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

ALLAN JAZRAWI

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Abstract

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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

Allan Jazrawi, Department of Surgical Sciences, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden. Centre for Clinical Research, County of Västmanland, Västmanlands sjukhus Västerås, Uppsala University, SE-72189 Västerås, Sweden.

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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.

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Contents

Introduction 11

Background 13
- Breast carcinoma in situ 14
- Histopathological classification and intrinsic biological subtypes 15
  - Tumour differentiation (Elston–Ellis classification) 16
  - Tumour biology 16
  - Intrinsic biological subtypes 17
- Staging 17

Sentinel lymph node dissection 19
- Superparamagnetic iron oxide nanoparticles 20

Non-palpable breast tumours 21

General and specific aims 22
- Paper I 22
- Paper II 22
- Paper III 22
- Paper IV 23

Materials and methods 24
- Paper I 24
- Paper II 24
- Paper III 25
- Paper IV 25

Statistical analysis 27
- Paper I 27
- Paper II 27
- Paper III 27
- Paper IV 28

Ethical considerations 30

Summary of results 31
- Paper I 31
- Paper II 31
- Paper III 31
- Paper IV 32

Conclusions 33
Paper I 33
Paper II 33
Paper III 33
Paper IV 33
General discussion 34
Future perspectives 35
Sammanfattning på svenska 37
Mål med avhandlingen och delmål 38
Metod & Resultat 38
Acknowledgements 40
Appendices 43
Appendix 1: Supplement 2 for Paper III 43
Appendix 2: Supplementary material for Paper IV 45
Appendix 3: Errata for Paper I 54
Appendix 4: Errata for Paper II 54
References 55

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.

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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.

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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; OP-BCS, 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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