the tumour and its relation to the surrounding tissue, N corresponds to the prevalence of regional LN metastases, and M corresponds to distant metastases beyond the regional LN. The three factors in the TNM staging system are as follows (where clinical stages are given the prefix c):

- T – Tumour size. T1 (≤20 mm), T2 (21–50 mm), T3 (>50 mm) and T4 (invasion of surrounding tissue).
- N – Nodal status, which assesses the spread to local and regional LNs (pN0, no metastases; pN1, 1–3 LN metastases; pN2, 4–9 LN metastases; pN3, > 9 LN metastases or spread to the LN at the sternum or the clavicle).
- M – Absence or presence of distant metastasis, staged as M0 or M1, respectively. If this is not investigated, the case is staged as Mx.

There are two important aspects to breast cancer staging. The first is based on tumour biology, such as the intrinsic subtypes mentioned previously. The 8th edition of the American Joint Committee on Cancer TNM classification includes information from IHC tests and the genomic signature, and is the first to integrate the intrinsic biological subtypes with the TNM classification to classify the tumour and thus modify treatment recommendations underlining the important role of intrinsic subtypes and tumour biology. The second important aspect in the staging of breast cancer is the LN status, as breast cancer mainly spreads via the lymphatic system. While hematogenous spread can occur, the cancer in such cases primarily metastasizes to the liver, lung, brain and skeleton.

Sentinel lymph node dissection

Considering the axilla in patients with breast cancer, LN status has clinical significance. However, the handling of the axilla has changed from a routinely complete ALND evacuation where it was subsequently seen that a large proportion of the excised glands were healthy, while the accompanying morbidity for the procedure was significantly high (19).

Breast cancer is well known to spread primarily through the lymphatic system to the ipsilateral axilla in the first place. In addition, routine interventions in the axilla for the screening of metastases have a high degree of uncertainty (33, 34). Therefore, there has been a dual surgical approach regarding breast cancer, where a radical resection is sought while a diagnostic procedure is performed on the axilla. As mentioned above, ALND was the classical method in which the axilla was staged; however, this procedure resulted in a high morbidity with a consequence of postoperative complications, such as seromas, hematomas, infections, ipsilateral impaired arm mobility, impaired sensation and ipsilateral lymphedema (35).

The SLN is the first LN where the lymph from a breast cancer drains towards, which can consist of one or several LNs. When the cancer starts to spread, the malignant cells are first found in these nodes. SLND was introduced and validated during the 1990s (36, 37). It has since established itself as the gold standard method when it comes to staging the nodal status in the axilla. This method has significantly improved surgery in terms of staging the axilla and thus improved the best possible choice of treatment postoperatively. SLND has been shown to be as effective as ALND, but with a significantly lower morbidity (20–22).

SLNs are difficult to identify during surgery. Because of their size and location in the adipose tissue, the dual technique has long been considered the ‘gold standard’ for mapping and identification of SLNs. The dual technique uses the combination of RI Tc99 together with BD for visual aid. The isotope is given a few hours preoperatively, while BD is given perioperatively when the airways are secured, as it can produce anaphylactic reactions in a few patients. The isotope reaches the SLN via the lymphatic system and the surgeon uses a handheld gamma probe to locate the SLN and plan the incision in the axilla. The dual technique has a SLN detection of about 90%–99% (19, 38, 39).

However, there are some disadvantages with the dual technique. First, the handling of the RI requires nuclear medical facilities and special rules. The short half-life of the isotope (6 h) causes problems with the logistics and planning of operations, as patients usually need to present the same day or the day before to get their injection of the isotope. Against this background, access to isotopes is limited in developing countries. The BD is also an allergen and can produce an allergic reaction in 0.1%–1% of cases. In addition, it usually leaves skin discoloration, especially at the injection site (23).

Superparamagnetic iron oxide nanoparticles

For several decades, superparamagnetic iron oxide nanoparticles (SPIO) have been used as a contrast agent in magnetic resonance imaging (MRI) examinations. The first time the substance was used within SLND was in the 2010s (40). SPIO is currently sold under the commercial name Magtrace® and was formerly called Sienna® or Sienna XP®. This is a sterile water suspension of SPIO coated with carboxydextran molecules. These carboxydextran molecules emit the superparamagnetic effect in contact with the magnetic fields from the handheld SentiMag device. The size of the particles together with the coating is 60 nm, which enables them to pass in the lymphatic system to LNs where it is filtered, making it ideal for SLN detection. In addition, the SPIO solution is dark brown and usually stains the LN, which can provide visual assistance during surgery.

In recent years, scholars have shown that the magnetic technique is non-inferior towards the dual technique for SLN detection (41). In the Central-European SentiMag study, SPIO was injected 20 min before the start of SLND, but in the Nordic SentiMag study, SPIO was injected up to 20 min preoperatively and similar results were reported (42, 43). Furthermore, SPIO can be given several weeks preoperatively based on the longer half-life of the substance and it has been possible to give it up to 30 days before surgery (44). Another advantage of SPIO involves substance handling because it is not radioactive, which in turn means that there is no need for nuclear medical facilities. In theory, the management and administration of SPIO could be performed by all health-care professionals. In addition, SPIO also colours the LNs dark grey/brown.

However, there are some disadvantages with SPIO. The main argument against its use is primarily the risk of potential artefacts on postoperative MRI examinations. However, this can be largely avoided, as recent studies show that by reducing the volume of SPIO injected and the use of different injection techniques from a more superficial retro-areolar injection to a deeper peri-tumoural injection, the bulk of SPIO can be removed after BCS and thereby reduces the risk of artefacts on MRI (45, 46). Similarly to BD, another disadvantage of SPIO is that it can cause skin discoloration (47).

Non-palpable breast tumours

In Sweden, about 50% of all tumours are diagnosed through mammography screening (16), which has led to earlier detection leading to lower mortality and morbidity as well as a better prognosis. All women between the ages of 40 and 74 years are offered mammography biennially. The earlier the tumours are detected, the smaller they are. Therefore, the challenge that the surgeon faces is that more of these tumours are not palpable. From a global perspective, non-palpable tumours represent about 30%–50% of all cases (11), which has led to an increased need for a safe and effective method for locating and identifying non-palpable tumours with adequate surgical margins. The gold standard method for locating and identifying non-palpable breast tumours is the use of wire-guided localization, which is well-used globally (48). The wire is used as guidance to the tumour for the surgeon during surgery and by the radiologist after the specimen is excised and sent for X-ray investigations to assess radiological radicality. Although it is a well-proven and well-used method, it has a few disadvantages. The main problem with the use of a wire is in the logistics, as the patient must receive the steel wire implant on the day of surgery or the day before. In addition, the patient experiences much discomfort and the surgeon risks injury during surgery, as well as the risk of dislocation during and after surgery. There are alternatives to wire-guided localization, but the following techniques are not as well established:

- Coal suspension
- Intra-operative ultrasound-guided lumpectomy
- Cryo-assisted techniques
- Magnetic seed localization (Magseed®)
- Radio-guided occult lesion localization
- Radioactive iodine seed localization

General and specific aims

The overall rationale for this thesis was to find and develop feasible methods using the magnetic approach in breast cancer surgery. Our research group has previously been instrumental in showing the non-inferiority of SPIO against the 'gold standard' dual technique regarding SLND. The next step is to find and develop practical applications for SPIO. Because this is an evolving new method for SLND, the procedure must be refined further, such as dose optimization, evaluation of the injection techniques and further investigation into the effects postoperatively.

The specific aims of the thesis were as follows:

Paper I

The aim of this feasibility study was to determine whether a preoperative workup with SPIO-guided MRI-lymphography (MRI-LG) and magnetic-guided axillary ultrasound (MagUS) and core biopsy of the SLN can accurately localize SLNs and predict SLN status, and whether such a technique has the potential of replacing SLND surgery in the future.

Paper II

The purpose of this study was to compare different doses of SPIO and BD regarding the incidence and size of skin staining, and how long staining remained in the skin.

Paper III

A randomized trial aimed to compare the combined magnetic technique with Magseed® for the localization of a non-palpable breast tumour, with guidewire localization in combination with SPIO for SLN detection. The aim was to compare and evaluate the reoperation rate due to positive oncologic margins and the resection ratio between the two techniques in a prospective study.

Paper IV

The aim of this prospective observational study was to explore the outcomes of postoperative MRI artefacts in patients that underwent BCS and SLND following a peri-tumoural SPIO injection.

Materials and methods

Paper I

Candidates for this study were enrolled at Uppsala University Hospital. All adult women with clinical and ultrasound node-negative early breast cancer (clinical stage, cN0) planned for SLND, from September 2017 to December 2020 were included. SPIO (Magtrace® 2 mL was injected peri-tumourally in all patients up to 14 days before MRI-LG for SLN mapping. Axillary ultrasonography was performed after MRI-LG, towards the area where the SLNs were identified on the MRI scans. A handheld magnetometer (SentiMag®; Endomag, Cambridge, UK) was used to identify the 'pre-incision hotspot', which is the area with the highest magnetic uptake on the skin. Subsequently, core needle biopsy (CNB) of the identified SLNs was performed. The CNBs were then examined for magnetic SPIO uptake with the SentiMag probe and for the presence of brown staining. Macro- and microscopic control images were then obtained for the retrieved SLNs after the SLND to identify any signs of previous biopsy. Standard histopathology of the SLN specimen served as a reference for the microscopic examination of the CNB.

Paper II

Candidates for this study were all women with primary breast cancer cT0–2cN0cM0, and Eastern Cooperative Oncology Group performance status 0–2, undergoing BCS and SLND. Patients were recruited from the SentiDose trial (2017–2019), which was conducted at six Swedish hospitals (49). Both SPIO and Tc99 were used in all women while BD was used in most patients, according to local routines. The SentiDose trial was a dose optimazing trial comparing SLN detection using 1.5 or 1.0 mL of SiennaXP®/Magtrace®, respectively, injected at different time points using different injecting techniques. The SPIO was injected in two ways. Either a 1.5 mL retro-areolar injection of Magtrace® was used at least 20 min before surgery on the same day, followed by a 5-min massage. Otherwise, a 1.0 mL Magtrace® peri-tumoural or retro-areolar injection was given 1–7 days before surgery. Massage was optional. As a backup, all women were also injected with Tc99 with or without BD according to local clinical routines. For BD, a 1.0 mL sub-intradermal, retro-areolar injection was used. The incidence of skin staining and the size in

square centimetres (cm^2) were self-reported by the patients at telephone interviews conducted at 6, 12 and 24 months after surgery. If there was no staining, or if the staining was gone, no further follow-up was made.

Paper III

Enrolment to this prospective randomized study took place between May 2018 and May 2022 at three hospitals in Sweden (Akademiska University Hospital, Uppsala; Västmanlands Hospital, Västerås and Sahlgrenska University Hospital, Gothenburg). Inclusion criteria were non-palpable DCIS or invasive breast cancer (T1–3) planned for BCS and SLND. Patients with small diffusely palpable tumours requiring preoperative localization or multicentric/multifocal tumour amenable to breast conservation were also included. Participants were randomized to a localization method, a magnetic seed or guidewire at the first visit in the outpatient clinic in blocks of 8 with an allocation ratio of 1:1. Patients randomised to magnetic seed localization received the seed guided by ultrasound or mammography, 1–30 days preoperatively at the same time as SPIO (Magtrace®) was administered by the radiologist. The magnetic seed was placed ventrally to the tumour and 1–1.5 mL SPIO was injected dorsal to the tumour. If randomized to guidewire, the patients received the SPIO 1–30 days preoperatively and the guidewire was inserted on the same day or the day before surgery. Blue dye was used at the surgeon's discretion.

Routine specimen radiography was performed to confirm radiological radicality and then SLND was performed with the SentiMag probe following a 10% of the maximum signal cut-off to complete the procedure.

Paper IV

From 2017 to 2022, patients aged >18 years with DCIS or T1–3 invasive breast cancer planned for BCS and SLND were included in this study. Patients planned for BCS and SLND received 1, 1.5 or 2 mL SPIO up to 4 weeks before surgery from the radiologist or surgeon. On the day of surgery, the transcutaneous magnetic signal as well as the presence of any skin stain was registered. Moreover, the presence of brown breast tissue and the residual cavity signal were documented. Transcutaneous signal and discoloration were also documented during the postoperative visit in the outpatient clinic, as well as in clinical follow-up after MRI and mammograms had been performed. A baseline breast MRI and mammogram were performed after 3–6 months postoperatively. Patients without artefacts, as assessed by the principal study breast radiologist, and postoperative transcutaneous signal were not followed up any

further. If there was an artefact, then follow-up was prolonged up to a maximum of 5 years with annual imaging using breast MRI and mammograms. Consequently, the presence of skin staining and magnetic signals were correlated to the images to see if there were factors that could predict the presence of artefacts on the MRI.

Apart from the principal study breast radiologist, the imaging (i.e. MRI–mammogram pairs) was assessed by three external dedicated breast radiologists, who all had extensive experience with MRI from large-volume institutes. To ensure objectivity, the review was performed independently and blinded to any patient or procedure-related data.

Statistical analysis

Paper I

The primary endpoint was determination of the MagUS SLN detection rate, defined as successful SLN detection of at least one SLN of those retrieved in the following SLND. In the calculation of sample size, the MagUS trial was conceived as a single stage phase 2 trial following the A'Hern design (50). For a one-sided test, a type one error ($\alpha = 0.025$ and 80% power), a sample size of ≥75 patients was required between a maximum proportion of 95% (corresponding to the proportion of successful SLN detection above which the method can be considered further) and a minimum efficacy of proportion of 85% (corresponding to the proportion of successful SLN detection under which the method should not warrant further investigation).

Paper II

Skin staining was analysed in women who had received BCS. Descriptive statistics were performed by means (95% confidence interval) or medians (range) for continuous variables. Depending on data distribution, the statistical analyses were based on median values. Continuous data were analysed using non-parametric tests. Dichotomous data were analysed with Pearson Chi-square for non-paired observations and McNemar's test for paired observations. Spearman's rho test was used to measure the correlation between predictive factors for skin staining.

Paper III

The available literature suggests that the resection ratio for guidewire-based excision ranges between 1.9–2.8 (51, 52). The resection ratio is defined as the actual resection volume (ARV)/optimal resection volume (ORV), where the latter is the assessed volume needed to excise the lesion with 1-cm margins. The ARV was derived from fresh specimen weight with concomitant volume calculation while the ORV was calculated based on preoperative radiology. The MagTotal pilot study suggested that the MagTotal technique had a resection ratio of 1.5 (44), whereas, in a non-randomized comparison of guidewires

and magnetic seeds with isotope-based SLND, Zacharioudakis et al. found comparable ratios (1.92 *vs* 1.67) with comparable re-excision rates (14% vs. 16%). In the absence of established reference values, we assumed a 2-sided equivalence of a 0.3 difference in resection ratio as clinically meaningful (corresponding to excision of excess volume of 30%), with a two-sided *P*-value set at 0.05 and power of 80%, corresponding to 191 patients per arm. This population also satisfied the hypothesis of non-inferiority in re-excision rates for a standard of 4% by a 5% margin. Despite that the primary outcomes did not require follow-up, an additional 10% was included for each arm, leading to a total sample size of 430.

Continuous variables were summarized as means with standard deviation or medians with interquartile range (IQR), depending on data distribution. Comparisons were performed with Student's *t*-test for means and the Mann–Whitney *U* test or the Kruskal–Wallis test for medians. Likert items were analysed as ordinal data (median, IQR) and compared with non-parametric tests, as appropriate. Categorical variables were summarized as numbers and proportions (%) with 95% CIs and comparisons were performed with Fisher's exact test for unpaired data (Wald test for differences) and McNemar's test for paired data. Multivariable regression analysis was performed if significant univariate associations between clinically relevant variables were demonstrated. Analyses were performed according to intention to treat and protocols for the primary and secondary endpoints. Effect sizes (odds ratios [ORs] for logistic regression and β coefficients for linear regression) were reported with 95% CIs.

Paper IV

As the ferromagnetic signal is present in all patients with skin staining, it should be expected that absence of discoloration or magnetic signal in the resection margins should imply SPIO-free parenchyma. Therefore, all pairs of observations (post-excisional intra-operative background count and postoperative MRI) should be concordant. To test for this hypothesis, with an anticipated discordance rate (α) of 0.05 and a tolerance probability (β) of 95%, a minimum sample size of 93 patients would be required (53, 54).

Continuous variables were summarized as medians with IQR. Categorical variables were summarized as numbers and proportions (%) with 95% CIs and comparisons were performed with the Wald test. Likert items were analysed as ordinal data (median, IQR) and compared with non-parametric tests, as appropriate. Agreement statistics were performed using the Konger κ for multiple raters with 95% CIs or Krippendorf's α for the Likert items and the intraclass coefficient. Individual rater outcomes were pooled in a panel and items

on the presence of artefacts were dichotomized ('yes' *vs* 'no' and 'unsure') for further analyses to avoid arbitrary weighting that would result in non-clinically relevant groupings. Weighted outcomes summarizing panel ratings were summarized as medians (IQR) and mean ranks. Primary analyses were performed for each imaging set (i.e. MRI and mammogram), whereas per-patient analyses were performed for patient-specific outcomes. Univariable and, if required, multivariable analyses were performed to investigate for associations with the SPIO injection volume, technique (i.e. free-hand *vs* image-guided), type of surgery and time from surgery to imaging to the questionnaire results. All tests were two-sided and a *P*-value of 0.05 was considered significant.

Ethical considerations

The studies were all approved by the Uppsala University regional ethics committee and performed according to the 1975 Declaration of Helsinki and the Swedish Act on Patient Insurance. The studies were sponsored by Uppsala University and Uppsala University Hospital, and supported by institutional grants from Uppsala University, Västmanlands Cancer Foundation, Swedish Breast Cancer Association and the Centre for Clinical Research Region Västmanland. Magseed® and Magtrace® were provided by Endomag (Cambridge, UK).

Summary of results

Paper I

In 79 patients, 48 underwent upfront surgery, 12 received neoadjuvant chemotherapy and 19 underwent surgery for recurrent cancers. MagUS traced the SLNs in all upfront and neoadjuvant cases, detecting all patients with macrometastases (n = 10), and missed only one micro-metastasis, outperforming baseline axillary ultrasound (AUS) (area under the curve, 0.950 *vs* 0.508; $P < 0.001$) and showed no discordance to SLND ($P = 1.000$).

Paper II

A total of 270 women received SPIO. Of these women, 204 also received BD. A total of 58 (21.5%) women had a SPIO stain 6 months postoperatively with a median size of 6.8 cm^2 ($P = 0.56$), while 51 (25%) had a BD stain with a median size of 8.5 cm^2 ($P = 0.93$). The incidence and size of SPIO and BD staining decreased over time reciprocally. At 24 months, for patients with an initial stain, the incidence and median size of SPIO was 23 (8.6%) and 4 cm^2, respectively. For BD, the incidence was 14 (6.3%, $P = 0.13$), and the median size was 3.5 cm^2 ($P = 0.18$). Therefore, there was no statistically significant difference in the incidence or size of skin staining between SPIO and BD over time.

Paper III

A total of 426 women were analysed and randomly assigned to two well-balanced arms with 213 women in each arm. The totally magnetic arm included 215 women, whereas the guidewire arm included 208 women in the per-protocol analysis. The overall re-excision rate was 2.90% (95% CI: 1.60–4.80) and the resection ratio was (median, IQR) 1.96 (1.15–3.44). No differences were found between the guidewire and the seed in re-excisions (2.84% *vs* 2.87%; difference, −0.03%; 95% CI: −3.20–3.20; $P = 0.99$) or resection ratio, 1.93 (1.18–3.43) *vs* 2.01 (1.11–3.47; $P = 0.70$). Overall SLN detection was 98.6% (95% CI: 97.1%–99.4%) with no differences between arms (98.1% *vs*

99.0%; difference, −0.9%; 95% CI: −3.6–1.8, $P = 0.72$). More failed localizations occurred with the guidewire (10.1% *vs* 1.9%; difference, 8.2%; 95% CI: 3.3–13.2; $P < 0.001$). The surgeons, radiologists and theatre co-ordinators had better experience with the seed.

Paper IV

The analysis encompassed 97 patients and a total of 159 MRI examinations were performed. The study showed a discordance among raters for 'any artefact' (range, 24.1%–74.4%; weighted average, 32.4%) and 'SPIO-specific artefact' (range, 12.0%–49.4%; weighted average, 20.9%). The median area of 'any artefact' was 9.24 mm^2 (IQR, 4.72–15.50) and SPIO-specific artefact 9.88 (IQR, 5.32–15.5). Likert scores indicated higher difficulty interpreting MRI (median, 3, IQR, 2–3.5) compared to mammograms (median, 1.5; IQR, 1–2; $P < 0.001$). All six patients with local recurrence were successfully diagnosed on MRI by all raters. Only one radiologist found that SPIO artefacts significantly impacted image interpretation in one case. Logistic regression consistently identified free-hand SPIO administration as associated with artefacts.

Conclusions

Paper I

The MagUS technique enables minimally invasive axillary mapping that might meet patients' tailored needs and reduce the need for diagnostic surgery.

Paper II

No differences in either incidence or size of skin staining were noted when comparing SPIO and BD after 6, 12 and 24 months of follow up following breast cancer surgery.

Paper III

The combination of SPIO and a paramagnetic seed performs comparably to SPIO and guidewire for BCS and results in more successful localizations, shorter operations and better experience.

Paper IV

Affirms that using SPIO as a tracer for SLN detection does not compromise MRI interpretation after BCS. The method proves to be safe without concerns for future artefacts affecting breast cancer recurrence assessment in MRI images.

General discussion

The shift towards using more BCS and SLND in breast cancer treatment leads to increased interest in the various tracers that can be used for SLN detection. In the past, RI has undoubtedly been the unchallenged first choice for the detection of SLN. However, the introduction of SPIO and the establishment of SPIO as a fully effective alternative with several advantages over the classic dual technique has sparked great interest in the new tracer. Above all, this thesis has been designed to optimize and refine the magnetic technique, as well as illuminate and close the knowledge gaps around the postoperative effect of SPIO.

Paper I was a feasibility study where the aim was to evaluate whether SPIO could be used for minimally invasive axillary mapping. The study showed promising results where MagUS detected all macro-metastases (n = 10) and missed only one micro-metastasis. MagUS might be a method for the future that allows for alternatives to SLND to meet patients' tailored needs and reduce the need for diagnostic surgery.

Paper II followed up the patients from the SentiDose study (49). This study was the first to compare the incidence and size between the two traces. After 24 months, the incidence and median size for SPIO was 23 (8.6%) and 4 cm^2, respectively, and for BD they were 14 (6.3%) and 3.5 cm^2 (P = 0.13 and 0.18, respectively). There was no statistically significant difference regarding incidence and size of skin staining between SPIO and BD over time. Long-lasting skin staining had been reported previously. In a study by Rubio et al., a retro-areolar injection of 1.0–2.0 mL of SPIO was used and 70.3% reported discoloration 1 month after surgery (55). In this study, however, a lower SPIO dose resulted in a lower incidence, smaller size and faster diminishing of the staining. With the benefits of no nuclear medical facilities, similar detection rates to the dual technique and the possibility to inject well before surgery, SPIO appears to be an appealing choice of RI tracer for SLN surgery.

In **Paper III**, we used the combined magnetic technique and compared it to guidewire localization. This study showed no differences between the two cohorts regarding re-excision and resection ratio, which confirmed previous

findings (56–58). The fully magnetic technique for lesion removal and SLND outperformed the guidewire, offering shorter operative times and easier logistics. Concerns about larger specimen excision were unfounded, with the magnetic technique showing potential for precision surgery and smaller specimen resection. The fully magnetic technique was seen as more favourable compared to the guidewire in terms of better experience for health-care personnel, more favourable in regard to logistics and shorter operative time. Combining paramagnetic markers and SPIO proved successful, presenting a wire- and radioisotope-free technique.

In **Paper IV**, despite notable variability among radiologists in assessing artefacts, the Likert scores were consistently similar, and all six recurrences were accurately identified on MRI. Only one radiologist noted a significant impact on image interpretation due to SPIO artefacts in a singular case. The research demonstrated that a free-hand SPIO injection, compared with an image-guided injection, was linked to a higher prevalence of artefacts (73.7% vs. 9.1%; $P < 0.001$). However, the 5-year follow-up unequivocally confirms the safety of SPIO as an SLN tracer after BCS, as it does not compromise MRI interpretation or raise concerns about future artefacts affecting breast cancer recurrence assessment.

Future perspectives

The greatest increase in future breast cancers is expected to be observed in developing countries, where the availability of RI is limited. Therefore, there is a need and a vacuum in which SPIO can be useful. As the incidence of breast cancer continues to rise, the need for further development of different treatment and diagnostic options for breast cancer will continue to be a hot topic in the literature. I believe that the future above all depends on the 'MagUS' technique described in **Paper I**, as the necessity for correct axillary staging cannot be over-emphasized. Correct axillary staging is the basis by which physicians makes their decisions regarding tailored adjuvant treatments. The advantage that SPIO has over RI is the large timespan in which the substance can be given and the fact that it stays in the tissue for a long time, which makes SPIO an ideal tracer for tracking and localizing SLN. In addition to other advantages, such as easier logistics and the lack of a need for nuclear medical facilities, this makes SPIO an attractive tracer. Further studies regarding the MagUS technique should be conducted to develop this technique and the knowledge regarding minimally invasive axillary staging

so that a further de-escalation of surgery in the axilla may be seen in the future.

Two major concerns when using SPIO are above all the problem with skin staining and artefacts observed postoperatively on MRI. However, we have shown in this thesis that by changing the injection technique and the use of lower doses of SPIO, the problem of staining and artefacts can be reduced significantly. Our research group is currently investigating SPIO doses as low as 0.1 mL for SLN detection.

Sammanfattning på svenska

Bröstcancer idag är den absolut vanligaste cancersjukdomen som drabbar kvinnor. Introduktionen av mammografiscreening har lett till att ungefär hälften av alla bröstcancerfall som diagnosticeras idag inte är palpabla vid diagnostillfället. Traditionellt har identifieringen av icke palpabla bröstcancrar framför allt gjorts med ståltrådsvajer. Denna metod är den absolut vanligaste metoden för lokalisation av icke palpabla bröstcancrar. Metoden är väletablerad men har sina nackdelar, ståltrådsvajer placeras samma dag alternativt dagen innan operationsdagen. Den vanligaste operationsmetoden idag är bröstbevarande kirurgi i kombination med portvaktskörtelbiopsi. Dock är en viktig grundsten i behandlingen den postoperativa tilläggsbehandling såsom strålning, antihormonell behandling, cytostatika samt biologiska läkemedel.

Bröstcancer kan sprida sig på framför allt tre olika sätt, lymfogen spridning vilket är det vanligaste men även hematogen samt lokal invasiv spridning. Vid en lymfogen metastasering så är 'portvaktkörteln' även kallad sentinel node (SLN) den första körteln cancern metastaserar till. Om denna körtel är 'frisk' det vill säga den saknar metastaserade tumörceller så betraktar man cancern som icke metastaserad. Den axillära stadieindelningen är en viktig punkt i beslutstagandet kring patienternas postoperativa tilläggsbehandling. För att identifiera SLN används vanligtvis radioisotopinjektion (Tc^{99}) och blå färg (Patent V Blue), denna metod kallas för dual technique och de båda ämnena används då i kombination. Metoden har en detektionsfrekvens på >90% och metoden betraktas som det 'gyllene standard' metoden. Metoden har dock sina nackdelar, den största begränsningen gäller den korta halveringstid som Tc^{99} har (6h), den strikta regleringen som följer vid hantering av radioaktiva ämnen, tillgängligheten globalt är begränsad samt risken för anafylaktiska reaktioner vid användningen utav blåfärg.

Genom de senaste åren har Superparamagnetisk järnoxid nanopartiklar (SPIO) etablerat sig som en likvärdig kandidat till ovanstående beskrivna metod. Flertalet nyligen genomförda studier visar att SPIO har en likvärdig detektionsfrekvens till 'gyllene standard' metoden. Ämnet används på samma sätt och injiceras i bröstet och sprider sig då via lymfbanan för att koncentreras i armhålans första lymfkörtlar. Med hjälp utav en handhållen magnetometer som mäter magnetism kan man då lokalisera SLN. De främsta fördelarna med

detta spårämne är framför allt en längre halveringstid (ca 30 dagar), ingen hantering av radioaktiva ämnen eller behov av faciliteter för detta och en större tillgänglighet globalt. Nackdelarna som följer med SPIO är framför allt problemet med artefakter vid undersökningar med magnetkamera postoperativt och liksom för blåfärg så kan SPIO medföra missfärgningar i huden.

Denna avhandling har grundat sig i att förfina och optimera den magnetiska tekniken samt att belysa de kunskapsluckor som finns gällande det postoperativa effekterna av SPIO vad gäller missfärgning i huden samt artefakter på magnetkamera.

Mål med avhandlingen och delmål

Syftet med denna avhandling har varit att optimera och förfina den magnetiska tekniken vid användning inom bröstcancerkirurgin.

Målsättningen med avhandlingsprojektet är att med utgångspunkt från kliniska studier belysa följande:

- Att undersöka nyttan i användningen av SPIO i den preoperativa upparbetningen vid stadieindelning av körtelstatus i axillen.
- Att jämföra olika injektionstekniker och volymer av SPIO med blåfärg med avseende på förekomst av hudmissfärgning och varaktigheten av dessa.
- Jämföra indikering av icke-palpabel bröstcancer med magnetiskt clip alternativt ståltrådsvajer hos patienter planerade för bröstbevarande kirurgi och SNB med SPIO som enda spårämne.
- Att undersöka kompatibiliteten av magnetkameraundersökning på patienter som genomgått bröstbevarande kirurgi med SPIO som spårämne för SLN detektion samt störningsfrekvens vid kontrollundersökning postoperativt efter så kallade magnetiska artefakter.

Metod & Resultat

Delstudie I var en singelcenter studie där SPIO användes i en ny minimal invasiv metod för stadieindelning av körtelstatus i axillen. Metoden var sådan att SPIO injicerades i bröstet, MRI-LG utfördes efter SPIO-injektion för lokalisation av SLN. Därefter utfördes ett axillärt ultraljud med hjälp utav magnetism samt en mellannåls biopsi av den lokaliserade SLN (MagUS). Studien omfattade inte bara patienter som planerats för primäroperation utan även patienter med recidiverande cancer efter tidigare operation samt patienter som

planerats för neoadjuvant behandling. Den senare gruppen genomgick minimal invasiv mellannålsbiopsi före starten av neoadjuvant behandling därefter utfördes operation i armhålan efter avslutad neoadjuvant behandling. Hos 79 inkluderade patienter upptäckte MagUS alla patienter med makrometastaser jämförbart med kirurgisk SNB. Slutsatsen blev att MagUS möjliggör en säker metod för minimal invasiv kartläggning av axillen som på sikt möjligtvis kan minska behovet av diagnostisk kirurgi i axillen.

Delstudie II var en prospektiv studie där vi jämförde incidens, storlek och varaktighet av hudmissfärgning hos patienter som genomgått operation med bröstbevarande kirurgi med användning av SPIO eller blå färg som spårämne. Vår studie är den första som jämför förekomsten och storlek av hudmissfärgning mellan de två spårämnena. Studien genomfördes på 270 kvinnor som opererades med bröstbevarande kirurgi och erhöll SPIO som spårämne. Av dessa erhöll 204 kvinnor även blåfärg. Efter 24 månaders uppföljning fanns det ingen statistiskt signifikant skillnad avseende vare sig storlek eller förekomst av hudfärgning mellan de två substanserna.

Delstudie III var en randomiserad prospektiv multicenterstudie som utfördes på tre sjukhus i Sverige. Totalt blev 426 kvinnor lottade till två grupper. Syftet med studien var att jämföra ståltrådsindikering mot magnetiskt clip indikering hos patienter med icke-palpabel bröstcancer planerade för bröstbevarande kirurgi med SNB. I denna studie erhöll kvinnorna SPIO som spårämne för SNB. Studien använde sig utav en datorgenererad randomisering i åtta block kuvert, patienterna blev då randomiserad till antingen ståltrådsindikering alternativt magnetiskt clip. Det primära utfallsmåttet med studien var reoperationsfrekvens samt resektionsration mellan de två grupperna. Studien visade ingen signifikant skillnad mellan grupperna avseende reoperationsfrekvensen eller resektionsration.

Delstudie IV var en prospektiv studie mellan 2017 och 2022. Patienter över 18 år med DCIS eller T1 till T3 invasiv bröstcancer planerad för BCS och SLND inkluderades till studien. Patienterna fick antingen 1, 1,5 eller 2 mL SPIO administrerat av radiologen eller kirurgen upp till fyra veckor före operationsdagen. Transkutan magnetisk signal och hudfärgning registrerades på operationsdagen, och vid de postoperativa besöken. Totalt deltog 97 patienter och 159 MR-undersökningar genomfördes. Trots varierande bedömning av förekomsten av artefakter på MR av radiologerna så var resultaten jämförbara, och alla återfall av bröstcancer diagnostiserades framgångsrikt. Studien visade att användningen av SPIO för SLN detektion inte påverkar tolkningen av MR bilderna postoperativ efter bröstbevarande kirurgi.

Acknowledgements

Words cannot express my gratitude to everyone who has supported me during my academic and clinical journey. Special thanks are owed to the following individuals:

My principal supervisor, Associate Professor **Andreas Karakatsanis**, 'the Greek boss': I am deeply indebted to you, as you believed in me even when I doubted myself. You have always supported me and made me feel like your equal, even though we both know that I had no idea what we were doing at times. When I look back at our journey, I realize that you are a man of patience, even though your whole aura reflects the opposite. I will forever be grateful for everything you have taught me. Thank you for everything!

My co-supervisor, Associate Professor **Staffan Eriksson**: You are an inspiration to me. You have taken me under your wings since day one. You have taught me so much both in life and in my professional career. When I hesitated, you gave me the motivation to dare and challenge myself. I must thank you for so many things, but most of all, thank you for being my friend. This endeavour would not have been possible without you, and I am so grateful for everything. You are a role model to follow, and I could not have wished for a better mentor.

My co-supervisor, Professor **Fredrik Wärnberg**: Thank you for your discipline, ideas and commitment. Always full of enthusiasm, you showed me that research could be fun. You were always pushing me to the limit, and you are a role model.

Centre for Clinical Research – Region Västmanland: Thanks are due for all the support and grants I have received from the Centre. Special thanks are owed to **Maria Dell Uva Karlsson**, **Maria Pettersson** and **Mariana Ehn** for making this process so smooth.

To everybody at the **Breast Unit** at Västmanlands Sjukhus, thank you so much for your support and help in creating this thesis. It has been a pleasure.

I would also like to thank all my colleagues at the Department of Surgery at Västmanlands Hospital for their support and understanding. In particular, I would like to thank **Maziar Nikberg**, **Vadim Neopikhanov**, **Yvette Andersson**, **David Boëthius**, **Ammar Nahhas**, **Ahmed Tarfy**, **Alexander Aroch** and **Henrik Andersson.**

The former heads of the Department of Surgery at Västmanlands Hospital: **Haile Mahteme**, thank you for believing in me and giving me the opportunity to become a surgeon. **Anders Nilsson**, thank you for giving me the opportunity to move home from Denmark and work in Västerås.

Abbas Chabok, you were the one who first told me that I should become a general surgeon, even when I almost signed another contract. I will never forget our 'short' walk that took only 85 minutes. Thank you for always being by my side. Thank you and your beautiful family. You have always been like a father to me in my professional career. You are a great surgeon.

To my current chief of doctors **Maria Mani** and all my colleagues at the **Department of Reconstructive Plastic Surgery, Akademiska Sjukhuset Uppsala**: Thank you for welcoming me with open arms and letting me live my dream.

To all my co-authors, thank you for your help and contribution. Special thanks to **Eirini Pantiora** for all your help and support.

Special thanks are owed to my friend and research colleague, **Abdi-Fatah Hersi**. You have been like a big brother to me in the surgical and academic world. We quickly realized that we came from the same background and had the same goals. I remember these moments in our room at work, dreaming about this day. Look at us now, we both made it! Thank you for all the laughs we shared.

Kevin Afshari, thank you for guiding me in my career. You have always given me wise advice when I needed it, and you have always been there for me. I am eternally grateful to you for that.

To my childhood friends: **Sebastian**, **Dilan 'Papi'**, **Andreas**, **Axel 'Swidi', Patrik** and **Arian**, thank you for standing by my side and for sharing the ups and downs of life with me. Thank you, my brothers, for always not believing in me. I would never have made it without your non-existent support.

To my beloved family: I will never forget our roots, our childhood, and where we come from. I remember how we always dreamed of things that we could never have and that others took for granted. I remember all the sacrifices we

had to make for each other. I will never forget the only Christmas that we almost had for the first time, but in the last second, it was taken from us. Nothing makes me happier than seeing all three of us succeed in life. The pride of being your big brother. **Diyar**, my little brother, I hope you can see a role model when you look at me. **Karin**, my beautiful sister, and my niece **Estelle**, I am so proud of you two.

To my dear mother, **Fatima Miro**: I have seen all your sacrifices. All of this is for you, my mother. You came to this country as a young woman, leaving everything so that your children could have a safer environment to grow up in. I have seen you struggle day by day. I have seen how you made sacrifice after sacrifice just so we could study and have a better life. You gave away large parts of your life so that my siblings and I could have a better life. Mother, I could never have become the man I am today without you. This is for you. Be proud, mother, this is your work.

Finally, to my own family, my dear beautiful wife **Azin**, words cannot describe what I want to say to you. We have been through so much together. You have always been by my side; you have always supported me even when I did not deserve it. Without you and your sacrifices, this would not have been possible. Thank you for being the best wife and the best mom to our treasure, **Alexis**, Baba loves you so much. Hopefully, one day you will read this book, I hope you will be proud of your baba. Alexis *baba ji bo te dimire evîna min. Jîyana min. Keça baba*.

To all the patients participating in these studies, Thank you.

Appendices

Appendix 1: Supplement 2 for Paper III

Pantiora E, Jazrawi A, Hersi AF, et al. Magnetic seed vs guidewire breast cancer localization with magnetic lymph node detection: A randomized clinical trial. JAMA Surg. 2024;159(3):239–246. https://doi.org/10.1001/jamasurg.2023.6520

eTable 1. Resection ratio for site and type of surgery

	Overall	Guidewire	Magnetic marker	*P*-value
Entire Trial	1.96 (1.14–3.46)	1.96 (1.22–3.48)	1.97 (1.11–3.46)	0.96
Uppsala	1.45 (0.78–2.13)	1.59 (0.77–2.15)	1.26 (0.78–2.07)	0.08
WLE (n = 170)	1.48 (0.85–2.13)	1.60 (0.98–2.17)	1.29 (0.76–2.05)	
OPBCS Level I (n = 47)	1.26 (0.68–1.73)	1.46 (0.69–1.81)	1.15 (0.69–1.60)	
OPBCS Level II (n = 18)	1.87 (0.88–7.40)	1.38 (0.49–41.79)	2.13 (1.08–13.21)	
Västerås	3.33 (2.13–5.39)	3.21 (1.60–4.79)	3.46 (2.50–5.75)	.92
WLE (n=105)	3.42 (2.19–5.21)	3.33 (1.82–4.79)	3.44 (2.47–5.78)	
OPBCS Level I	-	-	-	
OPBCS Level II (n = 2)	4.21 (2.85, 5.57)	-	4.21 (2.85–5.57)	
Gothenburg	2.87 (2.00–4.38)	2.88 (2.05–4.38)	2.77 (1.86–4.63)	0.91
WLE (n = 71)	2.78 (2.00–4.27)	2.88 (2.22–4.20)	2.57 (1.73–4.27)	
OPBCS Level I (n = 3)	3.18 (3.00–6.62)	-	3.18 (3.00–6.62)	
OPBCS Level II (n = 1)	5.27 (5.27–5.27)	-	5.27 (5.27–5.27)	

Note: Resection ratios for each received marker (i.e. per-protocol analysis) in subgroups by site and type of surgery. Resection ratio is summarized as median (interquartile range, IQR). OPBCS, oncoplastic breast-conserving surgery; WLE, wide local excision. *P*-value: Independent medians test.

eTable 2. Type of complication for each received localization device

(n.%)	Per-protocol intervention		*P*-value
	Guidewire	Magnetic marker	
None	193 (92.8)	194 (90.2)	0.53
Symptomatic breast seroma	3 (1.4)	1 (0.5)	
Breast hematoma	2 (1.0)	4 (1.9)	
Symptomatic axillary seroma	0 (0.0)	1 (0.5)	
Axillary hematoma	2 (1.0)	1 (0.5)	
Breast infection	5 (2.4)	3 (1.4)	
Axillary infection	1 (0.5)	2 (0.9)	
Delayed wound healing	0 (0.0)	3 (1.4)	
Postoperative bleeding in the breast	1 (0.5)	4 (1.9)	
Pain at SPIO injection site	1 (0.5)	1 (0.5)	
Superficial venous thrombosis	0 (0.0)	1 (0.5)	

eTable 3. Health-care practitioners' experience with each marker

	Paramagnetic	Guidewire	*P*-value
Ease of logistics and planning (theatre co-ordinators)	10 (10.10)	6 (4.8)	< 0.001
Ease of localization (radiologists)	7 (7.9)	7 (7.7)	< 0.001
Ease of intra-operative detection (surgeons)	9 (8.10)	7 (7.8)	< 0.001

Note: Responses to Likert items with range (0–10), where a higher score denotes higher satisfaction. Likert scores are summarized as median (IQR). *P*-value: independent sample medians test.

Appendix 2: Supplementary material for Paper IV

Table 2. Outcomes of the radiological assessment.

		R1	R2	R3	R4	*P*-value	κ	95% CI	Percent agreement	95% CI
n,%										
Any Artefacts in MRI	'Yes' *vs* 'No' *vs* 'Unsure'	116 (74.4)	38 (24.1)	54 (34.2)	91 (57.6)	<0.001	0.293	0.223, 0.363	58.3	53.5, 63.0
	'Yes' *vs* 'No' and 'Unsure'	116 (74.4)	38 (24.1)	54 (34.2)	91 (57.6)	<0.001	0.350	0.272, 0.429	66.0	61.5, 70.6
Artefact significance for any artefact	Artefact that does not impact image and interpretation at all	12 (10.3)	7 (18.4)	8 (14.8)	35 (38.5)	<0.001	0.355	0.288, 0.421	59.6	54.4. 64.4
	Artefact impairs the image interpretation somewhat but does not interfere with characterization and assessment	103 (88.8)	31 (81.6)	46 (85.2)	56 (61.5)					
	Artefact impairs image to make interpretation impossible	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)					
SPIO-specific Artefacts in MRI	'Yes' *vs* 'No' *vs* 'Unsure'	43 (27.2)	19 (12.0)	49 (31.0)	78 (49.4)	<0.001	0.255	0.198, 0.312	50.1	45.3, 54.9
	'Yes' *vs* 'No' and 'Unsure'	43 (27.2)	19 (12.0)	49 (31.0)	78 (49.4)	<0.001	0.293	0.214, 0.372	69.5	65.3, 73.8
Artefact significance for SPIO-specific artefacts	Artefact that does not impact image and interpretation at all	5 (3.2)	2 (1.3)	8 (5.2)	30 (19.5)	0.021	0.435	0.366, 0.504	91.5	89.7, 93.2

	Artefact impairs the image interpretation somewhat but does not interfere with characterization and assessment	37 (23.7)	17 (10.7)	41 (26.5)	48 (31.2)					
	Artefact impairs image to make interpretation impossible	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)					
How difficult was it to assess the MRI?*	Median (IQR)	3 (2,3)	2 (1,3)	3 (2,4)	3 (1,4)	0.323**	0.18***	0.14, 0.22***	33.8	30.2, 37.6
	Mean rank	2.42	2.15	2.86	2.58					
How difficult was it to assess the mammogram?*	Median (IQR)	1 (1,1)	2 (1,2)	2 (2,2)	1 (1,2)	<0.001**	0.067***	0.003, 0,131***	49.5	45.2, 53.7
	Mean rank	1.64	3.01	3.13	2.22					
Recurrence diagnosed in MRI	n (%)	6 (100)	6 (100)	6 (100)	6 (100)	1.000	1.000	1.000, 1.000	100.0	100.0, 100.0

All summary data in columns R1–R4 are n,% except for *, which are Likert items summarized as medians (interquartile range, IQR). All *P*-values correspond to marginal homogeneity (Stuart–Maxwell) test, apart from **, that correspond to Friedman's test for k medians. All κ values respond to Conger's κ, apart from ***, that correspond to Krippendorf's α. MRI: magnetic resonance imaging.

Questionnaire. For the participating radiologists

1. PostMAG MRI Radiology CRF and explanations

- Q1:

Do you think that there are artefacts or postoperative changes in this examination?

☐ Artefacts ☐ Postoperative changes ☐ No

'No' to be coded as 0, 'Artefacts' to be coded as 1, 'Postoperative changes' to be coded as 2.

To facilitate the grading, you can have the following classification as means to facilitate your work:

a. No artefact present.
b. Artefact does not impact the image at all and does not affect interpretation at all.
c. Artefact impairs the image hinders image interpretation somewhat but does not interfere with characterization and ability to see the extent of disease.
d. Artefact impairs image to make interpretation impossible.

- Q2

Please classify what suits best (1–4).

- Q3 (Applicable if answer to Q1 is 'Artefacts'):

Do you think that the artefacts in this examination are related to SPIO?

☐ Yes ☐ No ☐ Unsure

'No' to be coded as 0, 'Yes' to be coded as 1 and 'Unsure' to be coded as 2.

- Q4 (Applicable if answer to Q1 is 'Yes'):

Please define the size of the artefacts by providing the two largest diameters in mm:

……. x ……..

- Q5

How easy is it to assess this examination?

……..

Score from 1 to 10, as a Likert item, without using decimals (no 'halves') with 1 for 'No difficulty at all' and 10 'The examination is impossible to interpret'.

Supplementary Tables for Paper IV.

Supplementary Table 1. Marginal homogeneity tests for each rater separately, for item 1 (any artefact or postoperative change on MRI and mammogram, respectively).

Rater 1					
		Any artefacts on mammogram			
		No	Artefacts	Postoperative changes	Total
Any artefacts on MRI	No	2	0	1	3
	Artefacts	1	3	112	116
	Postoperative changes	0	0	37	37
	Total	3	3	150	156
Rater 2					
		Any artefacts on mammogram			
		No	Artefacts	Postoperative changes	Total
Any artefacts on MRI	No	17	0	3	20
	Artefacts	7	2	29	38
	Postoperative changes	0	0	90	90
	Total	8	1	123	155
Rater 3					
		Any artefacts on mammogram			
		No	Artefacts	Postoperative changes	Total
Any artefacts on MRI	No	8	0	3	11
	Artefacts	0	1	53	54
	Postoperative changes	0	0	90	90
	Total	8	1	123	155
Rater 4					
		Any artefacts on mammogram			
		No	Artefacts	Postoperative changes	Total
Any artefacts on MRI	No	13	1	0	14
	Artefacts	0	0	91	91
	Postoperative changes	0	0	49	49
	Total	13	1	146	154

Note: All Marginal homogeneity tests (Stuart–Maxwell), $P < 0.001$.

Supplementary Table 2. Marginal homogeneity tests for each rater separately, for item 1 (SPIO-specific artefacts *vs* any artefact or postoperative change on mammogram, respectively).

Rater 1					
		Any artefacts on mammogram			
		No	Artefacts	Postoperative changes	Total
SPIO-specific artefacts on MRI	No	2	0	29	31
	Yes	0	1	42	43
	Unsure	1	2	79	82
	Total	3	3	150	156
Rater 2					
		Any artefacts on mammogram			
		No	Artefacts	Postoperative changes	Total
SPIO-specific artefacts on MRI	No	23	1	89	113
	Yes	4	0	15	19
	Unsure	3	1	19	23
	Total	30	2	123	155
Rater 3					
		Any artefacts on mammogram			
		No	Artefacts	Postoperative changes	Total
SPIO-specific artefacts on MRI	No	8	1	80	89
	Yes	0	0	49	49
	Unsure	0	0	17	17
	Total	8	1	146	155
Rater 4					
		Any artefacts on mammogram			
		No	Artefacts	Postoperative changes	Total
SPIO-specific artefacts on MRI	No	13	1	0	14
	Yes	0	0	78	78
	Unsure	0	0	24	24
	Total	13	1	140	154

Note: All marginal homogeneity tests (Stuart–Maxwell) $p<0.001$.

Supplementary Table 3. Marginal homogeneity tests for each rater separately, for item 1 (SPIO-specific artefacts *vs* any artefact or postoperative change on mammogram, respectively).

Rater 1: Presence of any artefacts							
		Univariable			Multivariable		
		Yes	No	p-value	OR	95% CI	p-value
Age (y)*		62 (53–70)	54.5 (45–66)	0.021**	1.043	0.982–1.106	0.170
Lesion size (mm)*		14 (10–21.5)	23.5 (16, 40)	< 0.001**	0.980	0.938–1.024	0.373
SPIO injection technique***	Free-hand	19 (100)	0 (0)	0.001****	Ref [1]		
	Image-guided	51 (66.2)	26 (33.8)		0.250	0.073–0.864	0.028
Breast procedure	WLE	28 (77.8)	8 (22.2)	< 0.001****	Ref [1]		
	OPBCS Level I	37 (84.1)	7 (15.9		2.142	0.535–8.578	0.282
	TM	3 (75.0)	1 (25.0)		5.153	0.217–140.172	0.301
	CWPF	3 (23.1)	10 (76.9)		0.028	0.001–0.734	0.032
Post resection signal*		2500 (650–7690)	0 (0–2000)	< 0.001**	1.000	0.999–1.001	0.079
Signal on postoperative visit*		910 (61–4750)	0 (0.135)	< 0.001**	1.0002	1.0001–1.0003	0.021
Rater 1: Presence of SPIO-specific artefacts							
Age (y)*		66 (61–71)	57 (50–68)	0.004**	1.082	1.019–1.148	0.010
Days from SPIO injection to surgery		2 (0–5)	5 (1–8)	0.038**	0.917	0.807–1.041	0.181
SPIO injection technique***	Free-hand	10 (52.6)	9 (47.4)	0.002****	Ref [1]		
	Image-guided	13 (16.9)	64 (83.1)		0.174	0.051–0.589	0.005

Note: *: median (interquartile range, IQR and range for the signals); ** Mann–Whitney U test; ***: n, %; ****: Fisher's exact test (2 × 2) or Chi-square (2 × 3 or 2 × 4). CWPF, chest wall perforator flap; CI, confidence interval; OPBCS, oncoplastic breast-conserving surgery; OR, odds ratio; Ref., reference category; TM, therapeutic mammaplasty; WLE, wide local excision; y, years.

Supplementary Table 4. Factors of association between prevalence of 'any artefact' or 'SPIO-specific artefact for Rater 2' (R2)

R2: Presence of any artefacts							
		Univariable			Multivariable		
BMI (kg/m^2)*		23.5 (22.1–25.0)	26.4 (24.4–30.1)	0.005**	0.860	0.735–1.001	0.060
SPIO injection technique***	Free-hand	9 (47.4)	10 (52.6)	0.001****	Ref. [1]		
	Image-guided	18 (23.4)	59 (76.6)		0.510	0.122–2.124	0.355
Breast procedure***	WLE	15 (41.7)	21 (58.3)	0.038****	Ref. [1]		
	OPBCS Lev I	10 (22.7)	34 (77.3)		0.248	0.074–0.840	0.025
	TM	0 (0)	4 (100)		1 (empty)		
	CWPF	2 (15.4)	11 (84.6)		0.182	0.024–1.385	0.100
Post resection signal*		3048 (2438–9999)	780 (0–2536)	<0.001**	1.0002	1.0001–1,0004	0.023
Signal on postoperative visit*		2010 (650–6267)	129 (0–1370)	<0.001**	1.000	0.9999–1.0001	0.732
R2: Presence of SPIO-specific artefacts							
BMI (kg/m^2)*		23.3 (21.6–24.4)	26.4 (23.7–30.1)	0.003**	0.960	0.820–1.125	0.616
Breast volume (mL)*		357 (160–510)	484 (323–756)	0.047**	1.001	0.999–1.003	0.159
SPIO injection tech-nique***	Free-hand	7 (36.8)	12 (63.2)	0.010****	Ref. [1]		
	Image-guided	8 (10.4)	69 (89.6)		0.172	0.050–0.599	0.006
Post resection signal		8000 (2650–9999)	1090 (40, 3156)	<0.001**	1.000	0.999–1.001	0.331
Brown staining on cut surface	Yes	12 (52.2)	11 (47.8)	<0.001****	0.519	0.123–2.198	0.373
	No	3 (4.1)	71 (95.9)		Ref. [1]		
Signal on postoperative visit		3 (4.1)	243 (0–2135)	0.004**	0.9999	0.9998–1.0001	0.732

Notes: *: median (interquartile range, IQR and range for the signals); ** Mann–Whitney *U* test; ***: n, %; ****: Fisher's exact test (2 × 2) or Chi-square (2 × 3 or 2 × 4). BMI, body mass index, measured in kilograms divided by square metres (kg/m^2); CWPF, chest wall perforator flap; CI, confidence interval; OPBCS, oncoplastic breast-conserving surgery; OR, odds ratio; Ref., reference category; TM, therapeutic mammaplasty; WLE, wide local excision; y, years.

Supplementary Table 5. Factors of association between prevalence of 'any artefact' or 'SPIO-specific artefact for Rater 3' (R3).

Presence of any artefacts							
		Univariable			Multivariable		
		Yes	No	*P*-value	OR	95% CI	*P*-value
SPIO injection technique***	Free-hand	14 (73.7)	5 (26.3)	0.001****	Ref. [1]		
	Image-guided	29 (37.7)	48 (62.3)		0.261	0.077–0.883	0.031
SPIO volume (mL)***	1	28 (51.9)	26 (48.1)	0.081****			
	1.5	12 (42.9)	16 (57.1)				
	2	3 (20.0)	12 (80.0)				
Post resection signal*		2500 (772–8250)	698 (0–2536)	0.011**	1.000	0.999–1.001	0.518
Signal on postoperative visit*		1370 (290–7584)	35 (0–550)	< 0.001**	1.0002	1.000–1.0003	0.008
Presence of SPIO-specific artefacts							
SPIO injection technique***	Free-hand	16 (84.2)	3 (15.8)	< 0.001****	Ref. [1]		
	Image-guided	22 (28.6)	55 (71.4)		0.092	0.023–0.371	0.001
Post resection signal*		2500 (280–9999)	784 (0–2618)	0.025**	1.0000	0.9999–1.0002	0.276
Signal on postoperative visit*		1115 (125–6267)	125 (0–1460)	0.003**	1.0001	0.9999–1.0003	0.092

Notes: *: median (interquartile range, IQR and range for the signals); ** Mann–Whitney *U* test; ***: n, %; ****: Fisher's exact test (2 × 2) or Chi-square (2 × 3 or 2 × 4). BMI, body mass index, measured in kilograms divided by square metres (kg/m^2); CWPF, chest wall perforator flap; CI, confidence intervals; OPBCS, oncoplastic breast-conserving surgery; OR, odds ratio; Ref., reference category; TM, therapeutic mammaplasty; WLE, wide local excision; y, years.

Supplementary Table 6. Factors of association between prevalence of 'any artefact' or 'SPIO-specific artefact' for Rater 4 (R4).

Presence of any artefacts							
		Univariable			Multivariable		
		Yes	No	*P*-value	OR	95% CI	*P*-value
Age (y)*		61 (54–70)	55 (47–66)		1.034	0.992–1.079	0.116
Lesion size (mm)*		14 (11–22)	20 (15–40)	0.001****	0.972	0.940–1.005	0.093
SPIO injection technique***	Free-hand	17 (89.5)	2 (10.5)	0.015****	Ref. [1]		
	Image-guided	46 (59.7)	31 (40.3)		0.257	0.052–1.280	0.097
Post resection signal*		2469 (441–7690)	643 (0–2450)	0.002**	1.0001	0.9999–1.0002	0.283
Signal on postoperative visit*		1370 (290–7584)	35 (0–550)	< 0.001**	1.00001	0.9999–1.0001	0.716
Presence of SPIO-specific artefacts							
Age (y)*	61.5 (53.5–70)		56 (48–66)	0.026**	1.043	0.999–1.086	0.053
Lesion size (mm)*	14 (10.5, 22.5)		20 (14–30)	0.067**	0.979	0.947–1.013	0.231
SPIO injection technique***	Free-hand	16 (84.2)	3 (15.8)	< 0.001****	Ref. [1]		
	Image-guided	35 (45.5)	42 (54.5)		0.171	0.042–0.701	0.014
Post resection signal*		2469 (375–7690)	780 (0–2536)	0.018**	0.9999	0.9998–1.0001	0.994
Signal on postoperative visit*		1005 (116–4750)	76 (0–950)	0.002**	1.0001	0.9999–1.0002	0.295

Notes: *: median (interquartile range, IQR and range for the signals); ** Mann–Whitney *U* test; ***: n, %; ****: Fisher's exact test (2 × 2) or Chi-square (2 × 3 or 2 × 4). BMI, body mass index, measured in kilograms divided by square metres (kg/m^2); CWPF, chest wall perforator flap; CI, confidence intervals; OPBCS, oncoplastic breast-conserving surgery; OR, odds ratio; Ref., reference category; TM, therapeutic mammaplasty; WLE, wide local excision; y, years.

Appendix 3: Errata for Paper I

Heading 2.1, page 2, row 4. Correction: Patients planned for NAT were not excluded.

Heading 3, page 6–7, Table 1. Correction: Under the headings, previous axillary surgery and neoadjuvant treatment, right should be replaced with yes and left should be replaced with no.

Heading 3, page 7, row 13. Correction: FNR should be changed from 8.3% to 9.1%.

Appendix 4: Errata for Paper II

Abstract, page 1, row 22. Correction: For BD, the incidence should be changed from 14 to 13.

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Acta Universitatis Upsaliensis

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Article

Magnetic-Guided Axillary UltraSound (MagUS) Sentinel Lymph Node Biopsy and Mapping in Patients with Early Breast Cancer. A Phase 2, Single-Arm Prospective Clinical Trial

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Simple Summary: Superparamagnetic iron oxide nanoparticles (SPIO) have been shown to identify sentinel lymph nodes (SLNs) in patients with breast cancer. This study investigated whether a minimally invasive approach with MRI-LG after SPIO injection in the breast followed by a magnetic guided axillary ultrasound and core biopsy of the SLN (MagUS) could accurately stage the axilla. The study included not only patients planned for primary surgery but also patients with recurrent cancer after previous surgery, but also patients scheduled for neoadjuvant treatment (NAT). The latter underwent minimally invasive SLNB prior to treatment and had their SLN clipped; surgery in the axilla was performed after NAT. In 79 included patients, MagUS detected all patients with macrometastasis and performed comparably with surgical sentinel lymph node dissection (SLND). It also allowed for marking of the SLN in patients planned for PST and enabled tailored decision making in breast cancer recurrence.

Abstract: Lymph Node Dissection (SLND) is standard of care for diagnosing sentinel lymph node (SLN) status in patients with early breast cancer. Study aim was to determine whether the combination of Superparamagnetic iron oxide nanoparticles (SPIO) MRI-lymphography (MRI-LG) and a Magnetic-guided Axillary UltraSound (MagUS) with biopsy can allow for minimally invasive, axillary evaluation to de-escalate surgery. Patients were injected with 2 mL of SPIO and underwent MRI-LG for SN mapping. Thereafter MagUS and core needle biopsy (CNB) were performed. Patients planned for neoadjuvant treatment, the SLN was clipped and SLND was performed after neoadjuvant with the addition of isotope. During surgery, SLNs were controlled for signs of previous biopsy or clip. The primary endpoint was MagUS SLN detection rate, defined as successful SLN detection of at least one SLN of those retrieved in SLND. In 79 patients, 48 underwent upfront surgery, 12 received neoadjuvant and 19 had recurrent cancer. MagUS traced the SLN in all upfront and neoadjuvant cases, detecting all patients with macrometastases ($n = 10$). MagUS missed only one micrometastasis, outperforming baseline axillary ultrasound AUS (AUC: 0.950 vs. 0.508, $p < 0.001$) and showing no discordance to SLND ($p = 1.000$). MagUS provides the niche for minimally invasive axillary mapping that can reduce diagnostic surgery.

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Citation: Jazrawi, A.; Pantiora, E.; Abdsaleh, S.; Bacovia, D.V.; Eriksson, S.; Leonhardt, H.; Wärnberg, F.; Karakatsanis, A. Magnetic-Guided Axillary UltraSound (MagUS) Sentinel Lymph Node Biopsy and Mapping in Patients with Early Breast Cancer. A Phase 2, Single-Arm Prospective Clinical Trial. *Cancers* **2021**, *13*, 4285. https://doi.org/10.3390/cancers13174285

Academic Editors: Moriaki Kusakabe, Akihiro Kuwahata and Katja Pinker-Domenig

Received: 22 June 2021
Accepted: 20 August 2021
Published: 25 August 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Keywords: sentinel lymph node biopsy; breast cancer; superparamagnetic iron oxide; magnetic tracer; sentinel lymph node

1. Introduction

Primary tumor biology and axillary status guide therapeutic decisions in breast cancer treatment [1,2]. Sentinel Lymph Node Dissection (SLND) is considered the standard method of axillary staging, both in upfront surgery as well as after neoadjuvant treatment (NAT) [3–8].

Preoperative identification of patients with a negative SLN, or low-volume axillary disease that does not warrant further surgery, but guides therapeutic decisions, may allow for tailored approaches avoiding upfront SLND [6,9,10]. In patients scheduled for NAT, identifying those with a true negative axilla, but also those with low-volume disease, as de-escalation of axillary surgery after conversion from cN1 to cN0, could be safely attempted. [7,11,12].

At the same time, SLND is not an indolent procedure and is related to complications and considerable short- and long-term morbidity [13–16]. Therefore, non- or minimally invasive modalities have been proposed in order to address this problem. All of them are based on the principle of injecting a contrast interstitially in the breast in the same manner as when SLND is performed. The contrast will then be taken up by the lymphatics and reach the SLNs and will subsequently be visualized by a radiological modality. Previously, several methods such as single-photon emission computed tomography (SPECT), tridimensional computed tomography lymphography (3D-CTLG) or contrast enhanced ultrasound with microbubbles (CEUS) have been evaluated as alternatives to surgery [17–19]. Most of these have shown promising results, but larger studies are missing and, complicated logistics, need for access to nuclear medicine facilities and demanding learning curves are restricting their introduction into clinical practice.

Superparamagnetic iron oxide nanoparticles (SPIO) are used as a SLND tracer with comparable detection to the combination of radioisotope and blue dye, as shown in previous studies [20,21]. Additionally, when SPIO is injected in the breast, it can identify SLNs in axillary magnetic resonance imaging lymphography (MRI-LG) [22]. At the same time, SPIO yields the benefit that it resides in the tissue for a prolonged period of time without migrating to higher lymph node echelons and, thus, allows for the identification for SLNs during a much wider timeframe [23]. In this manner the SLNs that are identified during surgery should be visible in an MRI and, at the same time, transcutaneous signal detected by a magnetic probe, as in surgery, should be able to guide the axillary ultrasound to allow for transcutaneous identification and biopsy of the SLNs. Such a concept would have the perceived advantages of combining and tailoring modalities and at the same time, allowing for preoperative work up in a timeframe wider than the short halftime of Tc^{99} used for SPECT or that in the case of CEUS [19,24].

The development of an integrated technique bridging non-invasive and minimally invasive procedures for enhancement of the standard, axillary ultrasound-based diagnostic work-up is highly relevant [23–25]. The aim of this study was to determine whether the preoperative work-up with SPIO MRI-LG and Magnetic-guided Axillary UltraSound (MagUS), can accurately localize SLNs and predict SLN status and whether such a technique has the potential of replacing SLN surgery in the future.

2. Methods

2.1. Patients

Adult patients with clinically and ultrasound node-negative early breast cancer (cN0) planned for SLND at Uppsala University Hospital, from September 2017 to December 2020, were enrolled in the study after written informed consent. Patients with hypersensitivity to dextran compounds or SPIO, iron overload disease or planned for NAT and monitored

with breast MRI for tumor response, were excluded. If a diagnostic breast MRI was needed, it was performed separately, before SPIO injection and axillary MRI-LG. The study was approved by the Regional Ethics Board in Uppsala (DNR 2016/385).

2.2. MRI-LG

Patients were injected peritumorally in the breast with 2 mL of SPIO (Magtrace®, Endomag., Cambridge, UK) and underwent MRI-LG one to 14 days after the injection. MRI-LG was performed with the patient in a supine position and adduction of the ipsilateral arm. The examination was performed without iv-contrast and took ca 8 min to complete. In cases of previous breast and axillary surgery or parasternal cancers, the contralateral axilla was also included in the MRI-LG to identify aberrant lymphatic outflow [26]. The MRI images were obtained using a 1,5-T and 3-T system (Philips®, Amsterdam, The Netherlands) with T2W cor, T2* tra and T2* cor sequences. Any lymph node with SPIO uptake in a T1 sequence or SPIO related void artifact on T2 sequence was considered a SLN, as previously described imaging was reviewed and the number of identified SLNs was documented [22]. SLN localization was described according to the classification proposed by Clough et al. [27], in relation to the lateral thoracic vein and the second intercostobrachial nerve. SLN metastatic status was assessed according to criteria previously proposed by Motomura et al. [22]; a lymph node was considered non-metastatic if there was a homogenous low intensity signal uptake of SPIO and metastatic if the entire node or a focal area did not show low signal intensity uptake.

2.3. Magnetic Guided Axillary UltraSound (MagUS) and Core Needle Biopsy (CNB)

After reviewing of MRI-LG, the radiologist performed a second look axillary ultrasound in another session. The examination was focused to the area where the SLNs were identified on MRI (Figure 1). After a primary assessment for lymph nodes, a handheld magnetometer (Sentimag®, Endomag, Cambridge, UK) was used to identify the "pre-incision hotspot" which is the area with the highest magnetic uptake on the skin, and concordance with the MRI localization was registered.

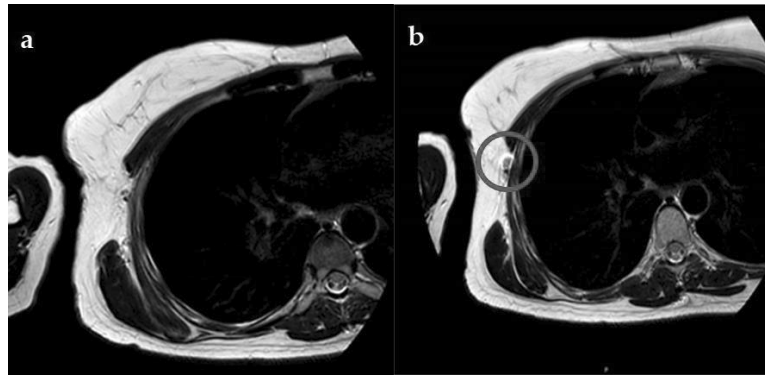

Figure 1. (**a**,**b**). Visualization of SLN with MRI before and after SPIO. In an enhancement of the SLN is visualized after injection of SPIO. The red circle visualizes the enhanced SLN after the injection of SPIO.

Subsequently, the identified lymph node(s) were assessed, and the percutaneous CNB of the SLN was performed with ultrasound guidance under local anesthesia (Figure 2). The CNB was evaluated for the presence of brown staining and magnetic uptake with the SentiMag probe (Figure 3). If more than one pathological lymph nodes were identified at this stage, the protocol stated that multiple efforts could be performed only after patient

consent; otherwise, if the bioptic material obtained was considered representative and adequate, only the most prominent node was biopsied. Standard histopathologic analyses to assess metastasis was also performed, including verification of SPIO presence in the SLN. In patients undergoing NAT, the SLN was clipped simultaneously after the CNB, at the same session. When CNB was completed, the area was scanned for bleeding.

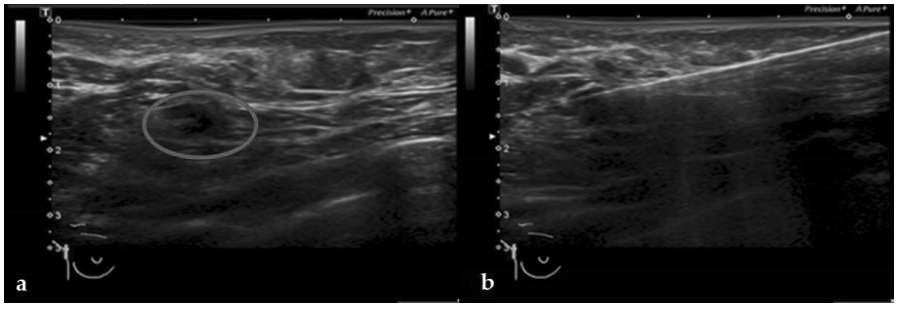

Figure 2. (**a**,**b**). MagUS with the SLN visualized in the red circle (**left**). Magnetic probe localizes the magnetic "hotspot" and after that CNB is performed (**right**). Monitor width 3.9 cm.

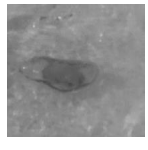

Figure 3. MagUS SLN-biopsy specimen (size 1 cm).

The study protocol ruled that the first five patients would undergo axillary MRI-LG before and after SPIO administration, and that MagUS and CNB was performed in the operation theatre, after the induction of anesthesia and right before surgery. In cases of recurrent breast cancer with aberrant SLN localization on MRI-LG and MagUS, a decision to attempt SLND was made at the multidisciplinary conference and after discussion with the patient. In patients undergoing NAT, a new axillary MRI-LG was performed after NAT, with no subsequent SPIO injection to see whether SPIO uptake in the SLNs was still visible. The number and localization of SLNs on MRI images was documented and axillary transcutaneous SentiMag signal was recorded. During subsequent SLND, concomitant radioisotope injection was administered and during surgery we registered which SLNs were magnetic, radioactive or both as well as the signal of the clipped node with both tracers.

2.4. Surgery and Specimen Pathology

During surgery, SLND was performed and the retrieved SLNDs were controlled macroscopically and microscopically for signs of previous biopsy, hematoma or the presence of clip, if placed. Standard pathology of the SLN specimen served as a reference to the microscopical examination of the CNB.

The entire MagUs flowchart is summarized in Figure 4.

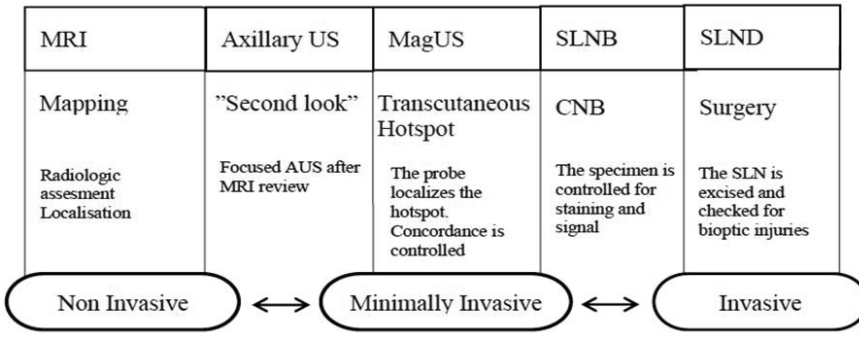

Figure 4. Flowchart showing the MagUS process.

2.5. Trial Design and Study Endpoints

To assess whether the MagUS concept has the niche to replace surgical axillary evaluation (SLND), it was necessary to ensure concordance and agreement across the different modalities. With other words, it was necessary to verify that the SLNs identified and retrieved during surgery, were the same lymph nodes visualized on the MRI and the same that were detected by the magnetic probe, identified by the ultrasound and subsequently biopsied with a core needle. The common denominator was the presence of SPIO in the node and how this is demonstrated throughout the different modalities (MRI, MagUS, Surgery). Therefore, the outcome of interest was a minimum agreement in the assessment obtained by the MRI/MagUS with the standard of care, that is surgery. For this, it was clinically relevant to assess if the technique at hand is feasible, before venturing on a large clinical trial. Subsequently, the MagUS trial was conceived as a single stage phase 2 trial following the A'Hern's design [28]. For a one-sided test a type one error a = 0.025 and 80% power, a sample size of 75 or more was required between a maximum futility proportion of 95% (corresponding to the proportion of successful detection above which the method can be further considered) and a minimum efficacy of proportion of 85% (corresponding to the proportion of successful detection under which, the method should not warrant further investigation).

The primary endpoint was determination of the MagUS SLN detection rate, defined as successful SLN detection of at least one SLN of those retrieved in the following SLND. Secondary endpoints were false-negative rate (FNR) of the MagUS technique, defined as no diagnosis of SLN metastasis (index test = negative) but presence of metastases by histopathology in any of the retrieved SLNs (reference test = positive) and overall accuracy, sensitivity, specificity and positive and negative predictive value (PPV, NPV).

Another aim of the study was to determine whether the MagUS technique could improve preoperative workup accuracy. For this, discordance in axillary evaluation from baseline clinical and ultrasonographical assessment was assessed.

Subgroup analyses were carried out to review the role of each component of the MagUS technique (MRI-LG. MagUS and MagUS core biopsy) and their potential role in tailored axillary mapping and inform on a future phase 3 trial.

The manuscript was prepared according to the Standards for the Reporting of Diagnostic Accuracy Studies (STARD) statement [29]. Descriptive statistics were performed by means of median (range) for continuous variables. Subsequently, non-parametric tests were used for comparisons. The McNemar's test was used for the assessment of discordance in paired observations. For diagnostic accuracy statistics, Receiver Operating Characteristics (ROC) curves were constructed and the area under the curve (AUC) is provided. Effect sizes are provided with 95% confidence intervals (95% CI). Data analyses were performed using SPSS (V 26.0. IBM Corp, Armonk, NY, USA) and Stata®, version 16 (StataCorp LP, College Station, TX, USA).

3. Results

The study is summarized in (Figure 5) and patient characteristics are presented in Table 1. In a total of 79 patients, 48 had early breast cancer and underwent upfront surgery, 12 underwent NAT and 19 had recurrent breast cancer after previous breast and axillary surgery.

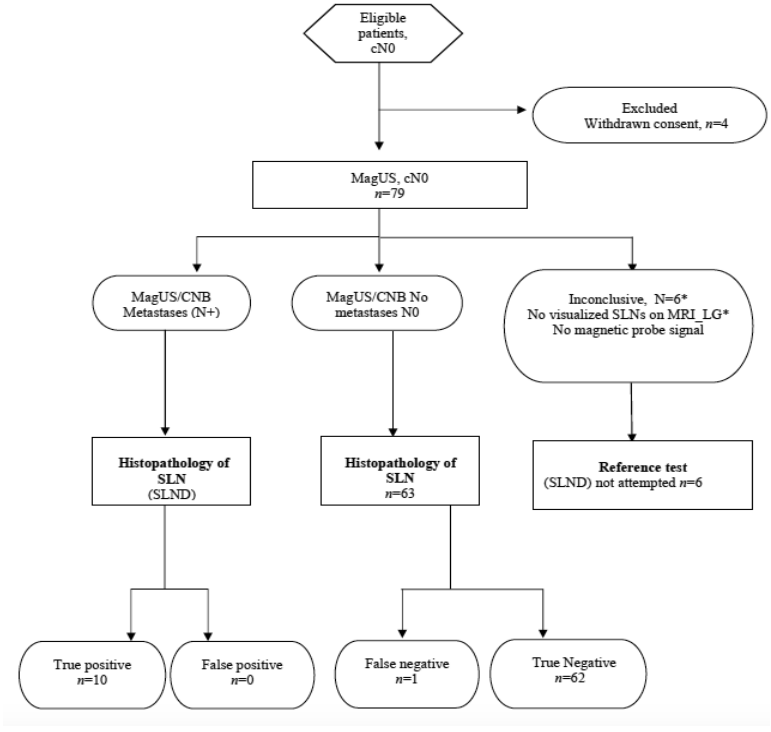

Figure 5. STARD flow diagram. * MRI_LG: Magnetic resonance imaging Lymphography. SLND: Sentinel Lymph Node Dissection.

Table 1. Patient characteristics.

Patient Characteristics.	
Patient age at operation (median, range)	64 (38–87)
Body mass index (median, range)	24.8 (19.1–43.8)
Preoperative tumor extent mm (median, range)	20 (5–120)
Days between injection and Surgery (median, range)	12 (0–140)
Laterality, number, %	
Right	41 (51.9)
Left	38 (48.1)
Previous breast surgery	
Right	21 (26.6)
Left	58 (73.4)
Previous axillary surgery	
Right	19 (24.4)
Left	59 (75.6)

Table 1. *Cont.*

Patient Characteristics.	
Neo adjuvant treatment	
Right	12 (15.2)
Left	67 (84.8)
Localization in the breast, number, %	
Upper outer	31 (39.2)
Upper inner	12 (15.2)
Lower outer	9 (11.4)
Lower inner	7 (8.9)
Central	7 (8.9)
Multicentric	11 (13.9)
Chest wall	2 (2.5)
Histological type (n = 79)	
Invasive ductal (n, (%))	66 (83.5)
Invasive lobular (n, (%))	11 (13.9)
Other Histology (n, (%))	2 (2.5)
Intrinsic Subtype (n = 79)	
Luminal A (n, (%))	36
Luminal B, erbb2− (n, (%))	20
Luminal B, erbb2+ (n, (%))	10
Non luminal erbb2+ (n, (%))	3
Triple negative (n, (%))	9
Type of surgery (n = 79)	
Wide local excision (n, (%))	28 (35.4)
Mastectomy (n, (%))	23 (29.1)
Oncoplastic breast conservation (n, (%))	28 (34.4)

MRI-LG was performed a median of 3 days after SPIO injection (range 1–12) and the MagUS with transcutaneous SLNB ± SLN clipping a median of 3 days (range 1–5) after MRI-LG. In all 73 patients where MagUS SLNB was performed, transcutaneous detection was successful and the SLN was located. Minimally invasive SLNB (MagUS CNB) retrieved lymphatic tissue with magnetic signal on the SentiMag® probe, and the presence of SPIO was confirmed on post-operative histopathology. At surgery, the node with signs of previous biopsy and/or clip was always retrieved. In one case, the lymph node that was biopsied was a non-sentinel node (i.e., ex vivo signal less than 10% of the signal of the SLN with the maximal signal), but the true SLN was just behind it and recovered during SLND.

Metastases on specimen pathology was found in 11 patients (11/73, 15.1%, 95% confidence intervals: 7.8; 25.4). MagUS identified all patients with SLN macrometastases (n = 10) and missed only one SLN with a micrometastasis, resulting in a FNR of 8.3% and an overall accuracy of 98.6% (Tables 2 and 3). In terms of diagnostic performance, when compared to the results of surgical pathology, MagUS performed very accurately (AUC: 0.955; 0.865, 1.000, $p < 0.001$) whereas AUS was not predictive at all (AUC: 0.505; 0.410, 0.601, $p = 0.916$).

Table 2. Comparison between MagUS and final pathology.

		Preoperative MagUS Assessment for Metastases		
		No *n*, (%)	**Yes *n*, (%)**	**Total *n*, (%)**
Metastases at histopathology	No	62 (98.4)	0 (0)	62 (84.9)
	Yes	1 (1.6)	10 (100)	11 (15.1)
Total		63 (100)	10 (100)	73 (100)

Mc Nemar's test, $p = 1.000$.

Table 3. Diagnostic performance of the MagUS technique.

	Rate	Lower 95% CI	Upper 95% CI
Sensitivity	90.9%	58.7%	99.8%
Specificity	100%	94.2%	100%
PPV	100%	69.1%	100%
NPV	98.4%	91.5%	99.9%
Accuracy	98.6%	92.6%	99.9%

The number of SLNs identified on MRI-LG (median 4, range 1–6) did not differ from the number of SLNs retrieved (median 3, range 1–6) (Wilcoxon signed rank test, $p = 0.331$) with high correlation (Cronbach's Alpha = 0.719; 0.481, 0.848, $p < 0.001$). Additionally, topographic concordance between MRI-LG, MagUS and SLND was 100%. In 63 patients (86%), the nodes were located medial to the lateral thoracic vein and caudal to the intercostobrachial nerve.

In patients receiving NAT, the MagUS allowed for accurate axillary mapping, identification and clipping of the true SLN prior to the initiation of NAT. After the completion of NAT, a median of 130 days (range 86–140) after SPIO injection, the SLNs were still visualized in MRI-LG and were detectable during surgery in all patients. There was excellent correlation between the number of SLNs identified on MRI (median 4, range 2–6) and the magnetic SLNs retrieved (median 3.5, range 1–6) with Cronbach's Alpha = 0.919; 0.699, 0.978, $p < 0.001$.

In patients with local recurrence after previous breast and axillary surgery ($n = 19$), MagUS showed either aberrant lymphatic outflow or no outflow in 9 patients (47.3%), preventing unnecessary ipsilateral axillary exploration. In the remaining 10 patients, both MagUS SLNB and subsequent surgery were successful.

4. Discussion

In this phase 2 trial, the MagUS technique (MRI-LG and MagUS) provided comparable results in accuracy and FNR with the standard of SLND. It was more accurate than the standard b-mode AUS in preoperatively detecting low-volume axillary disease. In this trial, it was demonstrated that accurate minimally invasive axillary staging can be achieved with a multimodal platform that can be modified to meet tailored patient needs.

SLND is not an indolent procedure and is related to short- and long-term morbidity such as postoperative pain, restricted shoulder range of motion, axillary web syndrome and lymphedema, as suggested in recent meta-analysis [13,14,30]. These findings indicate the need of establishing techniques for less invasive axillary staging that might result in less surgery, less subsequent postoperative complications and a reduction of costs and resources related with surgery [31,32]. Additionally, this MagUS workup can be performed in a wide timeframe and in an outpatient basis, as SPIO resides in the tissue a long period of time.

Recently, the necessity of surgical axillary mapping has been challenged in particular clinical scenarios. Observational data suggest that SLND may be safely omitted in older patients with primary tumors with small size and favorable biology [33–35]. The SOUND randomized trial examines whether a negative AUS can allow for the omission of SLND in patients with unifocal tumors < 2 cm planned for breast conservation and radiotherapy [36]. However, this approach does not take in consideration recent data that suggest that, in women with small tumors that are SLN negative, radiotherapy may be safely omitted nor that diagnosis of low-volume axillary disease, may allow for tailoring of radiotherapy or systemic treatment [6,9,37–39]. The results of the MagUS trial suggest that this technique may be used instead of SLND in selected cases.

It has been shown that 25% of patients considered as cN0 by AUS+/−FNAC will have a positive SLN in surgery. MagUS has the potential to correctly identify this low-volume axillary disease group, so that further treatment decisions may be tailored but without further axillary surgery, as it has been shown in landmark trials such as AMAROS,

ACOSOG Z0011 or, more recently, the RxPonder trial [6,9,40]. Reversely, in women with one positive lymph node on standard AUS, MagUS could assess the volume of axillary disease in a more accurate manner. This is a group that often harbors a higher nodal disease burden [41]. However, other studies show that this is explained by the fact that the sensitivity of AUS + FNAC increases significantly in patients with higher risk for nodal metastasis [42]. At the same time, up to 43.2% of this patient group, will be found to have two or less metastatic nodes, meaning that ALND will have been overtreatment [10]. If MagUS shows that there is only low-volume axillary disease, then the patient may have the possibility to avoid overtreatment and tailor treatment decisions may be made after discussion in the multidisciplinary meeting [43].

Subsequently, MagUS may also address issues regarding axillary staging in the setting of NAT, as it yields the potential of differentiating patients that are clinically node negative from those who are also SLN negative prior to NAT. In this manner, therapeutic decisions regarding the axilla, such as axillary radiotherapy may be better tailored, while its definitive role in this setting remains still to be elucidated [44,45]. At the same time, it may answer whether, in cN positive patients, the metastatic node is a sentinel or if, at presentation, there are non-sentinel metastases, which is suggestive of a higher axillary nodal burden. In this manner, it becomes safer to identify more appropriate potential candidates for axillary conservation post-NAT as recently suggested in the Lucerne toolbox [12]. Moreover, MRI-LG before and after NAT allows for an estimate of the number of SLNs in the axilla. This may address the problem of FNR after NAT, that has been discussed in landmark trials, such as Sentina and ACOSOG Z1071 [46–50]. In these trials FNR was shown to decrease with the removal of $\geq$3 nodes, including clipped nodes, if such, whereas double tracer was shown to increase detection rate [7,46–50]. In the present study, post-NAT MRI-LG showed uptake in the same SLNs, suggesting that SPIO did not migrate in higher nodal echelons during NAT. Intraoperatively, there was transcutaneous magnetic signal and SLNs were detected in all cases. It may be so that, a MagUS could be repeated after NAT to allow for more focused axillary evaluation, as standard AUS has not shown promising results in this setting [51]. As omission of axillary surgery post neoadjuvant is discussed in several breast cancer subtypes, provided that there is pathologic complete response (PCR) in the breast, MagUS could provide a safer manner to discuss omission of surgery, rather than, in case of non-PCR, performing SLND that will be subject to the risk of false negatives post NAT and after a previous excision in the breast [52,53]. A given restriction is that SPIO injection in the breast impairs the diagnostic accuracy of the MRI, suggesting that the tumor response should be performed with other modalities. Reassuringly, modalities, such as ultrasound and PET-CT have shown comparable accuracy in this setting, without the known risk of false positive findings from the MRI [54–57].

Evaluating nodal status for breast cancer after previous breast and axillary surgery is a challenge. SLN detection rate is lower and aberrant, extra-axillary lymphatic drainage is not unusual [26,58,59]. For this reason, the use of preoperative mapping by means of scintigraphy is recommended in this setting. However, whilst accurate, scintigraphy complicates logistics and this is why it recent data suggest that it is no longer necessary for patients without previous breast or axillary surgery undergoing upfront SLND [60]. MagUS has, in this setting, allowed for tailored patient treatment with flexibility, as the MRI-LG performed preoperatively, allowed in good time to know whether SLND would be attempted on the day of surgery. In this manner, logistics were facilitated, and treatment decisions could be tailored with more precision and accuracy.

The strictly controlled study design allowed for safe results, despite the absence of a control arm. However, this is a phase 2 trial and these results need to be refined and reproduced in a larger scale. Consequently, a phase 3 randomized controlled trial is needed prior to standardization and routine adaptation of the technique instead of surgical SLND. The results suggest that MagUS has the potential to provide a substantial niche to avoid axillary surgery. The cost of surgery is the most substantial, especially if one takes the expenses related with leave of absence, morbidity and complication risks into consideration.

Moreover, it is currently unclear whether the technique will always be implemented with the combination of an MRI and MagUS, something which might complicate and prolong the preoperative assessment of the patient. Finally if clinical MRI of the breast is intended, it should be performed first, to be followed by MagUs in another, different session. However, study results suggest that in women without risk factors for decreased ultrasound accuracy and transcutaneous magnetic probe detection (obesity, previous axillary surgery, etc.), MagUS and CNB were sufficient to accurately stage the axilla, suggesting that MRI is probably necessary in a small subgroup of patients (obesity, previous axillary surgery, etc.). This means that tailoring the technique to the specific patient will result in different routines and probably costs. Another substantial benefit is that this can be performed during the period between diagnosis a breast surgery, so that axillary mapping can be performed preoperatively and on an outpatient basis.

MagUS seems to be a method that can allow for alternatives to surgical axillary mapping. It comes to add to the armamentarium of other minimally invasive techniques that have previously been proposed [17,19,22,61] allowing for tailored axillary mapping in breast cancer. Its presumed advantages are the combination of different imaging modalities, together with that SPIO remains in the node a longer period, so as to allow for delayed SLND. Technique refinement and larger studies will allow for elucidation of the possibilities and its role in breast cancer diagnosis and treatment.

5. Conclusions

MagUS provides the niche for minimally invasive axillary mapping that can meet tailored patient needs and reduce diagnostic surgery. A phase 3 RCT is planned to further evaluate the technique.

Author Contributions: Conceptualization, A.K.; methodology, A.K.; software, A.K.; validation, A.K.; formal analysis, A.K.; investigation, A.J., E.P., S.A., D.V.B., F.W., S.E. and A.K.; resources, A.J., E.P., S.A. and D.V.B.; data curation, F.W. and A.K.; writing—original draft preparation, A.J.; writing—review and editing, A.J., E.P., D.V.B., S.A., F.W., H.L., S.E. and A.K.; visualization, A.J.; supervision, F.W., S.E. and A.K.; project administration, F.W. and A.K.; funding acquisition, A.K. All authors have read and agreed to the published version of the manuscript.

Funding: Institutional grants by Uppsala University and the Swedish Association for Breast Cancer ("Bröstcancerförbundet", www.brostcancerforbundet.se, ref. KDB1621/17) are acknowledged. Sponsors and funding bodies had no role in study design, data collection, analysis, or interpretation.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Helsinki Declaration of ethical principles involving human subjects and was approved by Uppsala University regional ethical committee (decision number 2017/063).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical considerations and data regulations.

Conflicts of Interest: The authors declare no conflict of interest.

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Paper II

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Article

A Comparison of Skin Staining after Sentinel Lymph Node Biopsy in Women Undergoing Breast Cancer Surgery Using Blue Dye and Superparamagnetic Iron Oxide Nanoparticle (SPIO) Tracers

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Citation: Jazrawi, A.; Wärnberg, M.; Hersi, A.-F.; Obondo, C.; Pistioli, L.; Eriksson, S.; Karakatsanis, A.; Wärnberg, F. A Comparison of Skin Staining after Sentinel Lymph Node Biopsy in Women Undergoing Breast Cancer Surgery Using Blue Dye and Superparamagnetic Iron Oxide Nanoparticle (SPIO) Tracers. *Cancers* **2022**, *14*, 6017. https://doi.org/10.3390/cancers14236017

Academic Editors: Nikolaos V. Michalopoulos and Nikolaos Arkadopoulos

Received: 3 November 2022
Accepted: 5 December 2022
Published: 6 December 2022

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Simple Summary: Both superparamagnetic iron oxide nanoparticles (SPIO) and blue dye (BD) have been reported to cause skin staining after breast-conserving surgery. SPIO is a novel tracer that has been shown to identify sentinel lymph nodes (SLNs) in patients with breast cancer. Our study was the first to compare the incidence and size of skin staining between the two tracers. We reported on these outcomes in a preplanned secondary analysis of a prospective clinical trial in which women received both SPIO and BD. This study investigated whether there was a difference in the incidence and size of skin staining between SPIO and BD after SLN-dissection. In all, 270 women were operated on with breast-conserving surgery and received SPIO, and 204 of these women also received BD. After 24 months of follow up, there was no statistically significant difference between the two tracers with regard to the size and incidence of skin staining.

Abstract: Superparamagnetic iron oxide nanoparticles (SPIO) are a tracer for sentinel lymph node (SLN) detection. In a preplanned secondary analysis of a prospective clinical trial (SentiDose) we reported on skin staining after SPIO and blue dye (BD) injections. For SPIO, either a 1.5 mL retroareolar injection on the day of surgery or a 1.0 mL peritumoral/retroareolar injection 1–7 days before surgery was given. A 1.0 mL sub-/intradermal periareolar injection of BD was also administered to all these women. Staining was then assessed at 6, 12 and 24 months after surgery. A total of 270 women received SPIO and were operated on with breast-conserving surgery. Of these, 204 women also received BD. A total of 58 (21.5%) women had an SPIO stain 6 months postoperatively with a median size of 6.8 cm^2 ($p = 0.56$), while 51 (25.0%) had a BD stain with a median size of 8.5 cm^2 ($p = 0.93$). The incidence and size of SPIO and BD staining decreased over time reciprocally. At 24 months, the incidence and median size of SPIO was 23 (8.6%) and 4 cm^2, respectively. For BD, the incidence was 14 (6.3%, $p = 0.13$), and the median size was 3.5 cm^2 ($p = 0.18$). There was, therefore, no statistically significant difference in the incidence or size of skin staining between SPIO and BD over time.

Keywords: sentinel lymph node biopsy; breast cancer; blue dye; superparamagnetic iron oxide; magnetic tracer; sentinel lymph node; skin staining

1. Introduction

Sentinel lymph node dissection (SLND) constitutes the standard of care for axillary staging in patients with clinically node-negative early breast cancer, as it is accurate and associated with a decreased morbidity compared to the historical standard of axillary lymph node dissection (ALND) [1–5]. Novel tracers have been developed to overcome the limitations of radioactive isotope technetium99 (Tc^{99})- and Patent Blue V® (BD)-based detection, such as a short Tc^{99} half-life, strict regulations in handling and disposal, access to medical facilities and allergic reactions [6–9]. SPIO is a tracer with a comparable performance in previous studies and meta-analyses [6–9]. It is logistically convenient to use, as the timing of its administration is flexible [10–12].

Skin staining after an SPIO injection has previously been a concern as has been the case with BD [10,11,13,14]. However, the bulk of studies reporting on SPIO investigated this outcome after a retroareolar superficial injection of 2.0 mL of SPIO diluted in 3 mL of NaCl [10,11]. Since then, other studies have reported on smaller doses of SPIO (undiluted 2.0, 1.5 and 1.0 mL) in different time frames and different injection sites (peritumoral) [15]. In the SentiDose multicenter trial [15], patients received either 1.5 mL in the subareolar area less than 24 h before surgery or 1.0 mL 1–7 days before surgery. The injection site was left to the discretion of the operating surgeon. All patients however received Tc^{99} +/− BD as a background control. In this study, the SLN detection rates at lower doses were found to be comparable to Tc^{99} +/− BD. Following a 1.5 mL retroareolar injection, the detection rate was 97.5%, and it was 100% after a 1.0 mL peritumoral injection. This was comparable to Tc^{99} +/− BD. The longitudinal follow-up of skin staining after breast-conserving surgery with SPIO and BD were predefined secondary endpoints.

The primary outcome of this predefined analysis from the SentiDose study (SentiColor subprotocol) was to report on the incidence, duration and size of skin staining in the SentiDose patient population injected with both SPIO and BD. The secondary outcomes were to determine the predictive factors for SPIO staining and to investigate if different injection techniques could prevent skin staining.

2. Materials and Methods

2.1. Patient Selection

Between 2017 and 2019, all women in the SentiDose trial [15] undergoing breast-conserving surgery (BCS) and SLNB at six Swedish Hospitals were recruited into this study. Both SPIO and Tc^{99} were used in all women, while BD was used as an adjunct according to local routines or surgeon preferences. Inclusion criteria were primary breast cancer (cT0–2cN0cM0) and Eastern Cooperative Oncology Group (ECOG) performance status 0–2. Women with previous ipsilateral breast or axillary surgery and/or radiation and neoadjuvant chemotherapy were excluded. Pregnant women, patients with iron sickness, patients who underwent mastectomy either primarily or within six months of initial surgery were excluded in this follow-up part of the SentiDose trial. The study was approved by the Uppsala University regional ethics committee (Decision Number 2017/063).

2.2. Methods

2.2.1. Procedure

In the SentiDose trial, SLN detection rates were compared after injecting two different doses of SiennaXP®/Magtrace® (Sysmex Europe, Hamburg, Germany) at different time points using different injection techniques [15]. SPIO was injected either as a 1.5 mL retroareolar injection at least 20 min preoperatively on the day of surgery or as a 1.0 mL peritumoral/retroareolar injection one to seven days before surgery. The 1.5 mL dose was followed by a five-minute massage, whereas the massage was optional for the 1.0 mL dose. As a control measure, all the women also received Tc^{99} +/− BD according to each site's routine. BD was given as a 1.0 mL sub-/intradermal or retroareolar injection.

The Sentimag probe was used during surgery to localize the SLN, and a gamma probe was subsequently used to identify any residual SLNs with radioactive signal. All magnetic,

radioactive, blue, or brown SLNs were excised. The conventional cutoff of 10% of the SLNs with the highest signal (SPIO or Tc^{99}) was implemented to define additional SLNs.

2.2.2. Data Collection

Patient and tumor characteristics are presented in Table 1. The incidence of skin staining and the size of the staining in square centimeters (cm^2) were followed up by telephone interviews 6, 12 and 24 months after surgery. If there was no staining, brown (SPIO) or blue (BD), on the first visit three to four weeks after surgery, no further follow-up was conducted. Follow-up was also ended when staining disappeared or at 24 months. Mean staining size calculations included only women with a stain at each time point. SLN detection rates have been reported elsewhere [15]. This manuscript was prepared according to the Strengthening the Reporting of Observational Studies (STROBE) Statement [16].

Table 1. Patient and tumor characteristics in women undergoing breast-conserving surgery and sentinel lymph node biopsy.

Patient Characteristics	
Patient age at operation (median, range)	64 (38–87)
Body mass index (median, range)	26.3 (16.8–49.3)
Menopausal status, *n* (%)	
Premenopausal	54 (20)
Postmenopausal	215 (79.6)
Missing data	1 (0.4)
Preoperative tumor size, mm median (range)	16 (0–93)
Tumor localization, *n* (%)	
Upper outer	130 (48.1)
Upper inner	51 (18.9)
Lower outer	32 (11.9)
Lower inner	24 (8.9)
Central	33 (12.2)
Histological type, *n* (%)	
Invasive ductal	197 (73)
Invasive lobular	41 (15.2)
Other histology	32 (11.8)
Type of axillary surgery, *n* (%)	
Sentinel lymph node dissection	265 (98.1)
Axillary lymph node dissection	5 (1.9)

2.2.3. Statistical Analysis

Staining was analyzed in women with BCS. Descriptive statistics were performed with means (95% confidence interval) or medians (range) of continuous variables, and, depending on data distribution, statistical analyses were based on medians. Continuous data were analyzed using nonparametric tests. Dichotomous data were analyzed with Pearson chi-square for nonpaired observations and McNemar's test for paired observations. Spearman´s rho test was used to measure the correlation between predictive factors for skin staining. Data analyses were performed using SPSS® (V 26.0. Armonk, NY, USA, IBM Corp.).

3. Results

In total, 271 women were operated on with BCS in the SentiDose trial, and one woman was later excluded due to a conversion to a mastectomy. Patient characteristics are presented in Table 1. SPIO was given to 270 patients. A total of 129 of these had a 1.5 mL retroareolar injection on the day of surgery, 71 patients had a 1.0 mL retroareolar injection 1–7 days before surgery, and 70 patients had a 1.0 mL peritumoral injection 1–7 days before

surgery. In addition, 204 of the 270 women also received BD (76%) (95% confidence interval 0.70–0.81).

At six months, 58/270 (21.5%) women had an SPIO skin stain with a mean size of 12.6 cm^2 (95% confidence interval 5.8–19.5) and a median of 6.8 cm^2 (range of 1–88). Between the 6- and 12-month controls, one woman had a mastectomy, and one died. Both of these women had an SPIO stain but no BD stain at 6 months. The corresponding data for SPIO staining at 12 and 24 months were 41/268 (15.3%) with a mean size of 5.9 cm^2 (95% confidence interval 1.3–10.4) and a median of 4 cm^2 (range of 1–28 cm^2) and 23/268 (8.6%) with a mean size of 6.4 cm^2 (95% confidence interval 0.7–12.0) and a median of 4 cm^2 (range of 0–20 cm^2) (Table 2). At six months, 51/204 (25%) women had a BD skin stain with a mean size of 10.8 cm^2 (95% confidence interval 4.4–17.1) and a median of 8.5 cm^2 (range of 3–25 cm^2). Between the 6- and 12-month controls, one woman with a BD stain but without an SPIO stain was lost to follow-up. The corresponding data for BD staining at 12 and 24 months were 32/201 (15.9%) with a mean size of 4.2 cm^2 (95% confidence interval 1.4–7.1) and a median of 4 cm^2 (range of 0–9 cm^2) and 13/201 (6.3%) with a mean size of 4.9 cm^2 (95% confidence interval 0.4–9.4) and a median of 3.5 cm^2 (0–15 cm^2) (Table 2). When comparing the incidence and size of the skin staining with SPIO and BD after 6, 12 and 24 months, there was no statistically significant difference between the two tracers (Table 2). There was a significant reduction in the incidence of SPIO-induced skin staining between 6 vs. 12 months (21.5% vs. 15.3%; *p*-value of >0.0005) and 12 vs. 24 months (15.3% vs. 8.6%; *p*-value of >0.0005). The trend was similar for BD with the following corresponding numbers 25% vs. 15.9% and 15.9% vs. 6.3% with a respective *p*-value of >0.0005 for both comparisons.

Table 2. Incidence and median size of postoperative skin staining in women treated with breast-conserving surgery and injected with superparamagnetic nanoparticles of iron oxide (SPIO) and Blue Dye (BD) for sentinel lymph node detection.

Staining	SPIO *n* = 270	BD *n* = 204	*p*-Value
Incidence, number (%)			
6 months	58 (21.5%)	51 (25%)	0.556 [a]
12 months	41(15.3%)	32 (15.9%)	0.430 [a]
24 months	23 (8.6%)	13 (6.3%)	0.132 [a]
Median Size, cm^2 (range)			
6 months	6.8 cm^2 (1–88)	8.5 cm^2 (3–25)	0.925 [b]
12 months	4 cm^2 (1–28)	4 cm^2 (0–9)	0.345 [b]
24 months	4 cm^2 (0–20)	3.5 cm^2 (0–15)	0.176 [b]

[a] McNemar test; [b] Wilcoxon signed rank test.

With regard to SPIO dosing, there was a significant difference between the 1.5 mL and 1.0 mL SPIO cohorts with reference to the incidence of skin staining at 6 months; there was an incidence of 34/129 (26.4%) versus 24/141 (17%) ($p = 0.011$) (Table 3). The corresponding numbers at 12 and 24 months were 23/128 (18%) and 13/128 (10.2%) versus 18/140 (12.9%) and 10/140 (7.1%) ($p = 0.05$ and $p = 0.034$, respectively). The median size of the skin staining for the separate volumes and injection techniques for SPIO are shown in Table 3. Only a small portion of women had a 1.0 mL peritumoral injection, but, in this subcohort, we noted the lowest incidence and the smallest stains (see Table 3). However, due to the low numbers, we did not look at the statistical differences between the 1.5 mL injection cohort and the 1.0 mL retroareolar injection cohort.

Table 3. Incidence and median size of SPIO staining postoperatively by injection site and volume.

	Injection Site		
SPIO stain	Retroareolar 1.5 mL, *n* = 129	Retroareolar 1.0 mL, *n* = 71	Peritumoral 1.0 mL, *n* = 70
6 months	34/129 (26.4%)	16/71 (22.5%)	8/70 (11.4%)
12 months	23/128 (18%)	12/70 (17.1%)	6/70 (8.6%)
24 months	13/128 (10.2%)	8/70 (11.4%)	2/70 (2.9%)
Median Size, cm^2 (range)			
6 months	8.5 cm^2 (1–64)	6 cm^2 (1–88)	8.5 cm^2 (1–25)
12 months	4 cm^2 (1–28)	4 cm^2 (1–28)	6.8 cm^2 (3–9)
24 months	5 cm^2 (0–20)	4 cm^2 (0–20)	4.0 cm^2 (4)

A low BMI showed a statistically significant positive correlation with skin staining at 6 months of 0.176 (Spearman's rho) (95% confidence interval 0.054–0.292) ($p = 0.004$), but the difference had disappeared at 24 months to 0.098 (Spearman's rho) (95% confidence interval 0.026–0.218) ($p = 0.111$). Figure 1 below shows a patient who had both SPIO and BD staining at 6 months.

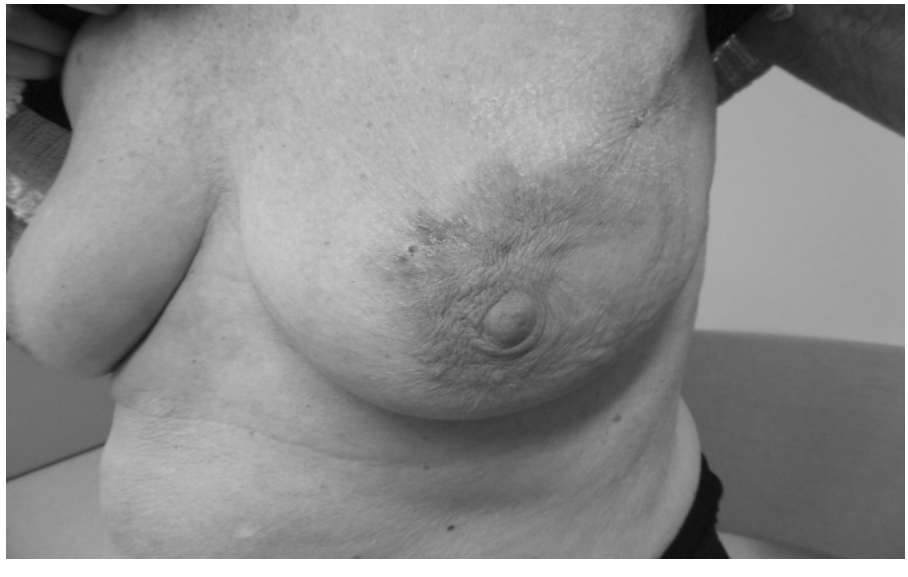

Figure 1. Illustrates a woman 6 months postoperative who received both SPIO and BD. The blue color represents BD staining. The brown color represents SPIO staining.

4. Discussion

This study is, to our knowledge, the largest prospective study in which the incidence of skin staining with both SPIO and BD were followed up in parallel. In this study, more than 200 women received both SPIO and BD. There were no significant differences in incidence or size of skin staining when comparing SPIO and BD as tracers for SLNB. After 6 months, the incidence was 21.5% with a median size of 6.8 cm^2 for SPIO and 25% with a median size of 8.5 cm^2 for BD. Both SPIO and BD stains diminished in a similar fashion over time. The incidence and median size of SPIO staining at 24 months was 8.6% (4.0 cm^2) and 6.3% (3.5 cm^2) for BD.

A lower volume of SPIO resulted in lower rates and smaller sizes of skin staining that disappeared faster. A peritumoral injection resulted in a lower incidence of skin staining compared to a retroareolar injection. The lowest incidence and smallest skin stains were noted when a 1.0 mL peritumoral injection was used. However, no statistical analysis was carried out in this subgroup due to the low number of women in the group. Further, a low preoperative BMI was a predictive factor for an increased risk of skin staining. A possible explanation is that the dispersion of SPIO in a larger breast seems to result in less uptake by the skin lymphatics and the removal of the bulk of SPIO when performing the BCS.

In earlier studies, 35 to 41% of women were reported to have BD staining at 12 months along with 8.6% after 36 months. However, none of the women reported a cosmetic or psychological problem relating to the BD staining [13,14]. Long-lasting skin staining has previously been reported after injection of SPIO. In the SUNRISE study by Rubio et al., [17] a retroareolar injection of 1.0–1.5–2.0 mL of SiennaXP was used, and 70.3% of the women undergoing a breast-conserving surgery reported discoloration one month after surgery. The staining diminished over time, and the majority of women did not regard the SPIO discoloration as a problem. It has also been shown that by modifying the injection technique, discoloration can be reduced. In an earlier cohort we showed that a deeper peritumoral injection reduced SPIO staining compared to a retroareolar injection: 37.8% and 67.3%, respectively [11].

The preoperative injection of SPIO compared to perioperative administration was associated with the identification of more SNs, and no extra intraoperative time or a massage at the injection site was required for the tracer to migrate to the axilla. This resulted in a shorter operating time with no consequent problems relating to tracer spillage or the diminished visualization of the SN as experienced with other methods such as t ICG [10].

A further concern with SPIO usage is the likelihood of MRI artifacts due to SPIO´s paramagnetic properties [18,19]. A peritumoral injection results in the excision of most of the SPIO used during surgery as discussed by Ghilli et al. [20]. The latter may result in a reduction of MRI artifacts although this remains to be proven. Our research group is currently addressing this issue in the prospective POSTMAG MRI trial [21].

A potential limitation in our study was the possible risk of reporting bias. The follow-up was conducted by telephone interviews. The initial status of staining at the first follow-up at two to four weeks after surgery was documented in the clinical register form (eCRF). If the medical record clearly stated that no stain was visible, then no further follow-up was initiated. Furthermore, the study protocol did not mandate the use of BD. While this restricted the numbers in the study, it was more in line with real world data that show that surgeons familiar with the isotope are often hesitant about adding BD to avoid its known adverse effects. It might have been that the use of BD in all the patients could have affected the reported outcomes, but this was a conscious decision of the investigators to align with optimal patient outcomes.

5. Conclusions

To summarize, no differences in either incidence or size of skin staining were noted when comparing SPIO and BD after 6, 12 and 24 months of follow up. For both tracers, the staining diminished or disappeared in a similar manner over time. A lower dose of SPIO resulted in a lower incidence, a smaller size and faster diminishing of staining. With the benefits of no need for nuclear medical facilities, detection rates similar to the dual technique and the possibility to inject well before surgery, SPIO appears to be an appealing tracer choice for sentinel lymph node surgery.

Author Contributions: Conceptualization, F.W.; methodology, F.W.; software, A.K.; validation, A.K.; formal analysis, A.K. and A.J.; investigation, A.J., F.W., M.W. and A.K.; resources, A.J., S.E. and M.W.; data curation, F.W. and M.W.; writing—original draft preparation, A.J.; writing—review and editing, A.J., M.W., C.O., A.-F.H., L.P., A.K., S.E. and F.W.; visualization, A.J.; supervision, F.W., S.E. and A.K.;

project administration, F.W. and A.K.; funding acquisition, S.E. and F.W. All authors have read and agreed to the published version of the manuscript.

Funding: Sysmex Europe GmbH and Endomagnetics, Cambridge, UK, provided the SentiMag® device and Magtrace® vials for the trial. Institutional grants by the Centre of Clinical Research, Region Västmanland, Uppsala University and the Västmanland Cancer Foundation are acknowledged. The study was partially funded by the Swedish Association for Breast Cancer (Bröstcancerförbundet). Sponsors had no role in the study design, data collection, analyses, or interpretation.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Helsinki Declaration of ethical principles involving human subjects and was approved by the Uppsala University regional ethical committee (decision number 2017/063).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical considerations and data regulations.

Conflicts of Interest: The authors declare no conflict of interest.

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Paper III

JAMA Surgery | Original Investigation

Magnetic Seed vs Guidewire Breast Cancer Localization With Magnetic Lymph Node Detection
A Randomized Clinical Trial

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IMPORTANCE Guidewires have been the standard for breast lesion localization but pose operative and logistic challenges. Paramagnetic seeds have shown promising results, but to the authors' knowledge, no randomized comparison has been performed.

OBJECTIVE To determine whether the combination of a paramagnetic seed and superparamagnetic iron oxide (SPIO) is equivalent to guidewire and SPIO for breast cancer localization and sentinel lymph node detection (SLND).

DESIGN, SETTING, AND PARTICIPANTS This was a phase 3, pragmatic, equivalence, 2-arm, open-label, randomized clinical trial conducted at 3 university and/or community hospitals in Sweden from May 2018 to May 2022. Included in the study were patients with early breast cancer planned for breast conservation and SLND. Study data were analyzed July to November 2022.

INTERVENTIONS Participants were randomly assigned 1:1 to a paramagnetic seed or a guidewire. All patients underwent SLND with SPIO.

MAIN OUTCOMES AND MEASURES Re-excision rate and resection ratio (defined as actual resection volume / optimal resection volume).

RESULTS A total of 426 women (median [IQR] age, 65 [56-71] years; median [IQR] tumor size, 11 [8-15] mm) were included in the study. The re-excision rate was 2.90% (95% CI, 1.60%-4.80%), and the median (IQR) resection ratio was 1.96 (1.15-3.44). No differences were found between the guidewire and the seed in re-excisions (6 of 211 [2.84%] vs 6 of 209 [2.87%]; difference, −0.03%; 95% CI, −3.20% to 3.20%; *P* = .99) or resection ratio (median, 1.93; IQR, 1.18-3.43 vs median, 2.01; IQR, 1.11-3.47; *P* = .70). Overall SLN detection was 98.6% (95% CI, 97.1%-99.4%) with no differences between arms (203 of 207 [98.1%] vs 204 of 206 [99.0%]; difference, −0.9%; 95% CI, −3.6% to 1.8%; *P* = .72). More failed localizations occurred with the guidewire (21 of 208 [10.1%] vs 4 of 215 [1.9%]; difference, 8.2%; 95% CI, 3.3%-13.2%; *P* < .001). Median (IQR) time to specimen excision was shorter for the seed (15 [10-22] minutes vs 18 [12-30] minutes; *P* = .01), as was the total operative time (69 [56-86] minutes vs 75.5 [59-101] minutes; *P* = .03). The experience of surgeons, radiologists, and surgical coordinators was better with the seed.

CONCLUSIONS AND RELEVANCE The combination of SPIO and a paramagnetic seed performed comparably with SPIO and guidewire for breast cancer conserving surgery and resulted in more successful localizations, shorter operative times, and better experience.

TRIAL REGISTRATION ISRCTN.org Identifier: ISRCTN11914537

Visual Abstract

Invited Commentary

Supplemental content

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JAMA Surg. doi:10.1001/jamasurg.2023.6520
Published online December 27, 2023.

Breast cancer screening, along with the improvement of imaging, have led to an increase in breast cancer diagnosis at a presymptomatic stage.[1] In the majority of these cases, breast-conserving surgery is feasible, but preoperative tumor localization is required.

The guidewire has been the most extensively used method of breast tumor localization due to its low cost and ease of use.[2,3] However, complications such as dislocation, migration, and patient discomfort have been described.[4-7] Apart from these complications, guidewire localization is restricted to the day of surgery, posing logistical challenges. These issues have led to the development of novel, wire-free localization devices[8] such as radioiodine seeds,[9-11] radar reflectors,[12,13] radiofrequency tags,[14,15] and paramagnetic/magnetic seeds.[16,17]

Most of these patients are clinically node negative and undergo sentinel lymph node dissection (SLND), which has traditionally been performed with a radioisotope (RI) with or without blue dye (BD). Although highly reliable, this combination poses challenges due to restricted access to nuclear medicine facilities, strict regulations, and risk of allergic reaction to BD, whereas the short half-life of the RI limits administration on the day of surgery or the day before, complicating logistics. Superparamagnetic iron oxide (SPIO) nanoparticles have shown comparable performance with an RI with or without BD with the additional advantage of a wider time frame of preoperative administration.[18-20] Perceived drawbacks of the method are skin staining and artifacts on postoperative magnetic resonance imaging (MRI)[21,22]; a recent meta-analysis,[20] however, suggests that peritumoral SPIO administration could address these concerns, without any compromise of SLN detection outcomes.

Previous large cohort studies have shown that paramagnetic seeds are advantageous in terms of operating time and ease of logistics compared with the guidewire and with comparable re-excision rates and specimen sizes; this, however, has not been validated in randomized clinical trials (RCTs).[16,23] At the same time, combining seeds with SPIO for a totally magnetic technique encompassing tumor localization and SLN detection has been investigated in small studies.[24,25] The technique was found feasible with the possible advantages of simplified logistics, as the localization procedure and tracer injection are detached from the day of surgery and, possibly, increased patient and physician satisfaction. Furthermore, both seed and SPIO are detectable by the same probe, avoiding multiple equipment in the operating room. Therefore, an RCT would elucidate these questions.

Key Points

Question Is the combination of paramagnetic seed and superparamagnetic iron oxide (SPIO) equivalent to guidewire and SPIO for breast cancer localization and sentinel lymph node detection (SLND)?

Findings This randomized clinical trial including 426 patients from 3 hospitals in Sweden found that a totally magnetic technique was equivalent to the combination of guidewire and SPIO in re-excision frequency, specimen volumes, and SLND. In addition, seed and SPIO resulted in shorter operative times and increased satisfaction among health care practitioners.

Meaning A totally magnetic technique is an effective option for breast cancer localization and SLND.

Methods

In the interest of higher external validity, the Magnetic Marker to Detect Primary Lesion and Sentinel Node in Breast Cancer (MAGTOTAL) trial was designed as a phase 3, open-label, pragmatic trial including centers with different levels of experience with the magnetic technique (Supplement 1). The trial was approved by the Uppsala Regional ethics committee and registered to a publicly available database. Enrollment took place between May 1, 2018, and May 1, 2022, at 3 hospitals in Sweden (Akademiska University Hospital, Uppsala; Västmanlands Hospital, Västerås; and Sahlgrenska University Hospital, Gothenburg). Adult patients with nonpalpable ductal cancer in situ (DCIS) or T1 to T3 invasive breast cancer who were scheduled to receive breast-conserving surgery and SLND were eligible for inclusion in the trial. Patients with small, diffusely palpable lesions requiring preoperative localization or multifocal/multicentric lesions amenable to breast conservation were also included. Exclusion criteria included intolerance or hypersensitivity to iron or dextran compounds, iron overload disease, pregnancy and lactation, inability to provide informed consent, and pacemakers or implantable devices in the ipsilateral chest-wall or shoulder. Participant race and ethnicity were not collected because there is not any known interaction between these and the outcomes examined in the trial. This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines for pragmatic trials.[26]

After oral and written informed consent, participants were randomly assigned with an allocation ratio of 1:1 in blocks of 8. The randomization was performed using the randomizeR package of R statistical software, version 3.5.1 (R Project for Statistical Computing).[27] The sequence was concealed in opaque envelopes until the intervention was assigned. During the COVID-19 pandemic, the protocol was amended to allow for tolerance and ensure that scheduled surgery would not be affected by randomization.

In the experimental arm, lesion localization was performed with the Magseed marker (Endomag), a 5-mm paramagnetic seed used for the localization of breast cancer lesions, and in the control arm, with a guidewire (Bard Peripheral Vascular Inc). Regardless of randomization, because SPIO dose and injection timing do not affect SLN detection, patients received 1 to 1.5 mL of Magtrace (Endomag), a nonradioactive liquid tracer containing iron oxide nanoparticles, dorsally to the tumor, at any point between the preoperative visit for surgical planning to the day of surgery, either simultaneously with lesion localization or not.[20] Following trial pragmatism, the placement of the marker and the administration of SPIO were to be performed according to local routines or case-by-case convenience, meaning that surgeons or radiologists could insert the paramagnetic marker with or without simultaneous injection of the liquid tracer preoperatively, whereas guidewires were exclusively inserted by a breast radiologist on the day of

the surgery or the day before. Both methods of localization were performed under local anesthesia, and accurate localization was verified radiologically. There were no prerequisites such as medical professional level (resident, fellow, consultant), minimum experience, or a completed learning curve for participating radiologists and surgeons. Specimen radiography was performed as per routine, and SLND was performed with the SentiMag probe (Endomag), a probe that can detect both the paramagnetic marker and the liquid tracer, adhering to the 10% of the maximum signal cutoff rule, to complete the procedure. Due to the nature of the intervention, masking was not possible.

The primary outcome measure was resection ratio for each marker in patients with negative margins. The resection ratio was defined as the actual resection volume (ARV) divided by the optimal resection volume (ORV), the latter being the assessed volume needed to excise the lesion with 1-cm margins. The ARV was derived from the fresh specimen weight with concomitant volume calculation, and the ORV was calculated based on preoperative radiology; in cases of discordance between different modalities, the largest measurement was used. Negative margins were defined as "no tumor on ink" for invasive cancer and 2 mm for DCIS. Secondary outcomes included SLN detection rate, adverse events, time to specimen excision, operative time, and ease of implementation by all involved health care practitioners (surgeons, radiologists, surgical coordinators), assessed by Likert scales (scored 0-10, with a higher score denoting higher satisfaction). A prespecified longitudinal analysis of patient-reported outcomes and quality of life evaluation as well as patient-reported experience measures and cost-effectiveness analyses will be reported elsewhere.

Statistical Analysis

According to the Swedish Breast Cancer Registry, the 3 participating sites had comparable re-excision frequencies, with a documented average between 4% and 7%. Therefore, a clinically meaningful improvement based solely on a new device was not expected. However, placing the paramagnetic marker and injecting SPIO in the same location could cause an overlapping signal, possibly leading to excision of larger specimens, a concern that would not apply with the guidewire. Available literature suggests that the resection ratio for guidewire-based excision ranges between 1.9 and 2.8.[23,28] The MAGTOTAL pilot study suggested that the totally magnetic technique for nonpalpable tumor localization and magnetic SLND used in the trial had a resection ratio of 1.5,[25] whereas a nonrandomized comparison of guidewires and paramagnetic seeds with isotope-based SLND found comparable ratios (1.92 vs 1.67) with comparable re-excision rates (14 vs 16%).[23] In the absence of established reference values, we assumed a 2-sided equivalence of 0.3 difference in resection ratio as clinically meaningful (corresponding to a 30% difference in excised volume), with a 2-sided *P* value set at .05 and power of 80%, corresponding to 191 patients per arm. This population also satisfied the hypothesis of noninferiority in re-excision rates for a standard of 4% by a 5% margin, and an additional 10% was included per arm.

Table 1. Patient Characteristics

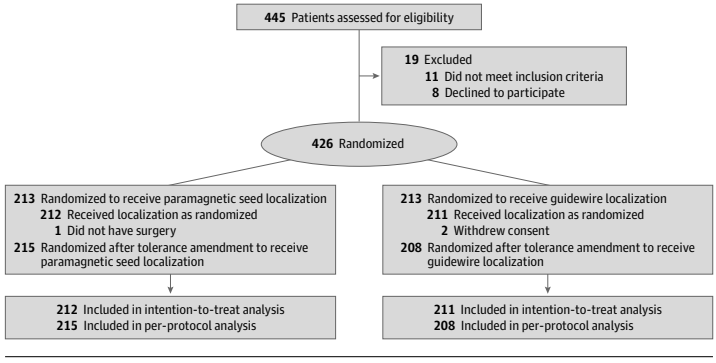
Characteristic	Allocation arm	
	Guidewire	Magnetic marker
Recruiting site, No. (%)		
Uppsala	121 (57.1)	115 (54.5)
Västerås	53 (25.0)	54 (25.6)
Gothenburg	38 (17.9)	42 (19.9)
Age, median (IQR), y	67 (56-72)	64 (56-70)
Body mass index, median (IQR)[a]	26.1 (23.8-29.8)	26.7 (24.1-29.8)
Screening detected lesion, No. (%)		
No	16 (7.6)	18 (8.5)
Yes	195 (92.4)	193 (91.5)
Palpable lesion, No. (%)		
No	199 (94.3)	196 (92.9)
Diffusely palpable	12 (5.7)	15 (7.1)
Preoperative MRI, No. (%)		
No	133 (75.1)	115 (66.5)
Yes	44 (24.9)	58 (33.5)
Lateralization, No. (%)		
Right breast	104 (49.5)	101 (47.9)
Left breast	106 (50.5)	110 (52.1)
Location, No. (%)		
Upper outer quadrant	119 (56.1)	115 (54.8)
Upper inner quadrant	33 (15.6)	40 (19.0)
Lower inner quadrant	22 (10.4)	20 (9.5)
Lower outer quadrant	29 (13.7)	20 (9.5)
Central/retroareolar	7 (3.3)	15 (7.1)
Multifocal/multicentric	2 (0.9)	1 (0)
Lesion size, median (IQR), mm	10 (8-15)	11 (8-15)
Histology, No. (%)		
IDC (NST)	170 (80.2)	174 (84.1)
ILC	27 (12.7)	16 (7.7)
DCIS	3 (1.4)	3 (1.4)
Other[b]	12 (5.7)	14 (6.8)
Nuclear grade, No. (%)		
Grade 1	52 (25.2)	63 (31.5)
Grade 2	123 (59.7)	105 (52.5)
Grade 3	31 (15.0)	32 (16.0)
Intrinsic subtype, No. (%)		
Luminal A	138 (69.0)	117 (59.7)
Luminal B, *ERBB2* negative	41 (20.5)	62 (31.6)
Luminal B, *ERBB2* enriched	4 (2.0)	6 (3.1)
Basal-like, *ERBB2* enriched	5 (2.5)	3 (1.5)
Triple-negative breast cancer	12 (6.0)	8 (4.1)
Primary systemic therapy		
Yes	7 (3.3)	7 (3.3)
No	205 (96.7)	204 (96.7)
Type of surgery		
Simple WLE	180 (84.9)	169 (81.3)
OPBCS level I	24 (11.3)	26 (12.5)
OPBCS level II	8 (3.8)	13 (6.3)

Abbreviations: DCIS, ductal cancer in situ; IDC (NST), invasive ductal cancer (nonspecific type); ILC, invasive lobular cancer; MRI, magnetic resonance imaging; OPBCS, oncoplastic breast-conserving surgery; WLE, wide local excision.

[a] Calculated as weight in kilograms divided by height in meters squared.

[b] Other refers to mucinous breast cancer, medullary breast cancer, tubular breast cancer.

Figure. MAGTOTAL Trial Consolidated Standards of Reporting Trials (CONSORT) Flow Diagram



Continuous variables were summarized as means with SD or medians with IQR, depending on data distribution. Comparisons were performed using a *t* test for means and the Mann-Whitney *U* test or the Kruskal-Wallis test for medians. Likert items were analyzed as ordinal data (median, IQR) and compared with nonparametric tests, as appropriate. Categorical variables were summarized as numbers and proportions with 95% CIs and comparisons were performed with Fisher exact test for unpaired data (Wald test for differences) and McNemar test for paired data. Multivariable regression analysis was performed if significant univariate associations of clinically relevant variables were demonstrated. Intention-to-treat and per-protocol analyses were performed for the primary end points, and per-protocol analyses were performed for the secondary end points. Effect sizes (odds ratios [ORs] for logistic regression and β coefficients for linear regression) were reported with 95% CIs. Analyses were performed with Stata 17 (StataCorp) and SPSS, version 28 (IBM Corp).

Results

Of the 445 assessed patients, 430 were deemed eligible. After consent withdrawal from 4 patients, 426 women (median [IQR] age, 65 [56-71] years; median [IQR] tumor size, 11 [8-15] mm) were randomly assigned to 2 well-balanced arms of 213 participants (**Table 1**). In the per-protocol analysis, the totally magnetic arm included 215 participants whereas the guidewire arm included 208 (**Figure**); however, the discordance was not significant (McNemar test: difference, −0.9%; 95% CI, −2.6% to 0.8%; *P* = .34).

Re-excision Rates, Resection Ratios, and SLND Outcomes

The overall re-excision rate was 2.90% (95% CI, 1.60%-4.80%). No differences were found between the guidewire and the paramagnetic seed (intention-to-treat analysis, 6 of 211 [2.84%] vs 6 of 209 [2.87%]; difference, −0.03%; 95% CI, −3.20% to 3.20%; *P* = .99 and per-protocol analysis, 6 of 206 [2.91%] vs 6 of 214 [2.84%]; difference, 0.07%; 95% CI, −3.10% to 3.30%; *P* = .95). Only the recruiting site was associated with re-excision rate in the univariable analysis (Uppsala: 0.9%; 95% CI, 0.2-2.7; Västerås, 3.8%; 95% CI, 1.3-8.7; Gothenburg, 7.6%; 95% CI, 3.2-15.0; *P* = .004), with logistic regression suggesting similar outcomes (1 [Reference] for free margins Uppsala; Västerås: OR, 0.219; 95% CI, 0.039-1.215; *P* = .08; Gothenburg: OR, 0.104; 95% CI, 0.020-0.529; *P* = .006).

The median (IQR) overall resection ratio was 1.96 (1.15-3.44). The outcomes were equivalent between the guidewire and the paramagnetic seed (intention-to-treat analysis: median, 1.93; IQR, 1.18-3.43 vs median, 2.01; IQR, 1.11-3.47; *P* = .70; per-protocol analysis: median, 1.96; IQR, 1.22-3.48 vs median, 1.97; IQR, 1.11-3.46; *P* = .82). In univariable analyses, resection ratio was associated with body mass index, recruiting site, diffusely palpable lesion, preoperative MRI, and type of breast conservation. In multivariable analyses, only body mass index, type of breast conservation, and recruiting site were found to affect the resection ratio (**Table 2**). Sites interacted with re-excision rates and were a surrogate of experience with the magnetic technique and (possibly) different operating styles; further analyses conducted showed that in the center with the longest experience with the probe, resection ratios and re-excision rates were the lowest. In this setting, the resection ratio for the paramagnetic seed was 0.3 lower than the guidewire (1.26 vs 1.57), but this did not reach statistical significance (eTable 1 in Supplement 2).

Overall SLN detection was (98.6%; 95% CI, 97.1%-99.4%). SLN detection rates were similar between the experimental and the control arms (203 of 207 [98.1%] vs 204 of 206 [99.0%]; difference, −0.9%; 95% CI, −3.6% to 1.8%; *P* = .72). A median (IQR) of 2 (1-3) SLNs were retrieved in both arms (*P* = .68). The prevalence of metastasis was also comparable (32 of 212 [15.1%] vs 21 of 204 [10.3%]; difference, −4.8%; 95% CI, −11.7% to 2.1%; *P* = .19) and did not affect detection rates or nodal yield.

Procedural Outcomes and Patterns of Implementation

Median (IQR) time to specimen excision was significantly shorter for the paramagnetic marker (15 [10-22] minutes vs

Table 2. Univariate and Multivariable Analysis for the Resection Ratio

	Univariate analysis		Multivariable analysis	
Site/variable	Resection ratio (IQR)	*P* value	β coefficient (95% CI)	*P* value
Per intention-to-treat analysis				
Magnetic marker	2.01 (1.11-3.47)	.70[a]	NA	NA
Guidewire	1.93 (1.18-3.43)			
Per-protocol analysis				
Magnetic marker	1.97 (1.11-3.46)	.82[a]	NA	NA
Guidewire	1.96 (1.22-3.48)			
Recruiting site			1.269 (0.763-1.775)	<.001
Uppsala	1.45 (0.78-2.13)	<.001[b]	1 [Reference]	NA
Västerås	3.33 (2.13-5.39)		2.478 (1.650-3.036)	<.001
Gothenburg	2.87 (2.00-4.38)		1.729 (0.805-2.653)	<.001
Body mass index[c]	0.307 (0.213-0.395)[d]	<.001[d]	0.181 (0.101-0.260)	<.001
Palpable lesion				
No	2.00 (1.18-3.52)	.03[a]	−0.957 (−2.491-0.577)	.22
Diffusely palpable lesion	1.60 (0.90-2.23)			
Preoperative MRI				
Yes	2.55 (1.50-4.27)	<.001[a]	−0.156 (−1.115-0.802)	.75
No	1.61 (0.95-2.83)			
Multifocal disease				
No	1.98 (1.18-3.46)	.13[a]	NA	NA
Yes	1.37 (0.56-3.15)			
Histology				
IDC (NST)	1.95 (1.15-3.54)	.53[b]	NA	NA
ILC	2.00 (1.04-2.81)			
DCIS	2.25 (1.57-3.06)			
Other	1.79 (1.07-2.85)			
Type of breast-conserving surgery			1.188 (0.475-1.901)	<.001
Simple WLE	2.07 (1.26-3.60)	<.001[b]	1 [Reference]	NA
OPBCS level I	1.37 (0.70-1.85)		−0.029 (−1.105-1.047)	.96
OPBCS level II	2.69 (1.05-5.57)		4.916 (3.367-6.466)	<.001
Overall	1.96 (1.15-3.44)			

Abbreviations: DCIS, Ductal cancer in situ; IDC (NST), invasive ductal cancer (nonspecific type); ILC, invasive lobular cancer; MRI, magnetic resonance imaging; reference category; NA, not applicable; OPBCS, oncoplastic breast-conserving surgery; WLE, wide local excision.

[a] Mann-Whitney *U* test.

[b] Kruskal-Wallis test.

[c] Calculated as weight in kilograms divided by height in meters squared.

[d] Spearman ρ (95% CI in parentheses).

18 [12-30] minutes; *P* = .01) as was the total operative time (69 [56- 86] minutes vs 75.5 [59-101] minutes; *P* = .03) (**Table 3**). These outcomes were associated with type of breast surgery on univariable analysis, too. Multivariable regression demonstrated that the use of a paramagnetic marker for lesion localization still resulted in shorter excision and operative times.

The rate of failed localizations in the trial was 5.9% (95% CI, 3.9-8.6). There were significantly more failed localizations in the guidewire arm compared with the paramagnetic marker (21 of 208 [10.1%] vs 4 of 215 [1.9%]; difference, 8.2%; 95% CI, 3.3%-13.2%; *P* < .001). From the 4 failed seed localizations, 1 was due to failed deployment and a guidewire was used instead; 3 were intraoperative due to superficial lesions, with the seed dislocated during dissection; in all cases, the tumor was identified with the SPIO magnetic signal. In the guidewire arm (n = 21), 8 localizations failed preoperatively due to tumor location or dense parenchyma and were replaced with a seed, and the remaining 13 were intraoperative dislocations, where resection was guided by the magnetic signal and brown staining of the SPIO. Re-excision was more common in failed localizations (2 of 25 [8%] vs 10 of 395 [2.5%]), but the difference was not significant (5.5%; 95% CI, −5.3% to 16.2%; *P* = .11) and did not differ per localization technique. Postoperative SPIO-induced skin staining at the postoperative visit was 10.5% (95% CI, 7.7%-13.8%) and was associated only with nonradiology-guided, free-hand peritumoral injection (17 of 108 [15.7%] vs 27 of 313 [8.6%]; difference, 7.1%; 95% CI, 0.04%-15.6%; *P* = .04; OR, 1.979; 95% CI, 1.032-3.795; *P* = .04). The rate of postoperative complications was 8.6% (95% CI, 6.1%-11.7%) and did not differ between the paramagnetic marker and the guidewire in frequency (9.8% vs 7.3%; difference, 2.5%; 95% CI, −3.3% to 8.3%; *P* = .45) or type (eTable 2 in Supplement 2).

There was significant variability in how lesion localization and SPIO administration were implemented (**Table 4**). However, none of these interacted with re-excision rates, resection ratios, or SLN detection. The localization time was shorter in the totally magnetic arm (median [IQR], 4 [3-5] minutes) than the guidewire arm (median [IQR], 5 [5-6] minutes) across all centers (*P* < .001).

Ease of Implementation

All the disciplines involved graded their experience on a Likert scale of 0 to 10 with higher scores denoting higher satisfaction. Overall, 15 surgeons, 4 radiologists, and 6 surgical coordinators were involved. Satisfaction was higher with the paramagnetic marker across all disciplines, with the difference

Table 3. Univariate and Multivariable Regression for Time To Specimen Excision and Operative Time

Marker/surgery type	Univariate analysis		Multivariable analysis	
	Median (IQR)	*P* value	β coefficient (95% CI)	*P* value[a]
Time to specimen excision, min				
Type of marker			3.768 (1.623-5.917)	.001
Magnetic marker	15 (10-22)	.01[b]	1 [Reference]	NA
Guidewire	18 (12-30)		3.763 (1.613-5.913)	.001
Type of breast-conserving surgery			4.913 (2.895-6.931)	<.001
Simple WLE	16 (11-24.5)	.01[c]	1 [Reference]	NA
OPBCS level I	20 (14-30)		5.079 (1.819-8.339)	.002
OPBCS level II	30 (11.5-36)		9.656 (4.831-14.479)	<.001
Total operative time, min				
Type of marker			10.227 (4.634-15.820)	<.001
Magnetic marker	69 (56- 86)	.03[b]	1 [Reference]	NA
Guidewire	75.5 (59-101)		10.442 (4.873-16.011)	<.001
Type of breast-conserving surgery			23.121 (17.782-28.460)	<.001
Simple WLE	69 (55-86)	<.001[c]	1 [Reference]	NA
OPBCS level I	78.5 (66-103)		15.505 (6.969-24.041)	<.001
OPBCS level II	115 (102-143)		54.236 (41.505-66.967)	<.001

Abbreviations: NA, not applicable; OPBCS, oncoplastic breast-conserving surgery; WLE, wide local excision.

[a] *P* value refers to the outcomes of the multivariable regression analysis (linear regression).

[b] Mann-Whitney *U* test.

[c] Kruskal-Wallis test.

Table 4. Patterns of Lesion Localization and Superparamagnetic Iron Oxide (SPIO) Administration

Localization/administration	Guidewire	Magnetic marker	*P* value
Localization modality, No. (%)			
Ultrasound	194 (93.3)	189 (92.2)	.71[a]
Stereotactic	14 (6.7)	16 (7.8)	
Days from localization to surgery, median (IQR)	0	5 (1-8)	<.001[b]
Time for lesion localization, median (IQR), min	5 (5-6)	4 (3-5)	<.001[b]
SPIO administration, No. (%)			
Surgeon[c]	86 (40.6)	22 (10.5)	<.001[a]
Radiologist	126 (59.4)	188 (89.5)	
SPIO volume, mL, No. (%)			
1.0	187 (89.0)	195 (92.9)	.23[a]
1.5	23 (11.0)	15 (7.1)	
Days from SPIO injection to surgery, median (IQR)	7 (0-15)	6 (1-8)	.04[b]
Single localization procedure (breast and axilla), No. (%)			
Yes	74 (34.9)	180 (85.3)	<.001[a]
No	138 (65.0)	31 (14.7)	

[a] Fisher exact test.

[b] Mann-Whitney *U* test.

[c] Surgeon denotes free-hand SPIO injection around the tumor.

being more pronounced for surgeons and coordinators (eTable 3 in Supplement 2).

Discussion

In this pragmatic, multicenter RCT, a paramagnetic marker was equivalent to the guidewire in terms of re-excision rates and excess tissue removal regardless of physician experience or localization routines. These results corroborate findings from previous cohort studies[16,23,29] and provide stronger evidence. Moreover, the implementation of a totally magnetic technique for lesion removal and SLND was favorable compared with the guidewire in terms of shorter operative times and easier logistics, as shown by the preferences of all health care practitioners that were involved.

One of the concerns expressed regarding the combination of a paramagnetic marker for lesion localization and a peritumoral SPIO injection was that the overlapping signal might lead to the excision of larger specimens.[24] Clearly, the combination is successful, regardless of SPIO injection location (subareolar or intraparenchymal in another quadrant of the breast), as smaller studies that tried to address this concern have suggested.[24,30] Reassuringly, resection ratios in this RCT were similar between the trial arms, regardless of previous physician experience or practice patterns, suggesting that adaptation is safe. Moreover, in the center with the highest experience, the resection ratio in the totally magnetic arm was 0.3 lower (1.26 vs 1.57) and one of the lowest reported in the literature with only 0.9% re-excisions. Although this did not reach statistical significance, it is indicative of how familiarization with the technique yields potential for precision surgery and

resection of smaller specimens. It seems that the totally magnetic technique for nonpalpable tumor localization used in the MAGTOTAL trial allows for the creation of a magnetic halo around the lesion, with the seed placed in the anterior aspect of the tumor, whereas the brown staining from SPIO in the surrounding tissue enables additional intraoperative visual navigation. This technique had lower failed localization rates than the guidewire, a finding similar to previous nonrandomized comparisons.[16] Furthermore, injecting SPIO close to the tumor, especially under ultrasonographic guidance, results in reduced skin staining because the bulk of SPIO is removed. This may contribute to minimizing postoperative MRI artifacts, which has been a concern with SPIO-guided SLND.[21,22] Currently, this hypothesis is being investigated in a prospective study from our group.[31]

Previous studies have investigated solely magnetic lesion localization and others solely magnetic SLN detection; the outcomes were comparable with the guidewire and, respectively, RI with or without BD.[16,20] Paramagnetic markers and SPIO both have the benefit of decoupling the respective procedure from the day of surgery[17,32,33]; however, if not combined, this benefit is not being fully utilized. In this RCT, the combination was successful and was positively met by all health care professionals involved in planning and performing breast cancer surgery. The present RCT showed that the totally magnetic technique for nonpalpable tumor localization is currently the only wire- and RI-free technique, to the authors' knowledge, where both lesion localization and SLN detection can be performed with the same probe, suggesting that the technique can be implemented in any setting.

Strengths and Limitations

Multiple, nonrandomized comparisons of the paramagnetic seed to the guidewire that had suggested similar outcomes served in providing baseline comparative evaluation. Therefore, an RCT was necessary for a definitive comparison of main efficacy and safety aspects, as suggested by the Idea, Development, Exploration, Assessment, and Long-term Follow-Up (IDEAL) Framework.[34] The trial did not investigate superiority, but equivalence, as the rationale that a device per se can improve outcomes had not been demonstrated in similar trials[11]; however, because the investigated technique had other presumed benefits, an RCT was necessary, as relevant literature suggests.[35] The pragmatic design ensures the external validity and that the intervention can be implemented with ease and flexibility and without expertise or previous familiarization.

On the other hand, the trial has several limitations. Differences in surgical style are hard to account for, which may be the reason for differences among sites, but, reassuringly, not between trial arms. Moreover, the inherent inability to mask the intervention may account for performance bias and the Pygmalion effect, but we chose end points that would minimize this as we investigated both re-excision and excess excision of healthy tissue at the same time.[36] Finally, cost efficacy analyses are still pending, but the shorter localization and operating time, along with the ease of preoperative planning, may compensate for the higher cost of the device.

Conclusions

In this RCT, a paramagnetic marker was equivalent to the guidewire in re-excisions and excised specimen volumes, with advantages of shorter operative time, safer localization, and preferable logistics. Additionally, familiarization with the technique may offer the potential for more precise surgery. Moreover, a totally magnetic technique for lesion localization and SLND relieves the health care system from the restrictions posed by guidewire localization or radioisotope-based methods, making it an attractive alternative for numerous and diverse clinical settings.

ARTICLE INFORMATION

Accepted for Publication: September 9, 2023.

Published Online: December 27, 2023. doi:10.1001/jamasurg.2023.6520

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Acquisition, analysis, or interpretation of data: All authors.
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Statistical analysis: Pantiora, Jazrawi, Wärnberg, Karakatsanis.
Obtained funding: Hersi, Wärnberg, Eriksson, Karakatsanis.
Administrative, technical, or material support: Pantiora, Jazrawi, Hersi, Abdsaleh, Ahlstedt, Molnar, Wärnberg, Eriksson.
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Conflict of Interest Disclosures: Dr Karakatsanis reported receiving grants from Endomag and honoraria from Pfizer, AstraZeneca, KUBTEC, and Resitu AB outside the submitted work. No other disclosures were reported.

Funding/Support: This work was supported in part by Uppsala University Hospital and supported by institutional grants from Uppsala University, Västmanlands Cancer Foundation, Swedish Breast Cancer Association, and the Centre for Clinical Research Region Västmanlands-Uppsala University grant LTV 851 021, 94 067, 967 823, 807 551.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 3.

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Paper IV

Prospective evaluation of MRI artefacts following breast conserving surgery and sentinel lymph node dissection with the magnetic technique.

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Abstract

Introduction:

Superparamagnetic Iron Oxide (SPIO) nanoparticles serve as a promising tracer for sentinel lymph node (SLN) detection in breast cancer. Concerns exist regarding potential artefacts on Magnetic Resonance Imaging (MRI) postoperatively. This prospective study explores the outcomes of postoperative MRI following breast conserving surgery (BCS) and SLND.

Materials and Methods:

Ninety-seven women with DCIS or invasive breast cancer underwent BCS and SLN detection with peritumoral SPIO injection with varying volumes (1, 1.5, or 2 ml), administered up to four weeks before surgery. Postoperatively, patients were followed with MRI and mammograms, and imaging outcomes were evaluated by four, independent breast radiologists, blinded to clinical data, using a predefined, standardized questionnaire.

Results:

The analysis encompassed 97 patients, revealing discordance among raters for "any artefact" (range: 24.1-74.4%; weighted average: 32.4%) and "SPIO specific artefact" (range: 12.0-49.4%; weighted average: 20.9%). The median area of “any artefact” was 9.24 mm^2 (iqr 4.72, 15.50) and SPIO specific artefact 9.88 mm^2 (iqr 5.32, 15.5). Likert scores indicated higher difficulty interpreting MRI (median: 3, IQR 2-3.5) compared to mammograms (median: 1.5, IQR 1-2, $p<$ 0.001). All six patients with local recurrence were successfully diagnosed on MRI by all raters. Logistic regression consistently identified free-hand SPIO administration as associated with artefacts.

Conclusion:

This prospective cohort study suggests that a targeted peritumoral SPIO injection can result in the removal of SPIO during lumpectomy and address the concerns for artefacts on postoperative MRI follow-up, in the selected patients that it may be required.

Introduction

Superparamagnetic Iron Oxide (SPIO) nanoparticles have extensively been evaluated as a tracer for sentinel lymph node (SLN) detection in breast cancer patients, performing comparably to the combination of Tc^{99} and blue dye (BD), without limitations in availability and access, strict regulations for handling and disposal or rare, but severe, anaphylactic reactions [1-3]. Multiple studies summarized in a recent meta-analysis have demonstrated that doses as low as 0.5 ml, injected peritumorally and >24 hours up to several weeks before surgery yield high detection rates, number of retrieved SLNs and accuracy [4]. Interestingly, this profile seems to result in reduced SPIO-induced skin staining compared to what had previously been described after a superficial, peri- or subareolar SPIO injection [5].

However, the presence of SPIO in the tissue results in artefacts on Magnetic Resonance Imaging (MRI) and, if this has to be performed as part of the diagnostic work-up, it has to precede SPIO injection [6-7]. The meta-analysis by Pantiora et al [4], pooled all the small retrospective series that addressed this and reported MRI artefacts in 70% of patients treated with breast conserving surgery (BCS) up to 46 months postoperatively. These studies, however, reported outcomes almost exclusively following a superficial SPIO injection and higher injection volumes than what nowadays constitutes the standard [6-9]. Moreover, they were retrospective, without standardized reporting of outcomes and sources of potential bias. Currently, there is no evidence to support the routine use of breast MRI following BCS [10] and MRI surveillance is considered in the preoperative setting for women with a known BRCA mutation and/or dense breasts [11-13]. However, compatibility concerns need to be addressed, for selected patients that MRI follow-up may be appropriate.

Previous research suggested that the presence of skin staining following a subareolar SPIO injection correlated strongly with the presence of transcutaneous magnetic signal [1]. Similarly, SPIO residue in the tissue should be expected to result in the present of artefacts on a postoperative MRI, as previous reports suggested. Therefore, absence of artefacts could be expected following a peritumoral SPIO injection, if the area with injected SPIO has been completely removed during surgery. Interestingly, this was demonstrated in a small series by Christenhusz et al, where no MRI artifacts were observed following an ultralow, intratumoral SPIO injection [7]. On the other hand,

available literature on the specificity of SPIO artefacts or the interpretation of a breast MRI following BCS and adjuvant treatment is lacking.

The aim of this prospective observational study was to explore the outcomes of postoperative MRI in patients that underwent BCS and SLND following peritumoral SPIO injection.

Methods

Patient selection

Patients >18 years of age with DCIS or T1-T3 invasive breast cancer planned for BCS and SLND treated between 2017-2022 were included in the study. Exclusion criteria were pregnancy, patients with pacemakers or other implantable devices in the chest-wall, or prosthesis in the shoulder, intraoperative or postoperative conversion to mastectomy, mental condition rendering the patient incapable of giving written informed consent, patient deprived of liberty or under guardianship. If patients had an indication for preoperative breast MRI and had consented to participation, the examination was performed before SPIO injection. The study was approved by the regional ethics board in Uppsala (Dnr: 2014.073, 2014.073.03, 2014.073.04). ICRTN registration number: 85167182

Methods

Data Collection and procedure

Patients planned for BCS and SLND received a peritumoral dose of SPIO (1, 1.5 or 2 ml) up to four weeks before surgery by the radiologist or the surgeon. On the day of surgery, the preoperative transcutaneous magnetic signal as well as any skin staining were registered. Surgery was performed according to routine, without aiming to excise the entire SPIO footprint around the excision area. The presence of brown discoloration on the cut surfaces and the residual cavity signal following tumour resection were documented. Transcutaneous signal and skin discoloration were also documented during the postoperative visit in the outpatient clinic, as well as in clinical follow-up after MRI and mammograms had been performed. A baseline breast MRI and mammogram were to be performed after three to six months postoperatively. Patients without artefacts, assessed by the principal study breast radiologist, and without postoperative transcutaneous signal were not followed up any further. For those with a

presumed artefact, clinical and radiological follow-up were prolonged up to five years with annual breast MRI and mammogram. The manuscript was prepared according to the Strengthening the Reporting of Observational Studies (STROBE) statement [14].

Mammogram and Breast MRI

Mammogram

Craniocaudal and Mediolateral Oblique projections were obtained per clinical routine.

MRI

All participants in the study were followed up with a postoperative MRI. Due to concerns for gadolinium induced kidney injury from the Swedish Ethical Review Authority, patients would undergo an MRI without contrast and contrast would be injected in the case of a clinical indication or concerns from the review of the synchronous mammogram [15].

The native scans were performed on either a 1.5 or 3 Tesla scanner (Ingenia R5.7; Philips Healthcare, Best, the Netherlands). Employing a standardized protocol without intravenous contrast, four sequences were generated, T1- and T2*-weighted images derived from a turbo spin echo, T1-weighted high-resolution isotropic volume examination (THRIVE) and a short tau inversion recovery (STIR) sequence. Scans with contrast were performed on a 3 Tesla scanner according to clinical routine.

Imaging assessment

Imaging was independently reviewed in separate, dedicated sessions by four experienced breast radiologists working in large volume centres from Sweden and the UK. Due to the lack of standardized classification of outcomes, the four radiologists agreed on the content and the formulation of a preformed questionnaire in a dedicated meeting with the study committee (Supplement 1). In summary, the questionnaire consisted of five items (questions, Q): Q1) presence of any artefact or postoperative change in the imaging, Q2) impact of this findings in imaging interpretation, Q3) whether the artefacts were deemed as SPIO specific, Q4) artefact size in two dimensions in mm (anteroposterior x mediolateral) and Q4) a Likert item (1-10) on the difficulty to assess the respective modality, with higher score denoting greater difficulty.

All imaging review sessions were supervised by a senior surgeon (AK) in a standardized fashion: each rater would review the MRI scan first followed by the synchronous mammogram; return to MRI was allowed, but not change in assessment or scoring, and the radiologists were blinded to any patient- or procedure-related data.

Sample Size Calculation and Statistical Analysis

Since ferromagnetic signal is present in all cases with skin staining, it should be expected that absence of discoloration or magnetic signal in the resection margins should imply SPIO-free parenchyma. This means that one would anticipate that all pairs of observations (post-excisional intra-operative background count and post-operative MRI) to be concordant. To test for this hypothesis, with an anticipated discordance rate (α) of 0.05 and a tolerance probability (β) of 95%, a minimum sample size of 93 patients would be required [16-17].

Continuous variables were summarized as medians with interquartile range (iqr). Categorical variables were summarized as numbers and proportions (%) with 95% confidence intervals (95% CI) and comparisons were performed with the Wald test. Likert items were analysed as ordinal data (median, iqr) and compared with non-parametric tests, as appropriate. Agreement statistics were performed using the Konger kappa for multiple raters with 95% confidence intervals (95% CI), Krippendorf's alpha for the Likert items and the intraclass coefficient (ICC) for continuous variables. Individual rater outcomes were pooled in a panel and items on the presence of artefacts were dichotomized ("yes" vs "no" and "unsure") for further analyses, to avoid arbitrary weighting that would result in non-clinically relevant groupings. Weighted outcomes summarizing panel ratings were summarized as medians (iqr) and mean ranks. Primary analyses were performed per imaging set (MRI and mammogram), whereas per patient analyses were performed for patient specific outcomes. Univariable and, if required, multivariable analyses were performed to investigate for associations with SPIO volume of injection, injection technique (free-hand vs image-guided), type of surgery, time from surgery to imaging to the questionnaire results. All tests were 2-sided and a p-value of 0.05 was considered significant. Data

analyses were performed using SPSS® (V 28.0. Armonk, NY: IBM Corp.) and Stata V17.

Results

Following written informed consent, 102 patients were recruited in the study. One patient underwent completion mastectomy and was subsequently excluded, one passed away before the first MRI was performed and three withdrew consent, leaving 97 patients and 159 breast MRI and mammogram examinations for analysis with a median of 15 months from surgery (range 3, 63). Patient characteristics are summarised in Table 1.

There was significant discordance among raters in the prevalence of both "Q1: any artefact" (individual ratings range 24.1-74.4%; weighted average 32.4%) and "Q3: SPIO specific artefact (individual ratings range 12.0-49.4%; weighted average 20.9%), with low to fair agreement outcomes (Table 2). A unanimous finding was that, what was described as artefact in MRI would most probably be interpreted as postoperative change on the mammogram, regardless of whether the presence of the artefact was considered to be SPIO specific, whereas there was high uncertainty on SPIO specificity (Supplementary Results, Tables 1&2). The median area (Q4) of "any artefact" was 9.24 mm^2 (iqr 4.72, 15.50) and SPIO specific artefact 9.88 (iqr 5.32, 15.5) for rater 1; the respective numbers were 4.38 (2.60, 10.00) and 4.20 (2.40, 9.00) for rater 2; 7.24 (3.00, 10.00) and 6.00 (2.56, 8.75) for rater 3; and 1.58 (0.09, 2.41) and 1.64 (0.12, 2.76) for rater 4. The intraclass coefficient was 0.694 (95% CI 0.608, 0.765).

With regards to how the artefacts affected MRI interpretation (Q2), the majority of responses was that the artefact did not affect characterization and assessment, affecting images "somewhat" or "not at all". Only in one MRI and for one single rater did the artefact impair image interpretation completely Table 2. The Likert score median for the difficulty of interpreting MRI was 3 (IQR 2, 3.5), significantly higher than the median for mammography (median1.5, IQR 1, 2, $p< 0.001$). Despite heterogeneity in Likert scores among individual raters, particularly R2, for MRI interpretation difficulty, all pairwise comparisons were not significantly different. Despite low inter-rater agreement on MRI difficulty assessment, the Likert scores were numerically comparable. On the other hand, all pairwise comparisons differed significantly in the assessment of the

mammograms. During follow-up, 6 patients presented with local recurrence; all were successfully diagnosed on MRI by all raters (Table 2).

Further on, we investigated the factors associated with the presence of "any artefact" and "SPIO specific artefact" for each rater individually, and for all raters, as a panel, using the weighted average of responses for the latter. The only factor that consistently retained significance on logistic regression for the panel (Table 3), but also for each rater individually (Supplement, Tables 3-6) was SPIO administration by a free-hand, non-radiology guided injection. Whilst neither intraoperative magnetic signal or postoperative transcutaneous magnetic signal retained significance on logistic regression, their absence always resulted in absence of SPIO specific artefacts.

Finally, due to discordance of interpretation among raters, a clinically meaningful follow-up of artefact prevalence or size over time was not possible; however, for raters 1 and 4, that had the highest "artefact rates" in their assessments, artefact prevalence reduced over time, though not in a significant manner whereas dimensions decreased in a statistically significant fashion (data not shown).

Discussion

In this prospective cohort study of breast MRI scans following BCS and SLND with a peritumoral SPIO injection, it was found that the weighted prevalence of SPIO related artefacts in the breast parenchyma was 20.9%, significantly lower than the 70% previously reported in the literature. Absence of residual SPIO signal in the resection cavity always resulted in absence of artefacts on MRI scans and the strongest predictor for artefacts was SPIO administration through a free-hand, non-radiology guided injection. Reassuringly, artefacts did not pose a challenge in image interpretation and all the local recurrences could be easily diagnosed by all participating radiologists.

Currently, no data suggest any survival advantage in the routine use of surveillance breast MRI after BCS over mammogram, thus its necessity is debatable, with the occasional exception of selected patients with very dense breast tissue or gene-mutation carriers treated with BCS [10-12, 18-20]. At the same time, mass-effect or surgical site contrast enhancement following BCS and radiotherapy are findings that do not have high specificity for benign vs

malignant pathology and may persist for years [21-23]. Moreover, the susceptibility artefact observed is iron specific, meaning that a haematoma could produce similar findings [24]. These facts may account for the observed interpretation heterogeneity among the raters with regards to the perception of artefact vs postoperative change, whether the artefacts were SPIO-specific or the artefact size. On the other hand, previous inter-rater agreement studies in the assessment for pathology have been shown to be, at best, moderate [25]. This suggests that the low agreement observed in the study should not be surprising, given that expert radiologists had to address a question previously unexplored in this fashion. Regardless, all raters felt that it was easy to interpret the MRI scans and, reassuringly, all recurrences in the study were easily identified in the MRI by all, suggesting that there was no clinically relevant hinder.

Previous reports on postoperative SPIO-related artefacts on follow-up MRI described outcomes following a periareolar SPIO injection, predominantly 2 ml that were diluted with 3 ml saline [4]. All were retrospective, institutional reports that raised awareness on this issue, but heterogeneity and differences in reporting did not allow for definitive conclusions. However, it seems that SPIO dose per se does not affect the prevalence of artefacts, if SPIO is not removed from the parenchyma. In a recent study from the Netherlands, 1 ml SPIO was injected in the parenchyma but in another quadrant, in an effort to avoid signal overlap with a magnetic marker that localized the tumour; in all fourteen patients, artefacts of a mean size of 41-44 mm were visible [26]. On the contrary, PostMag MRI is the first dedicated prospective study exploring whether resection of the bulk of SPIO may address artefacts. The study hypothesis coincided with the findings by Christenhusz et al., where, in the six patients that had received 0.1 ml SPIO intratumorally, no artefacts were present [7]. Moreover, the size of the artefacts was much smaller and absence of residual SPIO signal resulted in absence of SPIO-related artefacts; on the other hand, it seemed that small SPIO residuals, defined as discoloration of the cavity and intraoperative SPIO signal did not necessarily pose challenges to the interpretation. This is also supported by the fact that a free-hand parenchymal injection that is less accurate that radiology-guided injections, especially in impalpable lesions, was a risk factor for artefacts. This demonstrates that removing the bulk of SPIO injected is expected to address the issue of artefacts. This is expected optimally with a radiology-guided

peritumoral injection, so that the bulk of the SPIO footprint will be included in the resection specimen. All things considered, it seems that the optimal SPIO administration strategy should involve a radiology-guided peritumoral injection, as recently shown in the MagTotal randomised trial [27]. The possibility that smaller doses are explored is interesting as a smaller SPIO volume may be easier to be removed with the excision of the intended specimen volume. Therefore, the optimal SPIO profile involves defining a minimum effective dose for high SLN detection rates, intraoperative counts, nodal staining, and identifying an adequate median of SLNs to ensure low false-negative rates. Currently, a prospective study with a lower preoperative peritumoral SPIO dose is being planned by our research group.

The study has certain limitations: Firstly, intravenous gadolinium was not routinely administered in all patients, due to safety concerns from the Swedish Ethical Review Authority. Nevertheless, that did not affect image interpretation; on the contrary, raters felt that intravenous contrast facilitated interpretation. Moreover, the SARS-2 COVID pandemic affected the regularity of follow-up intervals, but this was a challenge that could not have been bypassed. Furthermore, the discordance among rater interpretation precluded any clinically meaningful time-to-event analysis that would allow for an exact estimate of how the artefacts behave over time, but this highlights the heterogeneity of interpretation of the findings. On the other hand, study strengths are that it is the first dedicated prospective study that informs on this research question, with predefined sample size, endpoints and possibility for a long follow-up, when deemed necessary. Instead of an arbitrary classification system, the radiological assessment was prospectively agreed upon among the study radiologists with regards to clinical relevance. The standardized, blinded assessment procedure ensured objectivity and reduced risk for bias. Furthermore, panel diversity, not by means of expertise, but different backgrounds and evaluation patterns, enhances the external validity of the present results. The latter is particularly noteworthy, given the variation in the routine performance of postoperative MRI compared to mammography [18]

In conclusion, this prospective cohort study suggests that a targeted peritumoral SPIO injection can result in the removal of SPIO during lumpectomy and address the concerns for artefacts on postoperative MRI follow-up, in the selected

patients that it may be required. Further studies should focus on the optimization of SPIO dose and administration profile, standardization of SPIO related artefact reporting, and adjusted sequences that may reduce the artefacts.

Author Contributions: Conceptualization, A.K ; methodology, A.K.; software, A.K.; validation, A.K.; formal analysis, A.K., A.J.; investigation, A.J., F.W., and A.K.; resources, A.J., S.E. and A.K.; data curation, A.K. and A.J.; writing—original draft preparation, A.J.; writing—review and editing, A.J., A.K., S.E., S.A., C-L.NG., A.Z., T.G. and F.W.; visualization, A.J.; supervision, F.W., S.E. and A.K.; project administration, A.K.; funding acquisition, S.E and F.W. All authors have read and agreed to the published version of the manuscript.

Funding and sponsorship: Institutional grants by the Centre of Clinical Research, Region Västmanland, Uppsala University, Västmanland Cancer Foundation and the Swedish Association for Breast Cancer (Bröstcancerförbundet) are acknowledged. Endomag, Cambridge, UK provided an institutional grant for the study. The study sponsors and funders had no role in the study design, data collection, analyses, or interpretation.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Helsinki Declaration of ethical principles involving human subjects and was approved by Uppsala University regional ethical committee (decision number 2017/063).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon reasonable request.

Conflicts of Interest: Dr Karakatsanis reported receiving institutional grants from Endomag and honoraria from Pfizer, AstraZenevea, KUBTEC and Resitu AB outside the scope of the submitted work. No other disclosures were reported. ICRTN registration number: 85167182

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Table 1. Patient characteristics

Age (years)*, median (iqr)		61 (50, 69)
Body Mass Index, BMI (kg/m^2)* (median, iqr)		25.1 (21, 29.6)
Breast volume (mm^3) *(median, iqr)		453 (309, 676)
Lesion Size (mm) *(median, iqr)		16 (12, 25)
Specimen Size (mm)*		38.8 (17.7, 60.4)
Lateralization, n,(%)	Right Breast	52 (53.6)
	Left Breast	44 (45.4)
	Missing data	1 (1)
SPIO injection site in the breast (n,%)	Periareolar	6 (6.2)
	Peritumoral	91 (93.8)
Massage after SPIO injection (n,%)	No	72 (74.2)
	Yes	25 (25.8)
Use of blue dye (n,%)	No	73 (75.3)
	Yes	24 (24.7)

Days between SPIO injection and surgery* (median, iqr)		5 (0, 7)
Histology (n,%)	IBC, Luminal A	48 (49.5)
	IBC, Luminal B, HER2-	22 (22.7)
	IBC, Luminal B, HER2+	7 (7.2)
	IBC, Basal-like, HER+	1 (1)
	IBC, Triple negative breast cancer	8 (8.2)
	DCIS/pLCIS	11 (11.3)
Location (n,%)	Upper Outer Quadrant	55 (56.7)
	Upper Inner Quadrant	12 (12.4)
	Lower Inner Quadrant	7 (7.2)
	Lower Outer Quadrant	13 (13.4)
	Central/Retroareolar	7 (7.2)
	Multifocal/Multicentric	3 (3.1)
Type of surgery (n,%)	Wide Local Excision	36 (37.1)
	Oncoplastic BCS (Volume displacement)	44 (45.4)
	Reduction mammaplasty	4 (4.1)
	Oncoplastic BCS (Volume replacement)	13 (13.4)
Successful SLN detection (n,%)	No	2 (2.1)
	Yes	87 (89.7)
	Not performed*	8 (8.2)
Number of SLNs retrieved* (median, iqr)		2 (2, 4)

Table 1. Patient characteristics. DCIS: Ductal cancer in situ. pLCIS: pleomorphic lobular carcinoma in situ. IBC: invasive breast cancer. iqr: interquartile range. SPIO: super paramagnetic iron oxide. BCS: breast conserving surgery. SLN: Sentinel lymph node. *DCIS cases in which SLND was not performed.

Table 3. Factors of association between prevalence of "any artefact" or "SPIO-specific artefact"

Presence of any artefacts							
		Univariable			Multivariable		
		Yes	No	p-value	OR	95% CI	p-value
BMI (kg/m^2)*		24.4 (22.95, 27.55)	26.7 (24.5, 30-1)	0.020**	0.949	0.847, 1.063	0.367
SPIO injection technique ***	Freehand	14 (73.7)	5 (26.7)	0.003****	Ref [1]		
	Image-guided	26 (33.8)	51 (66.2)		0.250	0.073, 0.864	0.028

Post resection signal*		2700 (852, 9200)	780 (0, 2536)	0.002**	1.0000	0.9999, 1.0002	3.318
Signal on postoperative visit*		2000 (393, 7584)	106 (0, 930)	<0.001**	1.0002	1.0001, 1,0003	0.021
Presence of SPIO specific artefacts							
		Univariable			Multivariable		
BMI (kg/m^2)*		23.7 (22.1, 24.8)	26.4 (23.6, 30.1)	0.009**	0.879	0.709, 1.091	0.244
SPIO injection technique ***	Freehand	14 (73.7)	5 (26.3)	<0.001****	ref. [1]		
	Image-guided	7 (9.1)	70 (90.9)		0.047	0.010, 0.217	<0.001
Post resection signal*		8000 (2000, 9999)	1090 (88, 2800)	<0.001**	1.0002	0.9999, 1.0004	0.120
Brown staining on cut surface	Yes	11 (47.8)		0.001****	1.588	0.290, 8.699	0.594
	No	10 (13.5)			ref. [1]		
Signal on postoperative visit*		2100 (200, 8900)	243 (0, 1810)	0.002**	1.0001	0.9999, 1.0003	0.196

*: median (interquartile range, iqr and range for the signals); ** Mann-Whitney U test; ***: n, %; ****: Fisher's exact test (2x2) or Chi-sqare (2x3 or 2x4). BMI: Body Mass Index, measured in kilograms divided by square metres (kg/m^2); CI: confidence intervals; ml: millilitres; mm: millimetres, OR: odds ratio; Ref.: Reference Category; y: years.

		R1	R2	R3	R4	p-value	kappa	95% CI	percent agreement	95% CI
n,%										
Any Artefacts in MRI	"Yes" vs "No" vs "Unsure"	116 (74.4)	38 (24.1)	54 (34.2)	91 (57.6)	<0.001	0.293	0.223, 0.363	58.3	53.5, 63.0
	"Yes" vs "No" and "Unsure"	116 (74.4)	38 (24.1)	54 (34.2)	91 (57.6)	<0.001	0.350	0.272, 0.429	66.0	61.5, 70.6
Artefact significance for any artefact	Artefact that does not impact image and interpretation at all	12 (10.3)	7 (18.4)	8 (14.8)	35 (38.5)	<0.001	0.355	0.288, 0.421	59.6	54.4. 64.4
	Artefact impairs the image interpretation somewhat but does not interfere with characterization and assessment	103 (88.8)	31 (81.6)	46 (85.2)	56 (61.5)					
	Artefact impairs image to make interpretation impossible	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)					
SPIO specific Artefacts in MRI	"Yes" vs "No" vs "Unsure"	43 (27.2)	19 (12.0)	49 (31.0)	78 (49.4)	<0.001	0.255	0.198, 0.312	50.1	45.3, 54.9
	"Yes" vs "No" and "Unsure"	43 (27.2)	19 (12.0)	49 (31.0)	78 (49.4)	<0.001	0.293	0.214, 0.372	69.5	65.3, 73.8
Artefact significance for SPIO specific artefacts	Artefact that does not impact image and interpretation at all	5 (3.2)	2 (1.3)	8 (5.2)	30 (19.5)	0.021	0.435	0.366, 0.504	91.5	89.7, 93.2
	Artefact impairs the image interpretation somewhat but does not interfere with characterization and assessment	37 (23.7)	17 (10.7)	41 (26.5)	48 (31.2)					
	Artefact impairs image to make interpretation impossible	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)					
How difficult was it to assess the MRI?*	median (iqr)	3 (2,3)	2 (1,3)	3 (2,4)	3 (1,4)	0.323**	0.18***	0.14, 0.22***	33.8	30.2, 37.6
	mean rank	2.42	2.15	2.86	2.58					
How difficult was it to assess the mammogram?*	median (iqr)	1 (1,1)	2 (1,2)	2 (2,2)	1 (1,2)	<0.001**	0.067***	0.003, 0,131***	49.5	45.2, 53.7
	mean rank	1.64	3.01	3.13	2.22					
Recurrence diagnosed in MRI	n (%)	6 (100)	6 (100)	6 (100)	6 (100)	1.000	1.000	1.000, 1.000	100.0	100.0, 100.0

Table 2. Outcomes of the radiological assessment. All summary data in columns R1-R4 are n,% except for *, which are Likert items summarized as medians (interquartile range; iqr). All p-values correspond to marginal homogeneity (Stuart Maxwell) test, apart from **, that correspond to Friedman's test for k medians. All Kappa values respond to Conger's kappa, apart from ***, that correspond to Krippendorf's alpha. MRI: Magnetic Resonance Imaging.