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Superparamagnetic iron oxide nanoparticles, a novel tracer in breast cancer surgery

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Abstract

Hersi, A.-F. 2021. Superparamagnetic iron oxide nanoparticles, a novel tracer in breast cancer surgery. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1778. 44 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1316-0.

The most common surgical choice of treatment in breast cancer is breast-conserving surgery (BCS) together with sentinel lymph node biopsy (SNB). Around 10% of breast cancer diagnosis are ductal carcinoma in situ (DCIS). Superparamagnetic iron oxide nanoparticles (SPIO) are a novel tracer for sentinel lymph node (SN) detection. The aim of this thesis was to investigate the unique applications and functionality of a magnetic approach in breast cancer surgery.

Paper I was a two-centre pilot study of 32 patients with non-palpable breast cancer who were scheduled for BCS together with SNB. They received SPIO for SNB and a magnetic seed (Magseed®) for localization of the breast tumour. All 32 patients underwent microscopically radical resection and SNB was successfully performed in all included patients.

Paper II was a multicentre prospective single-cohort study. It was a pre-planned interim analysis of 189 patients with "high-risk" DCIS who received SPIO at primary surgery but without performing SNB. If an invasive breast cancer was shown by the final histopathology report, the patient was scheduled for second surgery to undergo SNB. Because SPIO has a much longer half-life than the radioisotope, the magnetic signal at the second surgery was sufficient for detecting SNs; in fact, in patients with DCIS, it reduced from around 50% to 22%.

Paper III was a multicentre prospective trial. Two consecutive cohorts of patients with breast cancer scheduled for SNB (n = 328) were included. Lower doses of a refined SPIO suspension were tested in different time frames and injection sites. Analyses were performed as a one-step individual patient-level meta-analysis using patient-level data from a similar previous cohort (n = 206) as a third reference group. In 534 patients, the SPIO SN detection rates were comparable (97.5% vs. 100% vs. 97.6%, p = 0.11) and were noninferior to the dual technique.

Paper IV was a multicentre randomized pilot trial aimed to compare tumour localization in nonpalpable breast cancers using either Magseed® or guidewire in patients scheduled for BCS + SNB. All patients received SPIO for the SNB preoperatively. Patients who were randomized to the magnetic seed cohort received their Magseed® at the same time as the SPIO injection preoperatively while the guidewire placement was performed on the same day as surgery. In 207 patients, there were no significant differences in reoperation rate (3% in the magnetic seed cohort vs 7% in the guidewire cohort, p = 0.35).

Keywords: Breast Cancer, superparamagnetic iron oxide nanoparticles, SPIO, magnetic seed, magnetic surgery, sentinel node, sentinel node biopsy, nonpalpable, ductal carcinoma in situ, DCIS

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To my nieces and nephews, this is your uncle trying to run, so hopefully one day you can fly.

"Aqoon la'aani waa iftiin la'aane.
Waa aqal iyo ilays la'aane.
Ogaada ogaada dugsiyada ogaada.
Walaalayaal oo aada!"
-Abdillahi Qarshi

In loving memory of my dear little sister Jamila. Ilaahey ha o naaxaristo.

List of Papers

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

I. Hersi A-F, Eriksson S, Ramos J, Abdsaleh S, Wärnberg F, Karakatsanis A. A combined, totally magnetic technique with a magnetic marker for nonpalpable tumour localization and superparamagnetic iron oxide nanoparticles for sentinel lymph node detection in breast cancer surgery. *Eur J Surg Oncol*. 2019 Apr;45(4):544-549. doi: 10.1016/j.ejso.2018.10.064

II. Karakatsanis A* and Hersi A-F*, Pistiolis L, Olofsson Bagge R, Lykoudis PM, Eriksson S, Wärnberg F; SentiNot Trialist Group. Effect of preoperative injection of superparamagnetic iron oxide particles on rates of sentinel lymph node dissection in women undergoing surgery for ductal carcinoma *in situ* (SentiNot study). *Br J Surg.* 2019 May;106(6):720-728. doi: 10.1002/bjs.11110

III. Hersi AF, Pistiolis L, Dussan Luberth C, Vikhe-Patil E, Nilsson F, Mohammed I, Olofsson Bagge R, Wärnberg F, Eriksson S, Karakatsanis A. Optimizing Dose and Timing in Magnetic Tracer Techniques for Sentinel Lymph Node Detection in Early Breast Cancers: The Prospective Multicenter SentiDose Trial. *Cancers (Basel)*. 2021 Feb 9;13(4):693. doi: 10.3390/cancers13040693. PMID: 33572114; PMCID: PMC7914636

IV. Hersi AF, Jazrawi A, Laxander K, Abdsaleh S, Wärnberg F, Karakatsanis A, Eriksson S. A Randomised Clinical Trial comparing Magseed® with Guide Wire localization in nonpalpable breast cancer scheduled for Magtrace® assisted sentinel lymph node biopsy: The MagTotal RCT. *Manuscript*

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Contents

Abbreviations

AJCC	American Joint Committee on Cancer
ALND	Axillary lymph node dissection
BCS	Breast-conserving surgery
CIS	Carcinoma in situ
DCIS	Ductal carcinoma in situ
HER2	Human epidermal growth factor receptor 2
IBC	Invasive breast cancer
LN	Lymph node
LCIS	Lobular carcinoma in situ
MRI	Magnetic resonance imaging
SN	Sentinel lymph node
SNB	Sentinel lymph node biopsy
SPIO	Superparamagnetic iron oxide nanoparticles
Tc^{99}	Technetium-99 (medical radioisotope)
TNM	Tumor, node, metastasis (cancer staging system)
UICC	Union for international cancer control
WHO	World Health Organization

Introduction

Breast cancer is the most common malignancy in women worldwide. In Sweden as well as in the rest of the world, the incidence of breast cancer has been steadily rising. In 2017 in Sweden, there were 10 359 new cases of breast cancer diagnosed, which contrasts with around 2500 cases of breast cancer in 1960(1). Globally, the trend is similar; in 1960, about 600 000 breast cancer cases were diagnosed whereas around 2 million new breast cancer diagnoses were made in 2015(1-3). The incidence of ductal carcinoma in situ (DCIS) of the breast constituted about 10% of all breast cancers in Sweden in 2015(1). This preinvasive form of breast cancer should not be able to spread in theory, but the recommendations and guidelines in Sweden are to perform sentinel node biopsy (SNB), for staging purposes, when there are high-risk factors involved(1). The rationale behind this concept is not the belief that DCIS itself can spread but relates more to the observation that in 20% to 25% of cases with a preoperative diagnosis of DCIS, an invasive breast cancer (IBC) is discovered on final histopathology examination(1, 4).

Even though the incidence of breast cancer is much higher in the developed economies of the world, the cumulative mortality of breast cancer is still higher in the developing economies(3). This can be attributed to many factors such as the lack of facilities to enable early diagnosis and lack of adjuvant therapies among other factors.

Surgical treatment of breast cancer remains the primary treatment and staging method. However, an issue is determining the most effective and available method for performing SNB because this procedure is key in the staging of breast cancer and therefore in the choice of any subsequent adjuvant therapy. Sentinel lymph node detection has been performed using radioisotopes together with blue dye, which is widely regarded as the gold standard method(5, 6).

In the developed world, an issue has been emerging recently concerning breast cancer surgery. The tumours that are discovered are becoming smaller, primarily because of earlier detection. This means that at present 30% to 50% of breast tumours in the developed world are nonpalpable at diagnosis, meaning that the surgeon cannot feel the tumour that needs to be removed(7). This has

sparked a growing field of research on developing different methods to localize a nonpalpable tumours(8).

Background

Although the highest incidence of breast cancer is found in developed countries, the largest increase in incidence occurs within developing countries(3). At present, almost half of all new breast cancer cases and more than half of breast cancer-related deaths occur in under-developed countries(3). This steady increase in the incidence of breast cancer in the developing world is thought to be caused by increased awareness and detection, higher living standards, increased urbanization, fewer births per woman and adaptation of a more westernized lifestyle (e.g., higher body mass index, being older at conception, greater alcohol consumption)(3, 9, 10).

Mortality caused by breast cancer has been decreasing in Sweden, and the current overall 5-year survival rate is >90% and the 10-year survival rate is >80%(1). This reflects early detection but also the progress in adjuvant oncological therapy. The arsenal of adjuvant therapies has gone from selective oestrogen receptor modulators and basic chemotherapy regiments to more targeted therapy such as aromatase inhibitors and tumour-specific monoclonal antibodies. The use of adjuvant radiation therapy has also made headway; for example, the dosages and fields of radiation are more targeted.

This means that the morbidity associated with adjuvant therapy has decreased significantly in Sweden, which has allowed an expansion in the routine use of adjuvant therapy. Furthermore, improved adjuvant therapy allows older patients to be treated. Despite these changes, surgery remains the primary method for treating and staging breast cancer.

Breast cancer surgery has also evolved from the radical mastectomy, as described by Halsted, which was both mutilating and unnecessary in terms of the oncological resection margins now achieved by breast-conserving surgery (BCS), the predominant method of surgical treatment in Sweden today(11). The gold standard treatment of breast cancer is now widely regarded as being BCS together with SNB. This shift in surgical treatment is contingent on the patient receiving adjuvant radiation therapy; only this combination can provide oncological results equal to those of a mastectomy(12-14). Radical surgery with regard to IBC is defined as “no ink on tumor” meaning that there are no extra margins needed(15).

Breast cancer spreads predominantly via the lymphatic system. The sentinel lymph node (SN) is the node that first receives lymphatic drainage from the tumour area and is most likely to harbour metastasized tumour cells if the cancer has spread. This means that the SNB is a diagnostic and staging procedure. The concept of SNB was introduced and validated in the early 1990s(5, 6, 16) and its use has significantly reduced arm-related morbidity associated with axillary lymph node dissection (ALND)(17-19). SNB is now the standard technique used in patients with breast cancers with a clinically and radiologically negative axilla.

The gold standard technique for SN detection has been the "dual technique" of using a radioisotope together with blue dye. This technique has been validated in several studies and has a detection rate of 90% to 99%(16, 20). However, this method has several drawbacks, among which are the strict legislation on radioactive disposal, the short half-life of radioisotopes, and exposure of patients and health-care personnel to radiation. The blue dye is known to cause an anaphylactic reaction and can leave a temporary discolouration at the injection site in the breast(21).

Carcinoma in situ

Diagnosis of ductal carcinoma in situ (DCIS) constitutes around 10% of all breast cancers in Sweden and 10% - 20% of all breast tumours globally(11, 22). The term "carcinoma *in situ*" translates to "cancer in its place", meaning that, by definition, these cells display a cancer-like morphology, but the difference is that the tumour has not yet breached its cellular basal membrane, and therefore should not be able to spread. The natural development of these tumours, if left untreated, is thought to be that of invasiveness, although the time frame to develop invasive malignancy varies. There are only small differences in tumour biology between DCIS and invasive cancer, which supports the theory of its natural development(23).

There are two forms of carcinoma in situ (CIS): ductal and lobular. DCIS is the most common form. DCIS is the precursor to invasive ductal cancer while lobular carcinoma in situ (LCIS) is considered to increase the risk of developing IBC in the future(1). On the other hand, LCIS is more difficult to diagnose because of its inconspicuous growth pattern(1, 23, 24). There is a widespread consensus that DCIS should be surgically removed with >2 mm margin because of the known risk for invasiveness and for local recurrences associated with this precursor stage of invasive cancer(15, 25). However, there is no clear consensus about when a SNB should be performed. The national guidelines for this differ between many similar countries(1, 26-29). Most guidelines

consider the same factors but differ primarily in the way risk factors are weighted and prioritized(30).

DCIS is graded according to a classification system based on the histological appearance of the tumour cells. Two main aspects are key to the microscopic analyses: cytonuclear differentiation and architectural differentiation. The factors analysed include nuclear pleomorphism, number of mitoses, and nucleoli and chromatin appearance. The tumour is graded I-III: grade I is defined as tumour cells that display a high grade of differentiation, meaning that they resemble healthy cells the most, and grade III refers to a low grade of differentiation(31). The presence of necrosis and/or calcification is noted, and the size of the tumour is also considered. Swedish guidelines used to stipulate that "high-grade" DCIS, large tumour size and/or patients scheduled for mastectomy should be offered SNB. However, this has now been changed to a recommendation of confirming IBC before performing SNB (1, 32).

Histopathological classification and intrinsic biological subtypes

Invasive breast cancer can be classified according to the types of tumour cells (WHO classification), according to the degree of differentiation (Elston-Ellis classification), and according to the tumour biology (e.g., based on endocrine receptors, oncogenes, and cell proliferation).

WHO classification

- *Invasive carcinoma of no special type* (previously called *invasive ductal carcinoma*) accounts for 70-80% of all IBC. This form can have many different growth patterns (e.g., tubular, cribriform, solid) and is often associated with microcalcification(33).
- *Invasive lobular cancer* (e.g., solid, alveolar, pleomorphic) accounts for 5-15% of breast cancers and originates from the glandular lobule. It is the second most common form and is more difficult to diagnose because of its subtle growth pattern.(33)
- Malign stromal cancer (phyllodes tumour and sarcoma) accounts for 1-2% of all breast cancers and has a high risk of recurrence(33).

Histological differentiation (Elston-Ellis classification)

This 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 (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. Each variable is scored individually on a scale of 1-3 (1 being the best and 3 the worst). These scores are then combined into a cumulative score that correlates with the grade of differentiation: grade I (score 3-5), grade II (score 6-7), and grade III (score 8-9).

Tumour biology

This system uses immunohistochemical methods as part of the histopathology examination to identify the endocrine features displayed by the cancer cells and the degree to which these cells exhibit receptors for oncogenes and proliferation-associated proteins. The endocrine receptors assessed are for oestrogen and progesterone. According to the Swedish cut-off level, expression of these receptors in ≥10% of tumour cells is defined as "hormone positive"; the international cut-off is 1%(1).

Expression of the oncogene HER2 (also known as C-erbB2) is assessed and confirmed by in situ hybridization. HER2-positive cancers are now treated with targeted adjuvant therapy in the form of specific monoclonal antibodies.

A nuclear protein (antigen), Ki-67, is necessary for cellular proliferation and is used as a marker for assessing the proliferation rate of cancer cells. This marker is expressed as a percentage. Various cut-off levels for this marker exist, and each laboratory in Sweden sets its own standardized cut-off level.

Intrinsic biological subtypes

Because the tumour biology in breast cancer exhibits great heterogeneity, there has been a need for a better classification system that considers most of the biological factors. A landmark paper by Perou et al. in 2000 proposed a new system of classification based on extensive molecular assays(36). This system was refined by the Cancer Genome Atlas Network in 2012(37). The biological subtypes can be used to identify the specific tumour biology according to the above-mentioned biological markers, which enables clinicians to tailor the oncological treatment. The subtypes according to the 13th St

Gallen International Breast Cancer Conference (2013) Expert Consensus are as follows(38).

- "Luminal A-like" – Oestrogen and progesterone receptor positive, HER2 negative, low – intermediate Ki-67.
- "Luminal B-like" – Oestrogen receptor positive, progesterone negative or low, intermediate – high Ki-67; can be HER2 negative or positive.
- "HER2 over-expression" – Oestrogen and progesterone receptor negative and HER2 positive.
- Basal-like or triple-negative – Oestrogen and progesterone receptor negative, HER2 negative, high expression of Ki-67. This form has the worst prognosis.

Staging

As with most other malignancies, breast cancer staging follows the TNM staging system developed by the Union for International Cancer Control (UICC). This system assesses three individual factors and groups the cancer into five different stages (0 - 4). Stage 0 is CIS and stage 4 involves distant metastasis and spread of the cancer(39).

The individual factors considered in the TNM staging system of the UICC are as follows:

- T – Tumour size. T1 (<2 cm), T2 (>2 cm but <5 cm), T3 (>5 cm) and T4 (engaging adjacent tissue).
- N – Nodal status, which assesses the spread to local and regional lymph nodes (LNs). Nodal status is graded as N1 (cancer cells in local axillary LNs) to N3 (cancer cells in regional LNs).
- M – Absence or presence of distant metastasis, staged as M0 or M1, respectively.

There are two key unique aspects of breast cancer staging. The first is the tumour biological variables that play an important role in the staging, such as endocrine receptor status and the oncogenes and proliferation proteins evaluated. This means that the intrinsic biological subtypes previously mentioned are combined with the TNM staging when choosing between treatment options. The 8th edition of the AJCC TNM classification was the first to integrate these parallel systems(40). The second aspect is reliance on the diagnostic surgical staging of the axilla. Because breast cancer spreads mainly via the lymphatic system, the main method of staging involves the diagnostic procedure of harvesting 1-4 lymph nodes from the ipsilateral axilla, known as SNB.

Sentinel lymph node biopsy

The concept of staging the axilla in breast cancer emerged in the early 1990s as an alternative to the previously used ALND. It has long been known that breast cancers spread primarily through the lymphatic system and that the route most often involves the ipsilateral axilla. It is also known that clinical assessment of the axilla for metastasis screening purposes carries with it a high grade of uncertainty(41, 42). For example, patients with clinically palpable LNs have about a 30% chance of showing negative LN status in the final histopathology report. By contrast, around 45% of patients with clinically negative LNs will have metastatic cancer cells in LNs identified in the final histopathology report(43).

Given this physiological pathway for metastasis, surgeons treating women with breast cancers have long used a dual surgical approach: radical resection of the breast tumours together with diagnostic surgery for the axilla. There is also a certain therapeutic effect of axillary surgery because it reduces the rate of regional recurrence and, thus, can improve overall survival(44, 45).

ALND has been the established method of performing surgical staging of the axilla. This involves harvesting 10-20 LNs from the ipsilateral axilla for microscopic evaluation. This procedure carries with it a relatively high morbidity of 25% to 30% according to the literature(46, 47).

SNs are the first nodes draining the lymph from the tumour area and are therefore the first site of spreading of malignant cells. SNs can comprise 1-4 separate LNs and are most often found in the ipsilateral axilla. This method of staging the axilla by SNB has improved surgical staging and treatment immensely. It has been proven to be as effective as ALND for staging the axilla in patients with clinically negative axillary LN status but has a significantly lower morbidity than ALND(18, 19, 47, 48). A meta-analysis based on papers published up to 2003 reported a false negative rate of 7.7% for SNB(49).

Complications associated with axillary staging include seromas, hematomas, infections, ipsilateral paraesthesia, ipsilateral hyperalgesia, ipsilateral reduced arm mobility and lymphoedema(50).

SNB requires a technique that can distinguish the node(s) the surgeon needs to excise because LNs are generally very small and embedded in fat in the axilla. The gold standard method of identifying SNs is the use of two tracers: a radioactive isotope (Tc^{99}) together with a blue dye (Patent Blue V). This dual technique has a detection rate of 90-99%(16, 51, 52). The surgeon then uses a hand-held gamma probe intraoperatively to identify which nodes are SN.

However, this dual technique has several drawbacks. Tc^{99} is a medical radioactive isotope that has a short half-life (6 h), which means that only 6.25% of the radioactive signal remains after 24 h. Therefore, the patient must receive the injection the day before or on the same day as surgery. Tc^{99} is also not available in countries that do not have a nuclear plant, unless it can be imported, and strict regulations regarding chemical waste disposal also limit its availability. The blue dye is associated with an anaphylactic reaction in 0.1-1% of patients and can also leave a blue discolouration mark at the injection site of the breast(21).

Superparamagnetic iron oxide nanoparticles

Superparamagnetic iron oxide nanoparticles (SPIO) have been used previously as an intravenous contrast agent in magnetic resonance imaging (MRI), specifically in MRI of the liver and in stem cell labelling(53). In 2012, this material was launched as a novel tracer for SNB and received conformité européenne (CE) approval for use in 2011 together with a hand-held magnetic probe system (SentiMag®). The tracer is provided as a sterile suspension of SPIO nanoparticles coated with carboxydextran molecules. The coating together with the size of the nanoparticle suspension (60 nm) allows SNs to filter and trap them selectively. Superparamagnetic performance is characterized by a response to an external magnetic field while retaining no magnetic remnant in its absence. This behaviour makes SPIO nanoparticles ideal for SNB because their collective movement can be used to detect the LNs. The SPIO suspension was initially launched as Sienna+® and then an improved more concentrated suspension called SiennaXP® and is now marketed as Magtrace®

SPIO have been used as a tracer for SN detection in several studies and its noninferiority against the gold standard dual technique of radioisotope + blue dye has been established(54, 55). In the earlier studies on SPIO used as a tracer for SNB, the nanoparticles were injected in a perioperative setting(56). The Nordic SentiMag trial published in 2016 was a comparative multicentre prospective study in which SPIO were injected in a preoperative setting, and the trial reported equivalent detection rates for SPIO and the dual technique. That study also included a meta-analysis of seven previously published studies on the use of SPIO nanoparticles for SNB(57).

The use of SPIO nanoparticles as a tracer for SNB has several technical advantages over the dual technique. First is the availability; SPIO can be administered by any licensed medical staff or by the surgeon, which means that there is no need for nuclear facilities. This, together with fewer regulations concerning waste disposal, makes SPIO vastly more accessible than radioactive medical isotopes such as Tc^{99}. SPIO also have a longer half-life than Tc^{99} and have been shown to be detectable up to 30 days after the injection, as shown in **Paper II**. Moreover, SPIO stain LNs brown and the additional colouring function of the blue dye is not needed.

The inert qualities of SPIO allow for the design of pragmatic studies that take advantage of the characteristics of SPIO nanoparticles to improve surgical treatment options and to find new clinical applications in the context of breast cancer surgery. In **Paper I,** our research group used the longer half-life of SPIO (30 days) together with magnetic seed localization, which also can be injected up to 30 days before surgery, to improve logistics and minimize patient discomfort. Thus, we employed a totally magnetic technique, performing both tumour resection and SNB with the SentiMag® device. The hypothesis in **Paper II** was that the longer half-life of SPIO could reduce the rate of SNB procedures in patients with DCIS, who according to Swedish guidelines should undergo SNB(32). With **Paper III,** we aimed to investigate whether we could reduce the SPIO dose administered for SNB without compromising the SN detection rate. Furthermore, we sought to evaluate whether we could minimize the drawbacks of the SPIO suspension by comparing different injection techniques. In **Paper IV**, our research group compared the total magnetic approach investigated in **Paper I** with guidewire localization in nonpalpable breast cancers with regard to the reoperation rate of the breast due to positive tumour margins.

There are two main potential drawbacks of using the SPIO tracer: discolouration and MRI artefacts. As with the blue dye tracer, discolouration occurs at the injection site. SPIO nanoparticles injected into the interstitial space instead of intravenously can cause MRI artefacts. The short- and long-term effects of these potential drawbacks are currently being investigated(58).

Nonpalpable breast tumours

The early detection of breast cancer decreases mortality and morbidity, and this has led to the development of national mammography screening programmes. In Sweden, such national screening offers all women aged 40-74 years mammography every 18 to 24 months, previously at a subsidized cost and now at no cost. At present, almost half of all breast cancers are detected by screening in Sweden(1). The tumours discovered now are much smaller than in the past, which means that some are nonpalpable at diagnosis; these types of tumours constitute 30% to 50% of all breast cancers in the developed world(7). This shift has stimulated a growing field of research to find and develop safe, effective, and feasible methods of localizing tumours, and to enable the surgeon to excise tumours with adequate oncological resection margins without removing unnecessary amounts of healthy breast tissue.

Localization methods

Wire-guided localization is the most frequently used localization method, both nationally and internationally, and is the gold standard method against which new methods are judged(8). However, wire-guided localization has some disadvantages; there are logistical difficulties because the wire must be placed the day before or on the same day as the operation; patient discomfort and unfavourable cosmetic outcome. Because of these drawbacks a number of new ways to localize occult breast lesions have been developed:

- Cryo-assisted localization
- Charcoal suspension
- Intraoperative ultrasound-guided
- Magnetic seed localization (Magseed®)
- Radio-guided occult lesion localization
- Radioactive iodine seed localization

It is anticipated that further localization methods will be developed in the future to allow clinicians to tailor methods for individual patients.

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 been instrumental in showing the noninferiority of SPIO against the gold standard dual technique. The next step is to find and develop practical applications of SPIO. Because this is an evolving new method for SNB, the procedure needs further refining such as dose optimization and evaluation of the injection techniques.

The specific aims of the thesis were as follows:

Paper I was the first published paper to combine SPIO nanoparticles for SNB with magnetic seed localization for nonpalpable breast cancers scheduled for BCS. The aim was to report the initial outcomes, feasibility, and implementation of this standardized, combined, total magnetic approach.

Paper II was undertaken to determine whether unnecessary SNB could be minimized in patients with DCIS by injecting SPIO nanoparticles during the primary breast operation and performing SNB in a second session only if IBC was found in the final pathology report from the primary operation.

Paper III aimed to compare the SN detection rate using lower doses of a newer refined suspension of SPIO (Magtrace®), employing different time frames of injection (perioperatively vs preoperatively), and different injection sites (subareolar vs peritumoural). Furthermore, we aimed to evaluate whether this was noninferior to the previous suspension of SPIO (Sienna+®).

Paper IV aimed to compare the combined magnetic technique described in **Paper I**; SPIO for the SNB and Magseed® for the localization of a nonpalpable breast tumour, with guidewire localization. The main aim was to compare and evaluate the reoperation rate because of positive oncological margins between the two techniques in a prospective study.

Materials and methods

Paper I

This was a prospective pilot study of 32 patients. Candidates for this study were patients with DCIS or IBC planned for BCS in need of preoperative tumour localization and SNB. SPIO were injected in the preoperative period up to 4 weeks before surgery.

Injections were made dorsal to the nonpalpable tumour by the radiologist, and this was guided by ultrasonography or mammography. At the same time, the radiologist inserted the Magseed® ventral to the tumour.

During BCS, the transcutaneous signals detected by SentiMag® in the breast and axilla, as well as the presence and size of skin staining, were registered. After excision of the primary tumour, specimen and background counts were measured. During the patient's postoperative visit to the outpatient clinic, the staining and magnetic signal in the breast were registered.

At the postoperative multidisciplinary team meeting, the need for a reoperation because of non-radical resection was registered. The number of SNs and non-SNs, and the occurrence of LN metastases were recorded.

Paper II

The study design was a multicentre prospective single-cohort trial. The initial number of patients needed for inclusion was calculated as 246 with a predetermined interim analysis after 3 years of inclusion. The interim analysis was performed on 189 patients. The inclusion criteria were DCIS nuclear grade III tumours of any size; DCIS nuclear grade II and preoperative size >20 mm on imaging; mass effects on imaging or clinical examinations; and any cases of DCIS planned for mastectomy.

SPIO nanoparticles were injected in association with the primary breast surgery, subcutaneously close to the tumour. Counts by SentiMag were measured transcutaneously in the axilla at the end of the procedure. The SN was loaded with SPIO but was not removed.

The patient was then scheduled for a visit to the breast unit within 2-3 weeks after surgery. If there was an invasive tumour component found on the final histopathology report, SNB needed to be performed at a second operation scheduled within 1-2 weeks. A preoperative injection of radioisotope (Tc^{99}) needed to be administered as a back-up to maximize the chance of detecting the SN.

Each SNB started with a registration of the magnetic and isotopic signals in the axilla, and the incision was placed in relation to the signal. If no activity was measured, an injection of 1 ml blue dye needed to be given in the area of the breast where the tumour was located. After a mastectomy, the lateral part of the earlier incision was used. If no SN was found, axillary clearance or sampling followed according to the surgeon´s decision.

The SN was sent for cryosectioning to avoid a third operation if SN metastases were present.

Paper III

This was a multicentre prospective trial enrolling patients scheduled for primary breast surgery including SNB at six Swedish centres. Inclusion criteria were breast cancers graded $cT_{0–2}cN_0cM_0$, and Eastern Cooperative Oncology Group (ECOG) performance status 0–2. The dataset of a previous cohort, the Nordic SentiMag trial(57), was used to derive reference values and for subsequent patient-level comparisons.

Magtrace® was administered in two different sequential settings: the first patient cohort received a periareolar injection of 1.5 ml SPIO on the day of surgery, not later than 20 min prior to the start of surgery, followed by a 5-min massage. The second patient cohort received 1.0 ml SPIO by subareolar or peritumoural injection into the interstitial tissue without massage, 1–7 days before surgery. All patients received Tc^{99} and blue dye (BD) injections, according to routine practice. During surgery, the surgeon initially used the Sentimag® to localize the SN and then used the gamma probe to confirm this, both before and after skin incision. All SNs detected intraoperatively with the Sentimag®, gamma probe or stained brown or blue were excised. The conventional cut-off of 10% of the SN with the highest signal (SPIO or Tc^{99}) was implemented. After excision, ex vivo counts for each lymph node were registered for both probes. SN status was then assessed by routine histopathology.

Paper IV

In this prospective randomized pilot trial, patients were recruited at three Swedish hospitals. Inclusion criteria were DCIS, or invasive breast cancers (graded T_{1-3}) requiring localization and scheduled for BCS together with SNB. The patients were randomized to a localization method at their first visit to the outpatient clinic: magnetic seed or guidewire. Patients who were randomized to magnetic seed localization received it from a radiologist 1-30 days preoperatively, guided by ultrasonography or mammography, at the same time as SPIO (Magtrace®) was injected. The magnetic seed was inserted ventral to the tumour and the SPIO suspension was injected dorsal to or in the periphery of the tumour in cases of microcalcifications, deeper-seated lesions or cancers with diffuse growth patterns. If patients had been randomized to the guidewire method, this was inserted on the day before or on the same day as surgery and patients received SPIO 1-30 days preoperatively, injected by the surgeon, close to the tumour. Blue dye was used at the surgeon's discretion.

The SentiMag® hand-held magnetic probe was used during surgery to locate and excise SNs in all patients. The conventional 10% cut-off of the SN with the highest signal was applied to define additional SNs. In patients allocated to magnetic seed localization, the same hand-held magnetic probe was used for tumour localization and excision. Resection of the magnetic seed-marked breast tumour was guided by the maximum signal on the SentiMag® probe, which we know from the manufacturer corresponds to 5 mm from the seed, as such residual tissues with remaining magnetic signals were not excised routinely. After excision of the primary tumour, a specimen count as well as a background count in the breast was performed. The presence and extent of postoperative skin staining were also registered. All breast specimens were subjected to intraoperative mammography to confirm successful localization. SPIO signal counts for each excised SN and for the specimen marked with the magnetic seed were recorded *in vivo* as well as *ex vivo*. In patients randomized to guidewire localization, the resection of the breast tumour was according to routine practice.

Statistical analysis

Paper I

This was a pilot feasibility study including 32 patients. Descriptive statistical analysis was performed.

Paper II

In the calculation of sample size, data from the Uppsala-Örebro regional breast cancer registry (2014) showed that about 50% of all patients with a true DCIS would be subjected to a SNB based on the preoperative core biopsy results. About 20% of these core biopsies would turn out to be false negatives, which means that the final histopathology report would reveal an invasive cancer. The exact percentage of false negatives in Sweden is unknown. For a 5% uncertainty rate, which corresponds to a confidence interval of ± 5%, 246 patients would be needed, if the true percentage is 20% to show that the percentage of patients with a true DCIS receiving SNB could be reduced from 50% to 20%. Given that the procedure is simple and not harmful to patients and that there is a possibility of fewer events than expected, it was planned to include 300 patients overall. An interim efficacy analysis of the primary endpoint using the O'Brien–Fleming procedure(59) was prespecified at 3 years after initial recruitment.

Paper III

The main objective was to evaluate whether Magtrace® was noninferior to Sienna+® for SN detection. We used the earlier detection rate of 97% with Sienna+® from the Nordic trial(57) and defined a noninferiority margin of 4%, resulting in a lower threshold of 93%, to declare noninferiority. For this, a sample size of 150 patients per cohort with a minimum of 146 successful magnetic SNB procedures was required, to ensure that the lower 95% confidence interval of the detection rate proportion would still be >93%. Allowing for a 10% dropout rate, 165 patients were required in each cohort. The detection rate per patient was also tested using a right-sided binominal test with the alternative hypothesis that the proportion of successful SNBs would be >0.93

for each tracer. A p-value of <0.05 would indicate that the null hypothesis was rejected. To allow for direct comparisons and to define factors affecting outcomes, patient-level data from the Nordic trial(57) were used as a third reference cohort and comparisons were performed as a one-step individual patient data (IPD) meta-analysis(60). Any differences in study design or inclusion criteria between the SentiDose protocol and the Nordic trial protocol were parametrized as independent input variables, to allow for harmonization of definitions and the conduct of multivariable regression analyses, as appropriate.

All end-points were analysed at two different cut-off points with regard to the Sentimag® signal of the SN, >0 and >20. The latter was selected to adjust for overlapping of detection methods (Tc^{99} vs SPIO), as nodes with a low signal on one probe and high on the other (while formally considered to be SNs detected with both methods) would probably not have been identified had the patient received only one tracer.

Comparisons of numeric outcomes were performed by one-way analysis of variance (ANOVA), whereas dichotomous outcomes were analysed by means of Pearson's $\chi 2$ test. Bonferroni adjustment for multiple comparisons was performed. Multivariable regression was performed if univariable associations with $p < 0.1$ were detected among clinically relevant variables.

Paper IV

The reoperation rate due to positive margins after excision reported in the literature varies widely (5% - 25%) when using guidewire-assisted excision(61, 62). In a published pilot study of 32 patients who underwent a total magnetic surgical approach when performing BCS + SNB, no patient underwent reoperation(63). We aimed to include 200 patients for this randomized pilot study. The size of a larger study with adequate power depended on results from this pilot. For example, a noninferiority study with an estimated difference of no more than 5% would need >2,000 patients(64). Comparison of numeric variables was performed by unpaired Student's t-test or Mann-Whitney nonparametric U tests depending on the assumption of normal distribution whereas categorical variables were analysed by means of Fisher's exact test. Univariate logistic regression analysis was performed for clinically significant variables, but no multivariate regression analysis was performed as there were few events in the explanatory variables affecting the accuracy of the model(65).

Ethical considerations

The studies were all approved by the regional ethics committee of Uppsala University, Sweden, and were performed according to the 1975 Helsinki Declaration and the Swedish Act on Patient Insurance.

The studies were sponsored by Uppsala University, as well as by the Centre for Clinical Research, Region Västmanland, Västmanlands Cancer Foundation, and the Swedish Breast Cancer Association. Magseed® and SPIO

(Magtrace®) were provided by Endomagnetics Ltd, Cambridge, UK.

Summary of results

Paper I

Radical excision with negative oncological resection margins was performed and SN detection was successful in all 32 patients.

Paper II

Invasive breast cancer was found in 47 patients, and secondary SNB was performed in 41 of 189 patients. Hence, 78.3% of patients avoided SNB ($p < 0.001$). This was an absolute reduction because the inclusion criteria of the study matched those for performing SNB according to national Swedish guidelines.

Paper III

In 534 patients, the SPIO SN detection rates were similar. The SN detection rate was 97.5% in the 1.5 ml cohort vs 100% in the 1.0 ml cohort vs 97.6% in the Nordic trial cohort ($p = 0.11$), and noninferior to the dual technique.
Significantly more SNs were retrieved in the preoperative 1.0 ml cohort compared with the 1.5 ml and Nordic trial cohorts (mean values 2.18 vs 1.85 vs 1.83, respectively; $p = 0.003$).

Paper IV

In 207 patients ($n = 91$ in the magnetic seed and $n = 116$ in the guidewire cohorts), there were no significant differences in reoperation rates (3.3% in the magnetic seed vs 7% in the guidewire cohort; $p = 0.354$). Furthermore, there was no significant difference in the SN detection rate (97.8% vs 100%, respectively; $p = 0.187$) and both groups had similar mean numbers of SNs retrieved (2.52 vs 2.62 nodes, $p = 0.763$).

Conclusions

Paper I

A total magnetic approach in nonpalpable breast cancers scheduled for BCS together with SNB is feasible and improves logistics.

Paper II

The use of SPIO allows for delayed SNB in patients with DCIS with confirmed IBC on the final histopathology report. This reduces the frequency of SNB significantly in patients with high-risk DCIS tumours.

Paper III

Magtrace® in lower doses is noninferior for SN detection in patients with breast cancer compared with Sienna+® and is highly concordant with the dual technique.

Paper IV

Magseed® in combination with SPIO nanoparticles is a flexible and oncologically safe alternative to the guidewire technique in patients with nonpalpable breast cancers planned for BCS together with SNB.

General discussion

With every new surgical method developed there are basic steps needed to ensure that the method is safe and feasible. It is also necessary to prove that the new technique is not worse than the current gold standard method. Once this has been established, clinicians will seek to explore, refine, and investigate the new method and search for possible applications and usage.
The studies that comprise this dissertation aimed to refine the technique of magnetic-assisted breast surgery, to investigate whether the inert qualities of SPIO can be used to improve surgical treatment in patients with breast cancers, and finally to investigate new and better ways to apply this technique.

With **Paper I,** our research group was the first to describe a combined totally magnetic technique in nonpalpable breast cancer scheduled for SNB. The use of the magnetic seed for tumour localization together with SPIO proved to be safe and improved the flexibility in scheduling surgery.

Paper II used the relatively long half-life of SPIO compared with Tc^{99} to avoid unnecessary SNB in patients with high-risk DCIS. By applying the SentiNot concept of marking the SN with SPIO at the first operation for patients with DCIS without performing SNB, and only performing the SNB when we found an invasive cancer on the final histopathology report, we managed to decrease the frequency of SNB markedly. Because this was an absolute reduction and not a relative one, this study helped to change the current Swedish guidelines for SNB in patients with high-risk DCIS tumours(1, 66). However, because the study did not have adequate power to assess the secondary endpoint of detection rate these results should be treated as hypothesis generating, until they are tested in other trials.

In **Paper III** our group aimed to refine the SPIO technique for SNB by lowering the dose administered by 25% and 50%, respectively, in two sequential cohorts and then comparing them with a similar published cohort.

In the largest patient dataset to date, lowering the SPIO suspension volume injected to 1.0 – 1.5 ml did not affect SN detection. The SN detection rate per patient was at least 96.7%, consistently similar to Tc^{99} ± BD and was unaffected by SPIO dose, time frame, or the injection site.

Moreover, different doses and different injection time frames and sites resulted in equally high SPIO-Tc^{99} concordance rates. These findings were consistent with results published by Alvarado et al.(67) and Rubio et al.(68). However, in those studies the SPIO suspension was administered intraoperatively and injected in the subareolar area.

In Paper IV, we used the combined magnetic technique described in Paper I and compared it with guidewire localization. There were no differences between the two methods with regard to the reoperation rate required by findings of positive oncologic margins in the breast. Our findings were consistent with those published by Micha et al.(69) and Zacharioudakis et al.(70) in their respective nonrandomized cohort studies comparing magnetic seed with guidewire localization. They found no significant differences regarding reoperation rates: Micha et al. reported a 17% reoperation frequency with Magseed® vs 16% in the guidewire cohort ($p = 0.40$) and Zacharioudakis et al. found 16% in the Magseed® cohort vs 14% in the guidewire cohort ($p = 0.69$).

Future perspectives

As the incidence of breast cancer continues to increase in most parts of the world, and especially in evolving economies, there is a need for surgical methods that are more accessible than at present. The importance of a correct axillary staging method cannot be stressed enough because this is the basis for the subsequent choice of adjuvant therapy combined with the tumour resection. SPIO nanoparticle suspensions as SN tracers are more widely available than Tc^{99} and have some favourable qualities, such as the long half-life. It is likely that the SPIO approach will improve breast cancer treatment in developing countries more so than in highly developed countries such as Sweden where resources are much better. Furthermore, I believe that the SPIO-based approach can further de-escalate the axillary mapping by more minimal invasive methods such as targeted magnetic-guided axillary ultrasound biopsy(71).

Because the incidence of breast cancers has a strong relationship with the socio-economic conditions of a society, future improvements in surgical treatment of breast cancer will differ in different parts of the world. In most patients with breast cancer, which will probably be found in developing economies, a major challenge will be to ensure that there is a readily available SN tracer enabling safe SNB, which in turn should enable better tailored adjuvant therapies. Meanwhile, in our part of the world, the steadily evolving alternatives of localizing a nonpalpable tumour will help surgeons to adjust and individualize the surgery for each patient according to their physical properties, tumour size, and tumour location. Another major challenge will be to minimize the invasive nature of axillary staging in patients with breast cancer.

Sammanfattning på svenska

Bröstcancer är den i särklass vanligaste cancersjukdomen som drabbar kvinnor såväl globalt som i Sverige. Ungefär hälften av all bröstcancer som diagnosticeras idag går inte att känna eller se med blotta ögat vid det planerade operationstillfället vilket gör att man måste indikera tumören preoperativt med hjälp av mammografi/ultraljud. Den absolut vanligaste metoden att indikera icke kännbara brösttumörer är med ståltrådsvajer. Kirurgin som utförs kan delas upp i resektion av den primära brösttumören (behandling) samt portvaktskörtelbiopsi (diagnostik). Vidare erhåller en majoritet av patienter tilläggsbehandling såsom antihormonell behandling, cytostatika, strålning samt biologiska riktade läkemedel.

I Sverige diagnostiseras cirka 800 patienter med duktal cancer in situ (DCIS) varje år. DCIS ska per definition inte kunna spridas då cancern ej har vuxit igenom basalmembranet än, men trots det har det utförts sentinel node biopsi (SNB) hos cirka hälften utav dessa patienter. Detta är pga. att det i cirka 15 % - 20 % av fallen upptäcks en invasiv cancerhärd på den postoperativa mikroskopiska analysen av preparatet. Sekundärt till detta faktum har man utvecklat kriterier för att särskilja ”hög risk DCIS” från ”låg risk DCIS” såsom storlek på tumören och histologisk differentieringsgrad. Högriskpatienterna har genomgått SNB då man utifrån kriterierna bedömt att det förelegat en hög risk för samtidig invasiv cancerhärd.

Bröstcancer sprider sig i första hand via lymfsystemet. Sentinel node (SN), även betecknad ”portvaktskörteln” är den första körteln/körtlar som dränerar tumörområdet i bröstet. Ifall tumören skulle sprida sig är det dessa körtlar som innehåller metastaserade cancerceller först. SN identifieras vanligtvis med hjälp av radioisotopinjektion (Tc^{99}) och blå färg (Patent V Blue), oftast i kombination. Denna metod av identifiering har betraktats som ”gold standard” då SN går att hitta i >95% av fallen. Denna teknik har sina nackdelar bland annat reglering kring hantering utav radioaktiva ämnen, kort halveringstid (1–2 dagar), låg tillgänglighet globalt samt anafylaktisk reaktion associerat med blåfärgen.

Superparamagnetisk järnoxid nanopartiklar (SPIO) är ett spårämne som närmaste år seglat upp som ett alternativ till radioisotop + blå färg för SNB.

SPIO har utvärderats i flertalet stora studier och bevisats ha lika bra SN detektionsfrekvens som ”gold standard” metoden. Principen är densamma, man injicerar ett spårämne i bröstet som färdas längst lymfbanor till armhålans lymfkörtlar där portvaktskörtel identifieras med hjälp av en handhållen magnetisk prob i stället för gammaprob.

Dock kommer man bort från all radioaktiv reglering/hantering då produkten är CE märkt enbart. Fördelarna med denna metod är längre halveringstid (30 dagar), inget behov av nukleär medicin då kirurgen själv kan injicera medlet, ökad tillgänglighet globalt då produkten regleras som övriga medicintekniska produkter.

Båda metoder för SNB har som nackdel en viss missfärgning av skinnet vid injektionsstället i bröstet, med SPIO mörkgrå och med radioisotop+blåfärg en blå missfärgning. Ytterligare nackdel med SPIO är att kvarvarande SPIO i bröstet kan störa framtida undersökningar med magnetkamera.

I de fyra ingående delarbeten har vi använt oss av SPIO:s egenskaper för att hitta nya användningsområden inom ramen för bröstcancerkirurgi, förfinat tekniken samt utvecklat en helmagnetisk teknik att operera icke palpabla brösttumörer tillsammans med SNB.

Mål med avhandlingen och delmål

Det övergripande syftet med avhandlingen har varit att studera samt förfina SPIO:s användning inom bröstcancerkirurgi. Vidare har syftet varit att med hjälp av den magnetiska tekniken förbättra och förenkla den kirurgiska vårdprocessen för bröstcancerpatienter.

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

- Undersöka ifall patienter med icke-palpabel bröstcancer planerade för bröstbevarande kirurgi + SNB kan opereras med magnetiskt clip för tumörindikering tillsammans med SPIO för SN identifiering. En metodbeskrivning.
- Undersöka om det är möjligt att undvika onödiga SN biopsier hos DCIS patienter med hjälp av SPIO:s längre halveringstid.
- Undersöka ifall det går att sänka dosen av SPIO för att kunna identifiera SN utan att kompromissa med den onkologiska säkerheten.
- Jämföra indikering av icke-palpabel bröstcancer med magnetiskt clip alternativt ståltrådsvajer hos patienter planerade för bröstbevarande kirurgi och SNB med SPIO som enda spårämne.

Metod & Resultat

Delstudie I var en prospektiv pilotstudie på 32 patienter totalt med icke-palpabel bröstcancer planerade för bröstbevarande kirurgi + SNB. Syftet var att undersöka ifall det var säkert att operera dessa patienter med en total magnetisk teknik, dvs magnetiskt clip indikering för tumören och SPIO för SNB. Samtliga brösttumörer exciderades radikalt med histopatologisk marginal på 6,5 mm i median (0–14 mm) och SNB var framgångsrik hos samtliga patienter med median två SN exciderade/patient (1–5). Slutsatsen var att en total magnetisk teknik är säkert och genomförbart.

Delstudie II var en multicenter prospektiv singelkohort studie. En förplanerad interims analys av 189 patienter med ”högrisk DCIS” som enligt dåvarande vårdprogram skulle genomfört SNB erhöll SPIO injektion vid primäroperationen då brösttumören exciderades men vi avstod från SNB. Om patologen fann invasiv cancer vid slutgiltiga histopatologiska undersökningen av bröstpreparatet genomgick patienten ytterligare en operation för SNB. Då SPIO:s halveringstid är avsevärt längre än radioisotop var den magnetiska signalen vid SN operationen kvar till den grad att SNB kunde genomföras. Resultatet blev en reduktion av andelen SNB som genomfördes i vår region från tidigare 50% till 22%. Slutsatsen blev att det var säkert att avstå SNB hos ”högrisk DCIS” patienter ifall SPIO användes.

Delstudie III var en multicenter prospektiv ”individual patient data” metaanalys. Två prospektiva efterföljande kohorter jämfördes mot en tidigare liknande kohort avseende doserna av SPIO samt injektionsförfarandet. I denna dosoptimeringstudie jämförde vi den ursprungliga lösningen SPIO (5 ml, 2 ml SPIO utspätt med 3 ml NaCl) hos en tidigare kohort på 206 patienter med en nyare mer förfinad lösning av SPIO. Den nya lösningen SPIO utvärderades hos två efterföljande prospektiva kohorter. Första kohorten (n=163) erhöll 1,5 ml SPIO intraoperativt medan den andra kohorten (n=165) erhöll 1,0 ml upp till en vecka preoperativt. Vi såg ingen statistisk signifikant skillnad mellan grupperna (5 ml vs 1,5 ml vs 1,0 ml) vad gäller SN detektionsfrekvens (97,6 % vs 97,5 % vs 100% p = 0.11) samtliga doser var jämförbara med ”gold standard” metoden (radioisotop+blå färg). Slutsatsen blev att SPIO i doserna 1,5 ml och 1,0 ml inte var sämre än 5 ml och inte heller sämre än ”gold standard”.

Delstudie IV var en randomiserad pilotstudie på 207 patienter 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 + SNB. Samtliga patienter erhöll SPIO enbart som spårämne för SNB. Patienterna randomiserades till indikering med magnetiskt clip för brösttumören eller ståltrådsindikering vid diagnosbesked,

randomiserandet var med hjälp av datorgenererad slumpmässigt urval i tio block kuvert. Primärt utfallsmått var reoperationsfrekvens pga. bristande radikalitet. Sekundära utfallsmått var SN detektionsfrekvens, antal SN exciderade (medelvärde) samt volymen bröstvävnad som reseceras. Vi såg ingen signifikant skillnad mellan grupperna avseende reoperationsfrekvens.

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A combined, totally magnetic technique with a magnetic marker for non-palpable tumour localization and superparamagnetic iron oxide nanoparticles for sentinel lymph node detection in breast cancer surgery

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ABSTRACT

Background: Surgery for non-palpable breast cancer may often be a challenging procedure. Recently, a magnetic seed (Magseed®) used for tumour localization has been developed. Superparamagnetic iron oxide nanoparticles (SPIO) for sentinel lymph node (SN) detection is a novel tracer that may be injected up to four weeks preoperatively. This study is the first combining the magnetic seed and SPIO.
Material and methods: Patients planned for breast conserving surgery and SN-biopsy (SNB) were recruited from two units in Sweden. Patients underwent lesion localization with Magseed® and SPIO injection (Magtrace™) by the breast radiologist in the preoperative period. Feasibility of successful lesion localization and excision together with a successful SNB detection was evaluated. Seed migration, number of SNs, specimen volume and calculated resection ratio (CRR) were reported.A survey of the physicians' experience was conducted.
Results: Localization was performed at a median of three days before surgery (range 0–25). All 32 patients underwent microscopically radical resection with a CRR of 1.49. No seed migration was noticed. SNB was successful in all patients. A median of two SNs was retrieved. Radiologists and surgeons reported the procedure easy to learn and outperformed guidewire localization in terms of localization and excision time. They thought the technique facilitated planning localization and surgery.
Conclusions: The combined magnetic technique provided accuracy in tumour localization and SN detection without excess tissue excision and with promising results for flexibility in delivery of care. Larger studies are needed to confirm these findings.

Introduction

Breast cancer is the leading form of cancer among women worldwide and with a rising incidence [1–3]. The introduction of mammography screening has led to diagnosis of tumours at an earlier clinical stage, often with a clinically negative axilla [4]. In Sweden, about 50% of breast cancers diagnosed annually are screening-detected [5]. These cancers are often asymptomatic at clinical examination, as they either are too small in size or seated too deep in the breast parenchyma to be detected at clinical examination as a palpable lump and are often referred to as non-palpable lesions.

Surgical treatment of non-palpable lesions is an example of interdisciplinary dependence in surgical oncology. The breast

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surgeon is called to excise a part of the breast, guided solely by the localization performed by the radiologist. A variety of techniques have been developed, with guidewire localization being viewed as the default method [6]. However, guidewire localization poses certain challenges. The guidewire is placed preferably on the day of surgery to minimize risk for dislocation. Furthermore, a guidewire case is hard to postpone and, if the theatre list only includes cases requiring localization, the first case can be delayed, which may have an impact on utilization of resources and costs. Guidewires may also limit alternatives for incision placement, thus affecting the possible aesthetic outcome [7].

A paramagnetic steel seed (Magseed®, Endomagnetics Ltd, Cambridge, U.K.) for localization of breast lesions has recently gained interest. The Magseed® is inserted under ultrasonographic or stereotactic guidance. At surgery the seed is localized with a magnetic detector probe (Sentimag®, Endomagnetics Ltd, Cambridge, U.K.). The probe is also used for sentinel lymph node (SN) detection when using superparamagnetic iron oxide nanoparticles (SPIO) at SN-biopsies (SNB). Reports from the U.K. [8] and U.S.A. [9] on the Magseed® use demonstrate safety, efficacy and ease of implementation and suggest that the preoperative insertion of the seeds may facilitate logistics. However, in the study where breast conserving surgery (BCS) was performed, there was a concern regarding magnetic transcutaneous probe detection of lesions located deeper than 3.5 cm [9]. On the other hand, if the primary is detected with the magnetic probe, but SNB is performed using radioisotope, then nuclear medicine facilities are still necessary, two different probes are used and therefore increased resources are required, exposure to radiation is not avoided and the flexibility that seems to be provided by the Magseed®, compared to the guidewire, is not fully capitalised on.

SPIO-guided SNB has been the standard at the breast unit at Uppsala University Hospital since 2014. Our research group has demonstrated comparable results to the isotope and blue dye (BD) combination as well as feasibility of a preoperative SPIO injection, up to one month before surgery [10]. Subsequently, SPIO-guided SNB was combined with tumour localization with the Magseed® for patients with occult breast lesions in a totally isotope-free technique with magnetic guidance both for the resection of the primary and for SNB. Aim of this study is to report initial outcomes, feasibility and implementations of this standardized, combined method.

Patients and methods

Patient selection

Patients with non-palpable, screening-detected lesions with a core cut biopsy diagnostic for breast cancer that were planned for BCS and SNB were identified at the multidisciplinary meetings at the two centres. Exclusion criteria were hypersensitivity to dextran compounds or SPIO, iron overload disease, pregnancy, or mental condition rendering the patient incapable of giving written informed consent. The study was approved by the Regional Ethics Board in Uppsala (Dnr: 2017/508).

Technique

Consecutive patients meeting the inclusion criteria, such as depicted in Fig. 1, were recruited for the study and scheduled for an operation during their visit at the outpatient clinic. At any time-point between that visit and the day of surgery, an appointment for tumour localization was booked at the mammography unit. The tumour was located with ultrasound or mammography. SPIO (Magtrace™,1.0–2.0 ml, Endomagnetics Ltd, Cambridge, U.K.) was injected on the dorsal surface of the tumour (Fig. 2a), or divided in four doses at the periphery of the lesion in cases with micro-calcifications, larger lesions seated deeply in the breast or lesions with diffuse growth pattern. A MagSeed® was placed at the ventral surface of the tumour (Fig. 2b). It is known from the manufacturer that the MagSeed® gives maximum signal on the Sentimag® detection system at a distance of five mm, meaning that placing the

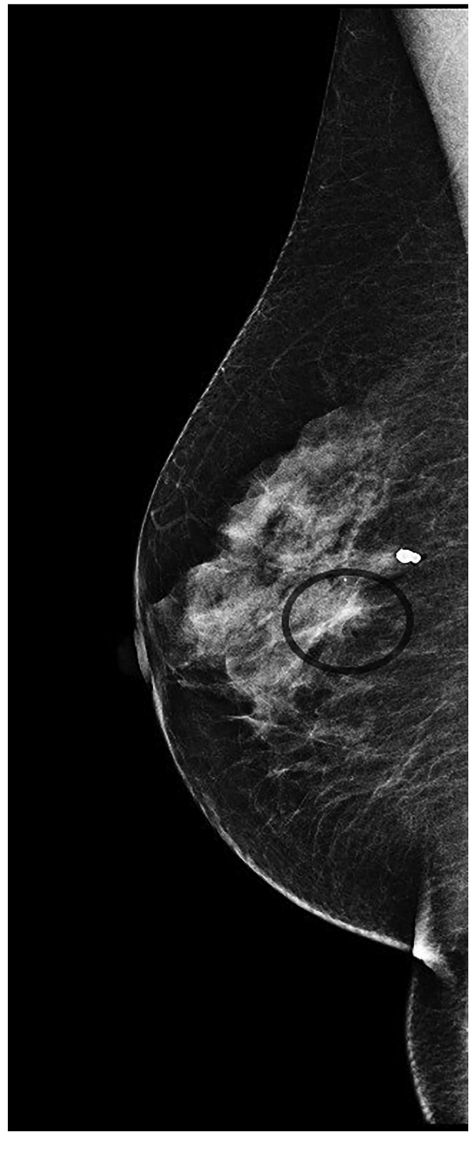


Fig. 1. Screening mammogram. Mediolateral oblique view of a right breast depicting a 15 mm invasive ductal cancer, oestrogen positive, in the lower outer quadrant, marked with a circle.

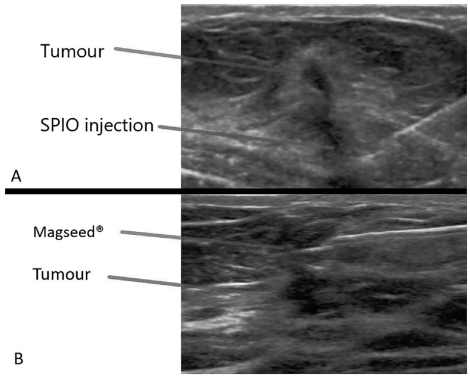


Fig. 2. A. Breast Ultrasound with the lesion and SPIO injected dorsally. B. The Magseed® is injected and left on the ventral surface of the tumour.

seed on the ventral surface of the tumour would allow for safe margins. Accordingly, SPIO injection at the periphery would result in the creation of a rim of maximum magnetic signal behind and around the tumour, both enhancing transcutaneous magnetic signal and surrounding the lesion, but without losing the focal signal of the MagSeed® on the anteroposterior axis of the breast, when the probe is accordingly placed. Mammography was conducted to confirm successful localization (Fig. 3) and the distance between seed and tumour was documented The optimal resection volume (ORV) for a given tumour size was calculated as described by Krekel et al. [11], including suspected areas of DCIS described by the radiologists. The distance between ultrasound probe or skin, in cases of stereotactic localization, and tumour was documented.

On the day of surgery, the seed was localized with the Sentimag® probe after imaging review. The axilla was scanned with the probe and BD was injected at the surgeon's discretion. Resection of the tumour was performed with help of the Sentimag® probe. Resection was guided by the maximum magnetic signal of the Sentimag® probe, without the need to resect all the tissue with magnetic signal. It is known from the manufacturer that maximum magnetic signal responds to a distance of five mm from the clip or the SPIO injection site. Time-to-specimen-excision and cavity residual signal counts were registered. Specimen radiography was obtained and radiologic margins were documented (Fig. 4). Distance between seed and tumour was also documented, to allow for comparison with the post-localization mammogram so as to see if there is seed migration. Cavity shavers were not routinely obtained. Specimen volume was obtained after weighing the specimen and assuming a molecular weight of 0.958 g/cm^3, which is known to correspond to a 1:1 proportion of gland-fatty-tissue [12]. SNB was subsequently conducted using the probe and detection rate, number of SNs and presence of metastases were documented. The calculated resection ratio (CRR) was calculated by dividing the ORV to the surgical specimen volume, to allow for an objective estimate of the outcome. A background comparison with the "true" resected volume (TRV) was also performed, using the formula of the ellipse volume, as previously performed in the literature [11] and the estimated CRR (eCRR) was defined as TRV/ORV. Finally, surgeons and radiologists undertook a survey to assess the ease of the method in comparison to the use of guidewires and their experiences on the method. No learning curve patients were operated prior to the study.

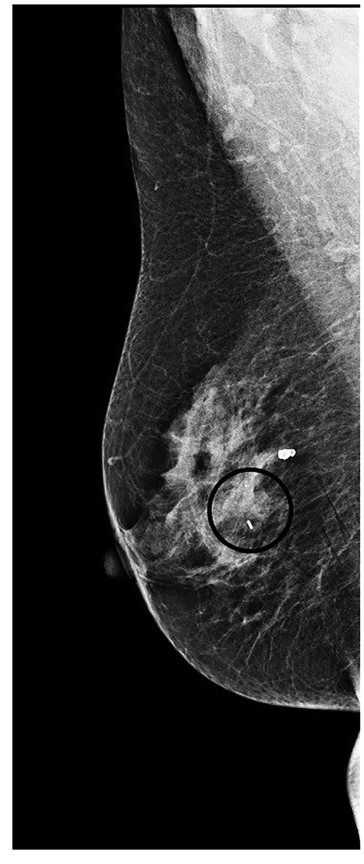


Fig. 3. Mediolateral oblique view of the mammogram confirming successful tumour localization.

Study endpoints

Primary endpoint was successful lesion localization and excision and successful SN detection. Secondary endpoints were Magseed® migration, number of SNs, localization time, excision time, CRR and a survey of the physicians' views on the technique.

Results

Thirty-two patients were included in the study. Patient demographics and tumour characteristics are presented in Table 1. Patients underwent localization and SPIO injection at a median of three days before surgery (range 0–25). The median minimum distance from the tumour to the skin was 17.5 mm (range 5–65). The most usual modality for localization was ultrasound (30 of 32 patients). Median time required for seed placement and SPIO injection per patient was six minutes (range 2–50); this time increased to twelve minutes (range 5–60) when the time required for the post-localization mammogram was added. Primary tumour resection was radical in all patients, with a median

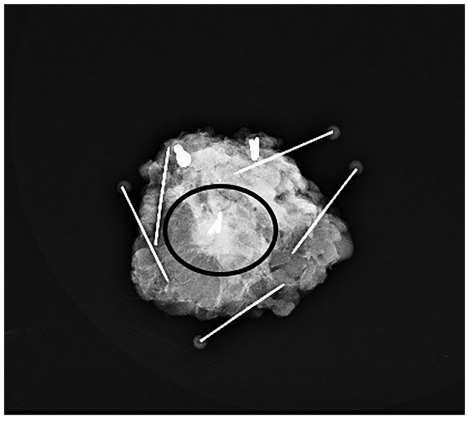


Fig. 4. Specimen radiography, anteroposterior view with the tumour and the Magseed® centrally located with adequate radiologic margins.

histopathological margin of 6.5 mm (0–14 mm). No seed migration was noted. Median time for specimen excision was eight minutes (range 4–35). SNB was successful in all cases with median of two retrieved (range 1–5). Blue Dye was added in eight cases, but did not prove to be necessary in any. Median operative time for tumour excision together with SNB was 64 min (range 38–113).

The comparison of medians for the different methods for specimen volumetry (direct volume estimate vs TRV) did not demonstrate any differences (42.19 vs 41.75 cm^3, Wilcoxon signed rank test, $p = 0.466$). Subsequently, no difference was seen between the medians of the "true" and the estimated CRR (1.82 vs 1.49, Wilcoxon signed rank test, $p = 0.681$).

The views of the physicians involved in the procedures are illustrated in Table 2. The response that everybody would be positive to use this technique and recommend it to others was unanimous. The operation theatre co-ordinators experienced that the combined method was an improvement, allowing for more flexibility in the schedule of the mammography unit and the theatre lists without delayed start. Finally, neither the SPIO nor the seed affected specimen pathology.

Discussion

The present study is the first report of a novel technique combining a magnetic seed for non-palpable lesion localization and SPIO for SNB, allowing for a totally magnetic, isotope and wire-free technique. Results and clinical outcomes are promising and seem to have the potential to improve current practice, providing flexibility in delivery of care as well as simplified logistics.

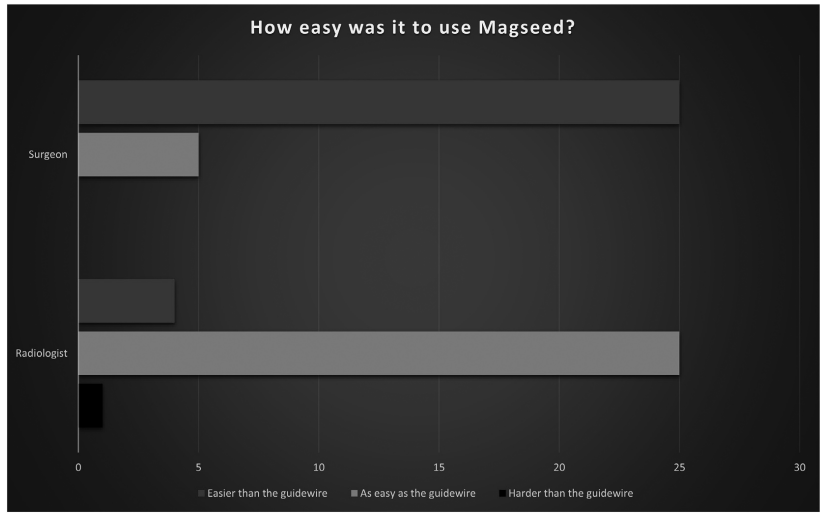
Three previous reports on the use of magnetic seeds have described safety and feasibility [8,9,13] but only Price et al. describe Magseed® in clinical use and focus on the advantages in logistics, the reliability in deployment and, the comparable re-excision rate to the use of guidewires [9]. Harvey et al. conducted a safety and feasibility study in mastectomy cases and concluded that the seeds could be placed accurately [8]. A Dutch group conducted a feasibility study using a similar magnetic marker comparing it to radioactive seeds and all fifteen cases could be identified with participating radiologists and surgeons reporting positive views of the technique [13]. The intratumoural injection of SPIO, both for

Table 1
Clinicopathological data of study patients (N = 32).

Age (years)		66 (41–82)
BMI (kg/m^2)		26.6 (19.2–39.9)
Side	Right	16
	Left	16
Localization in the breast	Upper Outer Quadrant	13
	Upper Inner Quadrant	9
	Lower Inner Quadrant	5
	Lower Outer Quadrant	4
	Central	1
Primary Systemic Treatment	No	30
	Yes	2
Primary tumour size*		13.5 (6, 47)
Histological type	DCIS	3
	IDC	27
	ILC	2
Nuclear grade	In situ, grade 2	3
	1	7
	2	17
	3	5
Receptor status	ER + HER2-	23
	ER + HER2+	2
	ER-HER2+	2
	ER-HER2-	2
	Not assessed (DCIS)	3
T-stage	T_{is}	3
	T_1	21
	T_2	8
Transcutaneous magnetic signal before incision	Yes	30
	No	2
Ex vivo signal on SN (median, range)		4300 (200,9999)
SNs retrieved (median, range)		2 (1,4)

Descriptives are presented as median (range), for continuous variables and numbers for nominal or ordinal variables. *: Based on the size of the largest focus. DCIS: Ductal Cancer In Situ, IDC: Invasive Ductal Cancer, ILC: Invasive Lobular Cancer, ER: Oestrogen Receptor, HER2: Human Epidermal growth factor Receptor 2.

Table 2
Bar plot with the views of the physicians who partook in the study.



tumour localization and SNB have been described [14]. Using a dose of 0.5 ml Sienna+ (Endomagnetics Ltd, Cambridge, UK), a SN was detected in 28 of 33 patients with SPIO only and in 32 of 33 in combination with BD. The CRR was 2.5 using and in two of 20 cases with non-palpable lesions the BCS was not radical.

The combination of magnetic marker and SPIO seems to be an improvement;, SPIO injected dorsally or around a lesion seems to amplify the transcutaneous magnetic signal in tumours located deep in the breast; the surgeon may intraoperatively be guided by the maximum focal signal provided by the Magseed® placed ventrally to the lesion and SPIO diffusion creates a "halo" of magnetic signal around the tumour. In other words, the radiologist uses the SPIO and the seed to demarcate the dissection plane for the surgeon with the additional benefit of simultaneously injecting the tracer for SNB. Injecting the SPIO on the lesion margins is additionally expected to result in surgical removal of the majority of the SPIO in the breast resulting in turn to less skin staining or artefacts in a postoperative MRI. This technique may account for the fact that all resections in the cohort were microscopically radical with satisfactory pathological margins, without removing a large excess of breast tissue. In fact, the estimated CRR was only 1.49, which is markedly lower than CRRs reported for other techniques, ranging from 2.5 for the MagSNOLL[15] up to 3.8 for the radioiodine seeds[12]. The low CRR obtained implies that the totally magnetic technique may yield promising results for smaller resection volumes and therefore potential for improved cosmesis [15]. That was particularly useful for larger, deep seated tumours in the present series, where the demarcation of tumour spared the resection of excess tissue. No seed migration was noted, nor did the SPIO spread diffusely the breast tissue. The exact association of SPIO volume injected and grade of diffusion at the area of injection as well as the minimum dose for a successful SNB is a question of clinical interest currently investigated by our group.

In this study, SPIO-guided SNB was successful in all patients. Blue Dye was injected either due to a low transcutaneous magnetic signal or the surgeon's decision. However, in all cases the SN was clearly magnetic when entering the axilla, and blue dye injection could have probably been avoided, a finding which is in agreement with previous findings of our group [10]. As far as the timeframe of the Magseed® insertion and the SPIO injection is concerned, a maximum of 40 days prior to the operation has been previously reported for the Magseed® insertion [9] and successful SPIO-guided SNB up to 47 days after SPIO injection have been conducted by our group [16], implying that long-term application may be feasible, which may be of interest for the marking of primary tumour and SN prior to primary systemic therapy, in cases that MRI during that period is not required. Results from the ongoing SentiDose trial, regarding the effectivity of a lower SPIO dose are expected to further refine the parameters of this combined technique.

Breast radiologists almost unanimously felt that localization was faster with the combination of Magseed® and SPIO compared to guidewire and that the procedure was comparable or easier to guidewire placement, despite the lack of experience with the seeds. There was no steep learning curve and overall, the technique seemed to be more comfortable for the patients. Moreover, there was no difficulty in identifying the Magseed® and the lesion on specimen imaging. The surgeons felt that the combined magnetic technique was easier than the guidewire and reported shorter time for excision, even when oncoplastic procedures were performed with incisions not right over the seed, the explanation being that dissection and raising of skin flaps all the way to the guidewire entrance under the skin was not necessary. Additionally, guidewire displacement or tip anchoring in the fascia were avoided by using the magnetic technique. Planning was more flexible both for the mammography unit and for the operating theatres, whereas late starts or rescheduling could be avoided. The implication of this technique on health economy related to the procedure was not an endpoint in the study due to small number of participants and lack of a control arm, but possibly shorter operating times and avoidance of late starts may be of value.

The present report demonstrates that the totally magnetic technique with a combination of Magseed® and SPIO is feasible for non-palpable breast cancer localization and SNB. This novel technique seems to overcome the limitations of wire- or radioiodine seed-guided surgery, to provide radical excisions without the removal of excess breast tissue. Simplification of logistics and reduction of resources seems to make it attractive for the global setting, where co-ordination of radiologists and surgeons or access to nuclear medicine departments maybe very challenging. Additionally, no skills in intraoperative ultrasound are required, which is mandatory for ultrasound guided excisions. Finally, both the primary tumour and the SN can be excised by the use of a single-principle technique, diminishing the need for complex procedures that would involve steep learning curves. Albeit very promising, these first results need to be tested in larger randomized trials. Therefore, our research group is currently accruing data within the MagTotal trial [17], in order to reach to more robust conclusions on the implementations and advantages of this novel technique.

Role of the funding source

The study was sponsored by Uppsala University. EndoMagnetics Ltd (Cambridge, UK) provided the Magseed® and the SPIO (Magtrace™) for the study. The sponsor and the funding source had no role in study concept and design, data acquisition, analysis and interpretation, manuscript preparation or decision to submit for publication.

Conflict of interest

Authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2018.10.064.

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Paper II

Original article

Effect of preoperative injection of superparamagnetic iron oxide particles on rates of sentinel lymph node dissection in women undergoing surgery for ductal carcinoma *in situ* (SentiNot study)

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Background: One-fifth of patients with a preoperative diagnosis of ductal carcinoma *in situ* (DCIS) have invasive breast cancer (IBC) on definitive histology. Sentinel lymph node dissection (SLND) is performed in almost half of women having surgery for DCIS in Sweden. The aim of the present study was to try to minimize unnecessary SLND by injecting superparamagnetic iron oxide (SPIO) nanoparticles at the time of primary breast surgery, enabling SLND to be performed later, if IBC is found in the primary specimen.

Methods: Women with DCIS at high risk for the presence of invasion undergoing breast conservation, and patients with DCIS undergoing mastectomy were included. The primary outcome was whether this technique could reduce SLND. Secondary outcomes were number of SLNDs avoided, detection rate and procedure-related costs.

Results: This was a preplanned interim analysis of 189 procedures. IBC was found in 47 and a secondary SLND was performed in 41 women. Thus, 78·3 per cent of patients avoided SLND ($P < 0·001$). At reoperation, SPIO plus blue dye outperformed isotope and blue dye in detection of the sentinel node (40 of 40 *versus* 26 of 40 women; $P < 0·001$). Costs were reduced by a mean of 24·5 per cent in women without IBC (€3990 *versus* 5286; $P < 0·001$).

Conclusion: Marking the sentinel node with SPIO in women having surgery for DCIS was effective at avoiding unnecessary SLND in this study. Registration number: ISRCTN18430240 (http://www.isrctn.com).

*Members of the SentiNot Trialists Group are co-authors of this article and can be found under the heading Collaborators

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Introduction

Ductal carcinoma *in situ* (DCIS) accounts for approximately 10 per cent of breast malignancies in Sweden. It is mostly detected by screening mammography because it does not typically present with a palpable lump[1]. The risk of nodal metastases in pure DCIS ranges from 0·2 to 0·7 cent[2,3]. Approximately 20 per cent of patients with a preoperative diagnosis of DCIS are found to have invasive breast cancer (IBC), requiring sentinel lymph node dissection (SLND)[4,5]. Until now, there has been no accurate method for predicting which women will have IBC. Guidelines[4,5] suggest that SLND should not be performed routinely, but considered when a mastectomy is planned, as it will not be technically feasible afterwards, or in women at high risk of IBC treated with breast-conserving surgery

(BCS). However, the definition of high-risk DCIS is not clear. Nuclear grade 3, the presence of a mass lesion and a large area of microcalcifications are considered to be risk factors, but the size cut-off of 5 cm often suggested is arbitrary[5]; in a recent study[6], size larger than 15 mm has been shown to be independently predictive of invasion. Thus, many women with DCIS are overtreated, and the literature shows that unnecessary SLND is associated with morbidity[7].

Superparamagnetic iron oxide (SPIO) nanoparticles are a novel tracer for SLND, with detection rates comparable to those of the combination of radioisotope and blue dye. Injection of SPIO avoids use of nuclear medicine resources and does not involve a steep learning curve[8]. It has the additional advantage that the signal persists for up to 1 month after injection, allowing SLND at a later date, potentially after the first operation[9,10].

The hypothesis of the study was that use of SPIO might be beneficial for women with DCIS for whom SLND is recommended. The aim was to determine whether unnecessary SLND could be minimized by injecting SPIO at the primary breast operation, and performing SLND in a second session only if IBC was found in the pathology from the primary breast operation.

Methods

The SentiNot ('Senti'nel node biopsy in ductal cancer *in situ*; how to 'Not' do it) study[11] is a single-arm multicentre prospective cohort study recruiting women with a preoperative diagnosis of DCIS where SLND is planned. The concept is to avoid SNLD by marking the sentinel node with SPIO at the first operation, and removing it at a second operation only if IBC is confirmed. Inclusion criteria were: nuclear grade 3; nuclear grade 2 and preoperative size larger than 20 mm on imaging; mass effect on imaging or clinical examination; and any DCIS planned for mastectomy. These criteria were selected as they were predictive factors for axillary evaluation in patients with DCIS; more than 70 per cent of women with these characteristics had SLND performed according to the Swedish Breast Cancer Registry. Exclusion criteria were: suspected or verified microinvasion on core biopsy; intolerance or hypersensitivity to iron or dextran compounds; iron overload disease; and pregnancy or lactation. All patients received oral and written information and provided signed informed consent. The study was approved by the regional ethics committee (Uppsala University, Sweden), performed according to the 1975 Helsinki Declaration and the Swedish Act on Patient Insurance, and sponsored by Uppsala University. No external funding was needed. The study was undertaken at five centres in Sweden: three university hospitals and two regional hospitals. Recruitment started on 1 June 2015. The manuscript was prepared according to the STROBE statement[12].

Procedures

On the day of surgery, the women had an interstitial injection of 2 ml SPIO (Endomagnetics, Cambridge, UK) diluted with 3 ml of 0·9 per cent sodium chloride or local anaesthetic (10 mg/ml lidocaine) at least 20 min before the operation, followed by a 5-min massage to allow tracer migration. Breast procedures were performed as planned and, at the end, the transcutaneous magnetic signal in the axilla detected by a SentiMag® probe (Endomagnetics, Cambridge, UK) was registered. If no signal was detected, the axilla was explored and the woman excluded from the study. If IBC was identified at subsequent specimen pathology, a secondary SLND was performed in a separate operation. Radioisotope (^{99m}Tc) was injected at the time of the second operation and the concomitant use of blue dye was recommended. In women who had BCS, the injection site for radioisotope and blue dye was defined by local routine. If a mastectomy had been done previously, the radioisotope and blue dye were injected intradermally, near the scar.

Transcutaneous magnetic and radioisotope signals in the axilla were detected and registered. SLND was completed with the SentiMag® probe. After sentinel lymph node (SLN) retrieval, the presence of radioisotope signal or blue dye was also registered; thereafter, the axilla was explored for additional radioactive and blue nodes. Intraoperative frozen section was employed to avoid a third operation. If SLND failed, the protocol stated that axillary lymph node dissection (ALND) or axillary lymph node sampling could be performed based on the surgeon's discretion.

The primary endpoint was a 60 per cent reduction in the number of SLND procedures required. Secondary endpoints were: number of SLND procedures avoided at the primary operation in relation to SLND procedures performed in a reoperation; predictive value for IBC of factors considered to relate to high-risk DCIS; SLN detection rate on reoperation; and resources spared by the SentiNot policy.

Data collection and statistical analysis

The Uppsala–Örebro regional breast cancer registry had very high coverage of women with a preoperative diagnosis of DCIS that was upgraded to IBC, and was therefore considered appropriate for reference in calculation

of the study sample size. Registry data (2014) demonstrated that 20 per cent of women with a preoperative diagnosis of DCIS actually had IBC. In the same region, the Swedish guidelines for use of SLND resulted in approximately 50 per cent of women with DCIS undergoing SLND. A sample size of 246 women with a preoperative diagnosis of DCIS would allow confirmation that the true proportion of DCIS lesions that were IBC is 20 per cent, with 5 per cent uncertainty (corresponding to confidence limits of +/−5 per cent). Given that SLND is performed in an observed 50 per cent of procedures for DCIS (Clopper–Pearson 95 per cent c.i. 43·6 to 56·4 per cent)[13,14], the intervention should lower the SLND rate to 20 per cent, such that it will be done only if IBC is diagnosed. The sample size was adequately powered to demonstrate that an anticipated reduction in SLND by 60 per cent is significant (z-statistic 11·763, $P < 0·001$).

An interim efficacy analysis of the primary endpoint using the O'Brien–Fleming procedure[15] was prespecified at 50 per cent of recruitment. Owing to protocol redesign, the trial steering committee decided to perform the interim analysis at the completion of 3 years of recruitment. To adjust for this modification, the two-sided P value for the primary endpoint was subsequently lowered to 0·0006 instead of 0·0054. For all other comparisons, two-sided $P < 0·050$ was considered significant.

To investigate the impact of implementation of the SentiNot policy at a national level, synchronous data on women with a postoperative DCIS diagnosis were retrieved from the Swedish Cancer Registry (retrieval date 5 December 2017). The Swedish Cancer Registry was founded in 1958 and the coverage for patients with breast cancer is estimated to be 100 per cent[16]. Appropriate potential candidates for the SentiNot study were identified to assess how many SLNDs could have been spared. Additionally, costs of inpatient and outpatient care were retrieved from the respective hospital registries, corresponding to actual healthcare expenses from 2015 to the present day. Fixed estimates of costs for healthcare per year and per region were calculated using the pricing lists provided by the respective economic departments of the Swedish centres participating in the study, with a model provided by Uppsala-care[17]. Actual total cost per patient included outpatient or inpatient treatment, operation and anaesthesia per minute and SLND pathology, either standard or intraoperative frozen-section analysis. Results were reported according to the CHEERS statement[18].

Tests of normality were carried out, and descriptive values are presented as mean (95 per cent c.i.) or median (i.q.r.) as appropriate. Continuous variables were analysed using Student's t test or non-parametric tests, depending on data distribution. Dichotomous data were analysed with Pearson's χ^2 test or Fisher's exact test, and McNemar's test was used for paired observations. When differences for dichotomous variables were demonstrated on univariable analysis, multivariable regression analysis was undertaken, and the exponentiated coefficient (Exp(B)) was calculated with 95 per cent confidence intervals. Analyses of outcomes were performed per protocol. Statistical analyses were undertaken in SPSS® version 23.0 (IBM, Armonk, New York, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

Results

For the interim analysis, 189 women (76·8 per cent) were recruited (*Table 1*). Mean DCIS diameter was 39·6 mm; 40·7 and 31·2 per cent of women had lesions larger than 40 and 50 mm respectively. Breast conservation was possible in 129 patients (68·3 per cent); IBC was finally diagnosed in 47 patients. Women with IBC had a larger preoperative DCIS size (47·2 *versus* 37·1 mm; $P = 0·047$) but not pathological size (45·3 *versus* 39·1 mm; $P = 0·176$) than those who had pure DCIS (*Table 2*). In six women, the invasive tumour was smaller than 1 mm and no SLND was performed, after discussion at the multidisciplinary meeting. The incidence of upgrade to invasive cancer (24·9 (95 per cent c.i. 18·9 to 31·7) per cent) was comparable to the hypothesized 20 per cent ($P = 0·092$), as was the proportion undergoing SLND (21·7 (16·1 to 28·3 per cent); $P = 0·584$). In this cohort of women who met the criteria for SLND, 78·3 per cent avoided SLND ($P < 0·001$).

At reoperation after a median of 28 (range 9–46) days, a transcutaneous magnetic signal was present in all but one woman. In that patient, the protocol had been violated because, despite the absence of axillary signal at the end of the primary procedure, the patient did not have immediate axillary exploration but instead underwent SLND in a second session. This left 40 patients for analysis. The combination of SPIO and blue dye localized the SLN in all procedures, whereas radioisotope and blue dye were successful in 26 ($P < 0·001$, McNemar's test). SPIO alone detected the SLN in 37 women and radioisotope alone in 23 ($P = 0·002$). Investigating SLN detection in relation to type of surgery, in the 30 women who had BCS, SPIO performed better than ^{99m}Tc (SLN detected in 30 *versus* 16 women; $P < 0·001$), and the addition of blue dye did not improve this (30 *versus* 18; $P < 0·001$). Correlation analysis showed that ^{99m}Tc detection was affected by the size of the excised DCIS (Kendall's $\tau = -0·494$, $P = 0·002$). In the ten SLNDs after mastectomy, tracer-specific detection was successful for SPIO and radioisotope in seven

Table 1 Clinical and pathological characteristics of SentiNot cohort

	No. of patients* (*n* = 189)
Age (years)†	60·2 (58·6, 61·9)
Radiological DCIS size (mm)†	39·6 (35·0, 43·6)
SPIO as standard method of SLND or surgeon experienced in SPIO use	
Yes	99 (52·4)
No	90 (47·6)
Size cut-off (mm)	
< 50	130 (68·8)
≥ 50	59 (31·2)
Nuclear grade on core biopsy	
2	63 (33·3)
3	119 (63·0)
Unknown	7 (3·7)
Detection mode	
Screening	166 (87·8)
Clinical	23 (12·2)
Palpable lesion or radiological mass effect	
Yes	27 (14·3)
No	162 (85·7)
Type of breast surgery	
Breast conservation	129 (68·3)
Mastectomy§	60 (31·7)
Transcutaneous axillary signal at end of operation	
Yes	187 (98·9)
No	2 (1·1)
Pathological DCIS size (mm)†	40·6 (36·7, 44·5)
Nuclear grade on specimen	
2	46 (24·3)
3	142 (75·1)
Missing	1 (0·5)
Invasive cancer in specimen	
Yes¶	47 (24·9)
No	142 (75·1)
Invasive cancer size (mm)‡	7 (2–100)
SPIO-induced skin staining	
Yes	42 (22·2)
No	147 (77·8)
SLND as a second operation	
Yes	41 (21·7)
No	148 (78·3)

*With percentages in parentheses unless indicated otherwise; values are †mean (95 per cent c.i.) and ‡median (range). §Direct reconstruction in 21 women (11·1 per cent). ¶Microinvasive cancer smaller than 1 mm in six women (3·2 per cent). DCIS, ductal carcinoma *in situ*; SPIO, superparamagnetic iron oxide; SLND, sentinel lymph node dissection.

Table 2 Characteristics of women who had invasive breast cancer on pathological assessment

	No. of patients (*n* = 47)	*P*†
Age (years)*	61·2 (58·2, 64·2)	0·552‡
Radiological DCIS size (mm)*	47·2 (38·1, 56·2)	0·047‡
Nuclear grade on core biopsy		0·526
2	19	
3	27	
Unknown	1	
Detection mode		0·607
Screening	40	
Clinical	7	
Palpable lesion or radiological mass effect		0·148
Yes	10	
No	37	
Pathological DCIS size (mm)*	45·3 (37·5, 53·1)	0·176‡
Type of breast surgery		0·715
Breast conservation	32	
Mastectomy	15	
SPIO-induced skin staining		0·547
Breast conservation	12	
Mastectomy	0	
Transcutaneous axillary signal at end of operation	40	

*Values are mean (95 per cent c.i.). DCIS, ductal carcinoma *in situ*; SPIO, superparamagnetic iron oxide. †*Versus* non-invasive cancer group (Fisher's exact test, except ‡Student's *t* test).

women; both tracers were successful in five women, both failed in one, and the results were discordant in four. The combination of SPIO and blue dye resulted in successful detection in all ten procedures; blue dye, however, did not enhance the detection rate of the radioisotope. The nodes from the three procedures in which intraoperative SPIO-guided biopsy was unsuccessful (3 mastectomies) were assessed later by a pathologist and found to contain SPIO, just like the probe-detected nodes. Detection using SPIO was better in BCS (30 of 30 *versus* 7 of 10; $P = 0{\cdot}012$) and where a surgeon was familiar with the method (29 of 29 *versus* 8 of 11; $P = 0{\cdot}017$). None of these factors retained significance in multivariate regression analysis (type of surgery: exp(B) 0·17, 95 per cent c.i. 0·01 to 2·80, $P = 0{\cdot}216$; surgeon familiarity with SPIO: exp(B) 0·22, 0·02 to 3·90, $P = 0{\cdot}314$). Both tracers, radioisotope and SPIO, enabled retrieval of the same number of SLNs (median 2; $P = 0{\cdot}385$). One patient who had a mastectomy for a 100-mm human epidermal growth factor receptor 2-positive, grade 3 DCIS presented

Table 3 Characteristics of women with a postoperative diagnosis of pure ductal carcinoma *in situ* in Sweden (2015–2017)

	No. of patients* ($n = 1688$)
Age (years)†	60·0 (59·4, 60·6)
DCIS size (mm)†	30·8 (29·5, 32·1)
Mode of detection	
Screening	1226 (72·6)
Clinical	460 (27·3)
Unknown	2 (0·1)
Type of breast surgery	
Breast conservation	1094 (64·8)
Mastectomy	567 (33·6)
Missing	27 (1·6)
Nuclear grade	
1	122 (7·2)
2	535 (31·7)
3	612 (36·3)
Unknown	332 (19·7)
Missing	87 (5·1)
Axillary surgery	
None	669 (39·6)
SLND	981 (58·1)
ALND	24 (1·4)
Missing	14 (0·8)

*With percentages in parentheses unless indicated otherwise; values are †mean (95 per cent c.i.). DCIS, ductal carcinoma *in situ*; SLND, sentinel lymph node dissection; ALND, axillary lymph node dissection.

with palpable axillary lymph nodes at 2-year follow-up. Ultrasound-guided cytology revealed metastasis. In the subsequent ALND, 12 nodes were retrieved, of which two were palpable and metastatic, and contained SPIO at pathological examination.

No adverse effects were noted. The rate of SPIO skin discoloration was 22·2 per cent, exclusively in women having BCS, with a mean stained area of 3·6 cm^2. Long-term follow-up on discoloration will be reported elsewhere.

The SentiNot policy resulted in substantial cost containment of surgical care, with a mean reduction of €448 (95 per cent c.i. 151 to 746) per patient, corresponding to a reduction of 8·5 per cent (€4813 *versus* 5261; $P = 0·003$) for the entire cohort. Looking at women with DCIS (and not IBC) who would have been treated with SLND, the mean cost saving was €1296 (886 to 1706), resulting in a 24·5 per cent reduction (€3990 *versus* 5286; $P < 0·001$).

During the study interval, 1688 women in Sweden had a preoperative diagnosis of DCIS, and pure DCIS confirmed by specimen pathology (*Table 3*). In total, 1005 of these (59·5 per cent) underwent axillary staging. Predictive factors for having had a SLND in multivariable analysis (*Table 4*) were younger age (58·9 *versus* 61·2 years; $P < 0·001$), larger DCIS size (36·7 *versus* 22·0 mm; $P = 0·005$) and mastectomy (SLND in 85·4 per cent; $P < 0·001$). Nuclear grades 1 and 2 were associated with fewer axillary staging procedures (grade 1: 29·5 *versus* 70·5 per cent, $P < 0·001$; grade 2: 50·8 *versus* 49·1 per cent, $P < 0·001$). Women with grade 3 DCIS had a 77·5 per cent rate of axillary staging, but this did not reach significance as a predictive factor on its own (77·5 *versus* 22·5 per cent; $P = 0·083$); It was, however, the primary factor for axillary surgery when breast conservation was used; 68·7 per cent of patients with DCIS undergoing BCS had axillary surgery because of nuclear grade 3 disease. Thirteen patients (1·3 per cent of those sampled and 0·8

Table 4 Characteristics of women with a postoperative diagnosis of pure ductal carcinoma *in situ* in Sweden, 2015–2017, according to whether axillary surgery was undertaken

	Axillary surgery*			Multivariable regression analysis	
	Yes	No	Univariable *P*	Odds ratio†	*P*
Age (years)‡	58·9 (58·2, 59·6)	61·2 (60·3, 62·1)	< 0·001§	0·99 (0·98, 1·00)	< 0·001
DCIS size (mm)‡	36·7 (35·0, 38·5)	22·0 (20·4, 23·5)	< 0·001§	1·01 (1·01, 1·02)	0·005
Nuclear grade ($n = 1601$)			< 0·001¶		
1	36 (29·5)	86 (70·5)		0·24 (0·14, 0·43)	< 0·001
2	272 (50·8)	263 (49·2)		0·48 (0·32, 0·72)	< 0·001
3	474 (77·5)	138 (22·5)		1·44 (0·95, 2·19)	0·083
Unknown	191 (57·5)	141 (42·5)		0·54 (0·33, 0·87)	0·012
Type of breast surgery ($n = 1661$)			< 0·001¶		
Breast conservation	521 (47·6)	573 (52·4)		1·00 (reference)	
Mastectomy	484 (85·4)	83 (14·6)		4·39 (3·24, 5·94)	< 0·001

Values in parentheses are *percentages and †95 per cent confidence intervals unless indicated otherwise; ‡values are mean (95 per cent c.i.). §Student's *t* test and ¶χ^2 test.

per cent of the entire cohort) had SLN metastases. Of 24 patients who underwent ALND, three procedures were for SLN metastasis and the rest owing to SLND failure. Application of the SentiNot inclusion criteria would have reduced the axillary staging rate from 59·5 to 13·3 per cent ($P < 0·001$, McNemar's test), considering that women not fulfilling the inclusion criteria would not have been considered for SLND (DCIS grade 1 or 2 and smaller than 20 mm, treated with BCS).

Discussion

In the present analysis, SentiNot resulted in 78·3 per cent of patients undergoing surgery for DCIS avoiding SLND. Moreover, SLND as a second procedure proved to be safe and effective. The policy provided a substantial reduction in healthcare-related costs. The present results suggest that SentiNot implementation at a national level would result in a substantial decrease in unnecessary SLND procedures and a significant cost reduction.

Although there is common agreement that routine SLND should not be part of standard treatment for DCIS[4,5], patterns of clinical practice seem to vary considerably in the international setting, even within healthcare systems. Despite compliance with guidelines[2,3,19,20], the rate of axillary evaluation in the setting of pure DCIS ranges from 33 to 84 per cent[21,22]. This may be attributed to the fact that, although the recommendation to consider SLND in patients with DCIS at high risk of invasion is uniform, the definition of high-risk DCIS is not. Traditionally, factors such as nuclear grade, size and mass effect (clinical or radiological) have been associated with increased risk of IBC. The most recent update of the guidelines[23] has not changed the recommendation from 2005[24], but the definition of DCIS with high risk of invasion has not been clarified[2]. Current National Institute for Health and Care Excellence guidelines[25] in the UK support the same view and state that the risk of invasive disease 'can be estimated using a range of standardized tools and clinical expertise'. However, recent data from the UK also demonstrate wide variation and lack of consistency in practice for women with palpable DCIS in whom BCS is planned. In addition, high-grade and multifocal DCIS are factors affecting the decision towards an axillary procedure. The Association of Breast Surgeons at British Association for Surgical Oncology guidelines generally advocate that extensive DCIS, defined as multifocal or larger than 40 mm, should be treated by mastectomy, which means that SLND would then be employed[26]. Despite this, the general impression is that SLND is not performed for DCIS when BCS is planned, but the uncertainty regarding which patients may have invasive cancer probably accounts for the fact that the frequency of axillary evaluation varies greatly from 9·4 per cent in the UK[2], to 22·6 per cent in the USA[21], and up to approximately 55 per cent in Denmark[20] and Japan[22]. A recent meta-analysis[27] identified grade and size as prognostic factors for invasion, which was estimated to affect 23 per cent of patients, but there was heterogeneity and publication bias in the included data. The lack of an accurate predictive model may account for variable rates of axillary staging, especially among low-volume hospitals and surgeons[28]. The present study included no women with low-risk DCIS; mean lesion size was 39·6 mm, more than 75 per cent of patients had grade 3 lesions, and smaller grade 2 DCIS were mostly mass lesions, meaning they would probably be considered for SLND regardless of country. Yet, none of these factors was predictive for IBC. The SentiNot concept addresses this problem efficiently, by providing a different approach: to mark the SLN and remove it only if needed.

The feasibility of SLND after BCS has been demonstrated, such that, if specimen pathology reveals IBC, SLND can be performed at a second operation[29]. This strategy may be challenged, as a detection rate of near 90 per cent after previous BCS has been reported, and may be considered suboptimal[30]. The GATA study[31] reported a detection rate of 85·5 per cent for SLND after diagnostic excision. The same study also showed that an interval shorter than 36 days between primary breast operation and secondary SLND increased the risk of detection failure, which is worrying as the usual time frame for reoperation is no longer than 30 days. Although distortion of lymphatics is another concern, a study[32] that evaluated scintigrams before and after diagnostic excisions, comparing operated and non-operated sides, showed that concordance was 85·7 and 88·9 per cent respectively. Although the study was small, this led the authors to state that there was no effect. In the present study, SPIO was more effective than radioisotope after a larger resection had been performed. Most of these women had an oncoplastic procedure, usually therapeutic mammoplasty, often requiring mobilization of the nipple–areola complex and rearrangement of the breast parenchyma. These considerations are not an issue for the SentiNot concept, because the SLN is identified using SPIO at the first operation and stays marked for at least 30 days. However, the study did not have adequate power to assess the secondary endpoint of detection rates, so these results should be treated as hypothesis-generating, until they are tested in other trials.

The SentiNot concept is also promising in the setting of mastectomy for DCIS. The ability to perform SLND at subsequent operation is debated[33], and so a SLND is uniformly recommended at the primary operation. SentiNot could also address the dilemma of whether to do a sentinel node biopsy during risk-reducing mastectomy, where the rationale for SLND is to avoid a second procedure that may lead to ALND, if occult disease is identified. In this setting, several alternatives have been reported[34]. Another potential benefit of SentiNot is that, if no SLND has been performed originally, in the event of recurrence the axilla is still unoperated[35,36]. The morbidity and complications of SLND in women with DCIS have been well described; in a Surveillance, Epidemiology and End Results Program data set analysis it was associated with complications including lymphoedema, wound infection, seroma and pain within 9 months of diagnosis[37].

The SentiNot policy has the potential to reduce treatment-related costs. It is a simple and pragmatic concept, requiring only a magnetometer and the tracer. It also has the benefit of simplifying logistics, as SPIO has already been injected at the first operation and no extra visits are required. Cost reduction stems from sparing SLND procedures[38]. The present economic model does not take into account possible effects of the technique on avoiding morbidity; however, the immediate reduction in surgical care-related costs by almost 25 per cent is more concrete than a hypothetical health economic model[39].

A consideration for widespread implementation is that magnetic detection at reoperation proved to be challenging for surgeons unfamiliar with the technique, particularly after mastectomy. This may explain why SLNs found to contain SPIO on pathology were not identified during the procedure. Until surgeons become more familiar with the technique, concurrent use of at least blue dye is recommended.

The conduct of interim analyses within clinical trials is challenging. To avoid overestimating the effect size owing to small numbers, the O'Brien–Fleming procedure was selected[40]. Despite reaching the primary endpoint, the interim analysis was prespecified mainly as a means to evaluate the study concept, rather than to terminate the study prematurely. The interim results indicate that tailored treatment in DCIS is feasible, enabling intervention only when necessary, with favourable effects on healthcare resources, and thus a good example of healthcare value (better outcomes for women at lower cost). A protocol amendment is currently planned to include more sites, broaden the indications to include diagnostic and risk-reducing procedures, and recalculate the sample size to provide robust results on comparable SLN detection rate at reoperation, specifically after mastectomy. Finally, long-term follow-up for oncological outcomes, quality of life, patient-related outcomes and cost-effectiveness will be undertaken.

Collaborators

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Paper III

cancers

MDPI

Article

Optimizing Dose and Timing in Magnetic Tracer Techniques for Sentinel Lymph Node Detection in Early Breast Cancers: The Prospective Multicenter SentiDose Trial

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Simple Summary: Superparamagnetic iron oxide (SPIO) nanoparticles have comparable performance to the combination of radioisotope and blue dye (RI + BD) for sentinel lymph node (SLN) biopsy in breast cancer. In this multicenter prospective study, lower SPIO doses (undiluted 1.5 vs. 1.0 mL) in different timeframes (perioperative vs. 1–7 days preoperative) and injection sites (subareolar vs. peritumoral) were compared to the previous standard (diluted 2.0 mL perioperatively) from the earlier Nordic trial. RI + BD were co-administered as background. In total, 534 patients were analyzed. SPIO SLN detection rates were similar (97.5% vs. 100% vs. 97.6%, $p = 0.11$) and respectively non-inferior to the dual technique. Significantly more SLNs were retrieved in the preoperative 1.0 mL cohort compared with 1.5 mL and the Nordic cohorts (2.18 vs. 1.85 vs. 1.83, $p = 0.003$). Thus, SPIO at 1.5 and 1.0 mL was non-inferior to both Sienna+® and the dual technique for SLN detection.

Abstract: Superparamagnetic iron oxide nanoparticles (SPIO) are non-inferior to radioisotope and blue dye (RI + BD) for sentinel lymph node (SLN) detection. Previously, 2 mL SPIO (Sienna+®) in 3 mL NaCl was used. In this dose-optimizing study, lower doses of a new refined SPIO solution (Magtrace®) (1.5 vs. 1.0 mL) were tested in different timeframes (0–24 h perioperative vs. 1–7 days preoperative) and injections sites (subareolar vs. peritumoral). Two consecutive breast cancer cohorts ($n = 328$) scheduled for SLN-biopsy were included from 2017 to 2019. All patients received isotope ± blue dye as back-up. SLNs were identified primarily with the SentiMag® probe and thereafter a gamma-probe. The primary endpoint was SLN detection rate with SPIO. Analyses were performed as a one-step individual patient-level meta-analysis using patient-level data from the previously published Nordic Trial ($n = 206$) as a third, reference cohort. In 534 patients, the SPIO SLN detection rates were similar (97.5% vs. 100% vs. 97.6%, $p = 0.11$) and non-inferior to the dual technique. Significantly more SLNs were retrieved in the preoperative 1.0 mL cohort compared with 1.5 and the 2.0 mL cohorts (2.18 vs. 1.85 vs. 1.83, $p = 0.003$). Lower SPIO volumes injected up to 7 days before the operation have comparable efficacy to standard SPIO dose and RI + BD for SLN detection.

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

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1. Introduction

Sentinel lymph node biopsy (SLNB) is the standard axillary staging method in patients with breast cancer without clinically evident nodal spread [1] and is associated with similar oncologic outcomes but less morbidity than conventional axillary lymph node dissection (ALND) [2–5]. Traditionally, detection with a radioisotope tracer (RI) combined with blue dye (BD), with a detection rate of more than 95%, has been regarded gold standard [1,6–8]. This method of combining tracers is known as the "dual technique". However, several drawbacks such as limited access, rigid legislation on radioactive disposal, short half-life of RI [9] as well as anaphylactic reactions and skin staining at the injection site related to the use of BD [10,11] limit its usage.

Superparamagnetic iron oxide nanoparticles (SPIO) are a SLNB tracer with comparable detection rates as the dual technique but provide logistical advantages such as increased flexibility in the timeframe of administration [12–14]. An earlier version (Sienna+®, Endomagnetics Ltd., Cambridge, UK) required dilution (2 mL SPIO + 3 mL NaCl 0.9%). Adverse effects included patient discomfort, artifacts on magnetic resonance imaging (MRI) and brown skin staining [15,16]. Previous reports indicated higher detection rates if SPIO was injected 1–28 days before surgery, instead of on the day of surgery [14,17,18]. Recently, a new solution of SPIO (Magtrace®, 2 mL, Endomagnetics Ltd.) with no need for dilution, has been shown to be noninferior to the dual technique [19,20].

The aim of this study was to compare the SLN detection rate using Magtrace® at lower doses, with different timeframes and injection sites, and to investigate whether they were noninferior to the previous SPIO solution of Sienna+®.

2. Methods

This multicenter prospective trial enrolled patients scheduled for primary breast surgery including SLNB at six Swedish centers. Inclusion criteria were breast cancers graded $cT_{0–2}cN_0cM_0$, and Eastern Cooperative Oncology Group (ECOG) performance status 0–2. All patients provided oral and written consent. Patients with previous ipsilateral breast or axillary surgery and/or radiation and neoadjuvant chemotherapy were excluded. The dataset of the Nordic SentiMag trial [13] was used to derive reference values and for subsequent patient-level comparisons. The study was approved by the Uppsala University regional ethics committee (Decision Number 2017/063), registered in a prospective database (ISRTCN11156955) and monitored by an independent external agency.

2.1. Procedure

Magtrace® was administered in two different sequential settings: the first patient cohort received a periareolar injection of 1.5 mL SPIO on the day of surgery, not later than 20 min prior to the start of surgery, followed by a five minute massage. The second patient cohort received 1.0 mL SPIO by subareolar or peritumoral injection into the interstitial tissue without massage, 1–7 days before surgery. All patients received RI and BD, according to routine practice.

During surgery, the surgeon initially used the Sentimag® (Endomagnetics Ltd.) to localize the SLN and then used the gamma probe to confirm this, both before and after skin incision. All SLNs detected intraoperatively with the Sentimag®, gamma probe or stained brown or blue were excised. The conventional cut-off of 10% of the SLN with the highest signal (SPIO or RI) was implemented. After excision, ex vivo counts for each lymph node with both probes were registered. SLN status was assessed by routine histopathology.

2.2. Sample Size Calculation, Statistical Analysis and Data Collection

The main objective was to evaluate whether administration of Magtrace® as described above was non-inferior to Sienna+® for SLN detection. We used the earlier detection rate of 97% with Sienna+® from the Nordic trial [13] and defined a non-inferiority margin of 4%, resulting in a lower threshold of 93%, to declare non-inferiority. For this, a sample size of 150 per cohort with a minimum of 146 successful magnetic SLNB procedures was required,

to ensure that the lower 95% confidence interval of the detection rate proportion would still be >93%. Allowing for a 10% dropout rate, 165 patients were required in each cohort. Detection rate per patient was additionally tested in a right-sided binominal test with the alternative hypothesis that the proportion of successful SLNBs would be >0.93 for each tracer. A p-value of <0.05 would indicate that the null hypothesis was rejected. To allow for direct comparisons and to define factors affecting outcomes, patient-level data from the Nordic trial [13] were used as a third, reference cohort and comparisons were performed as a one-step individual patient data (IPD) meta-analysis [21].

Demographic and clinical patient data, tumor characteristics, intraoperative magnetic and radioisotope signals, SLN-specific data, tracer-specific data, pre/postoperative histopathological data, possible adverse events and postoperative staining were recorded.

The primary endpoint was the proportion of successful magnetic SLN procedures divided by the total number of SLN procedures performed (detection rate per patient). A procedure was defined as successful for the respective tracer if at least one SLN was identified and retrieved. Secondary endpoints were (a) nodal detection rate, defined as the number of magnetic SLNs identified, divided with the number of SLNs detected with both modalities, (b) the average number of excised SLNs per patient, (c) the proportion of pathologically positive SLNs per patient and per node (malignancy rate) and (d) the SPIO-RI SLN concordance rate per patient and per node, defined as the proportion of patients or nodes detected by both SPIO and RI to the patients or nodes detected by RI.

All endpoints were analyzed at two different cut-off points with regards to the Sentimag® signal of the SLN, >0 and >20. The latter was selected to adjust for overlapping of detection methods (RI vs. SPIO), as nodes with low signal on one probe and high on the other, while formally considered as SLNs detected with both methods, would probably not have been identified had the patient received only one tracer.

The manuscript was prepared according to the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) statement [22], and the Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data (the PRISMA-IPD) statement was followed for database formation and statistical analyses [23]. Subsequently, any differences in study design or inclusion criteria between the SentiDose protocol and the Nordic trial protocol were parametrized as independent input variables, to allow for harmonization of definitions and the conduct of multivariable regression analyses, as appropriate. This resulted in an individual patient-level dataset comprising of the Nordic trial population (retroareolar/interstitial injection of 2 mL Sienna+® (Endomagnetics Ltd.) diluted with 3 mL of NaCl 0.9% or local anesthetic, administered perioperatively) used as a historic reference and the two prospectively collected cohorts of the SentiDose trial, as described above.

Comparisons of numeric outcomes were performed by one-way analysis of variance (ANOVA), whereas dichotomous outcomes were analyzed by means of Pearson's χ^2. Bonferroni adjustment for multiple comparisons was performed. Multivariable regression was performed if univariable associations with $p < 0.1$ were detected among clinically relevant variables. Background within-patient comparisons between SPIO and RI $\pm$ BD were performed to ensure non-inferiority and patient safety, but were not intended in the statistical analysis plan and thus, the published endpoints of the Nordic trial were not repeated.

2.3. Staining

All patients were prospectively followed for postoperative skin staining by SPIO or BD. Herein, patients with a brown/grey skin discoloration up to 6 months post-surgery were recorded. Long-term follow-up will be reported elsewhere.

3. Results

Consecutive patients were recruited, with the 1.5 mL cohort (n = 165) completed between August 2017 and April 2018 and the 1.0 mL cohort (n = 165) between May 2018

and September 2019. Protocol violation led to the exclusion of two patients from the 1.5 mL cohort. In total, 534 patients were analyzed and their characteristics are described in Table 1. There were no significant differences between the cohorts with regards to age, body mass index (BMI), tumor size, tumor type, tumor biology, or the proportion of patients with SLN metastasis. The SPIO injections were well-tolerated and no adverse effects were reported in the groups.

Table 1. Patient and tumor characteristics.

		Nordic Trial (2 mL)	Sentidose Trial (1.5 mL)	Sentidose Trial (1.0 mL)	*p*-Value
Patients, *n* = 534		206	163	165	n.a.
Age, years (mean)		62	64	63	0.101 *
BMI, kg/m^2 (mean)		27.9	27.2	26.5	0.568 *
Tumor size, mm (mean)		19	20	20	0.751 *
Histology	DCIS	12	9	4	0.694 #
	IDC	158	122	121	
	ILC	26	21	28	
	Other	10	11	12	
ER-status	Positive	170	138	145	0.831 #
	Negative	20	13	16	
	Missing	16	12	4	
HER2-status	Positive	20	9	16	0.217 #
	Negative	172	142	144	
	Missing	14	12	5	
Ki67 (%) (mean)		26.6	23.5	25.2	0.349 *
No. patients with metastasis		54	33	29	0.120 #
Previous ipsilateral breast surgery	Yes	17	0	0	<0.001 #
	No	189	163	165	
Previous ipsilateral axillary surgery	Yes	3	0	0	0.114 #
	No	203	163	165	
Type of surgery	BCT	154	130	141	0.038 #
	Mastectomy	52	33	24	
SPIO Injection site	Peri-/Sub-areolar	198	157	68	<0.001 #
	Peritumoral	3	6	97	
	Missing	5	0	0	

BCT: breast conserving therapy, BMI: body mass index, DCIS: ductal cancer in situ, ER: estrogen receptor, HER2: Human epithelial growth factor receptor type 2, IDC: invasive ductal cancer, ILC: invasive lobular cancer, n.a.: not assessed, SPIO: superparamagnetic iron oxide. *: analysis of variance (ANOVA), #: Pearson's χ^2 test.

3.1. Sentinel Lymph Node Identification—Per Patient

The overall magnetic SLN detection rate per patient was 97.6% in the Nordic trial, 97.5% in the 1.5 mL cohort and 100% in the 1.0 mL cohort (p = 0.110). Multivariable regression analysis showed a trend for significance for previous breast surgery with regards to the per-patient SLN magnetic detection rate at >0 magnetic tracer signal cut-off (b = 5.435, 95% confidence interval (CI) 0.925, 31.935; p = 0.061), and significance for previous breast surgery at >20 cut-off (b = 6.957, 95% CI 1.552, 31.192, p = 0.011). The detection rate of pathologically positive SLNs (malignancy rate) was 96.3% in the Nordic trial, 97% in the 1.5 mL cohort and 100% in the 1.0 mL cohort (p = 0.796). The SPIO-RI concordance rates

were 98% vs. 97.8% vs. 100%, respectively ($p = 0.115$). The concordance rate with regards to patients with pathologically positive SLNs was 98% in the Nordic trial, 97% in the 1.5 mL cohort and 100% in the 1.0 mL cohort ($p = 1.0$) (see Table 2).

Table 2. Sentinel lymph node identification—per patient.

n = 534	Nordic Trial (2 mL) *n* = 206	Sentidose Trial (1.5 mL) *n* = 163	Sentidose Trial (1.0 mL) *n* = 165	*p*-Value
SPIO SLN detection rate (%)				
If magnetic signal > 0	97.6	97.5	100	0.110 *
If magnetic signal > 20	97.1	95.7	100	0.016 *
SPIO SLN detection rate, malignancy (%)				
If magnetic signal > 0	96.3	97.0	100	0.796 #
If magnetic signal > 20	94.4	97.0	100	0.693 #
SPIO-RI SLN concordance (%)				
If magnetic signal > 0	98.0	97.8	100	0.115 #
If magnetic signal > 20	97.5	93.8	100	0.265 #
SPIO-RI SLN concordance, malignancy (%)				
If magnetic signal > 0	98.1	97	100	1.000 #
If magnetic signal > 20	96.2	100	100	1.000 #

#: Pearson's χ^2 test. * Detection rates compared with Fisher's exact test. Concordance calculated on cross-tabulations with use of the McNemar's test. SPIO: superparamagnetic iron oxide nanoparticles, SLN: sentinel lymph node, RI: radioisotope.

3.2. Sentinel Lymph Node Identification—Per Node

The nodal detection rate was 93.3% in the Nordic trial, 85.6% in the 1.5 mL cohort and 97% in the 1.0 mL cohort ($p = <0.001$). The mean number of SLNs retrieved in the three cohorts was 1.83 vs. 1.85 vs. 2.18 ($p = 0.003$). The SPIO malignancy rate per node was 93.8% in the Nordic trial, 79.5% in the 1.5 mL cohort and 100% in the 1.0 mL cohort. In multivariable analysis, preoperative injection (1–7 days) was associated with the retrieval of more SLNs and a higher nodal detection rate. Detailed per-node results are reported in Tables 3 and 4.

3.3. Effect of Injection Site and Injection Timing on SLN Detection

For a magnetic signal > 0, SLN detection after a periareolar injection was 97.9% vs. 100% after a peritumoral injection ($p = 0.301$), and for a magnetic signal > 20, 96.9% vs. 100%, respectively ($p = 0.174$). Regarding injection timing, a preoperative injection (1 to 7 days before surgery) was found to enhance SLN for a magnetic signal > 0 (100% vs. 97.6% for perioperative injection, $p = 0.063$). Looking into magnetic signal > 20, the difference was larger in favor of preoperative injection (100% vs. 96.5%, $p = 0.012$). This difference was retained in multivariable logistic regression. Regarding the number of SLNs retrieved, multivariable linear regression showed that periareolar injection was linked with a trend of retrieving less SLNs (b = 0.215, 95% CI −0.036, 0.465, $p = 0.093$), but the result was not statistically significant.

3.4. Skin Staining

The incidence and size of SPIO staining at 6 months in women undergoing breast conserving therapy (BCT) were not significantly different between the 1.5 mL cohort and the 1.0 mL cohort: 25.6% (33/129) vs. 18.4% (26/141) ($p = 0.15$), with mean sizes of 13.4

and 11.2 cm^2 ($p = 0.16$). In multivariable logistic regression, a peritumoral injection was associated with less skin staining.

Table 3. Sentinel lymph node identification—per node.

***n* = 534**	**Nordic Trial (2 mL) *n* = 206**	**Sentidose Trial (1.5 mL) *n* = 163**	**Sentidose Trial (1.0 mL) *n* = 165**	***p*-Value**
No. SPIO SLNs (mean)				
If magnetic signal > 0	1.83	1.85	2.18	0.003 *
If magnetic signal > 20	1.80	1.83	2.18	0.016 *
Nodal detection rate (%)				
If magnetic signal > 0	93.3	85.6	97	<0.001 #
If magnetic signal > 20	92	84.9	97	<0.001 #
No. SPIO SLNs, malignancy (mean)				
If magnetic signal > 0	1.11	0.8	1.18	<0.001 *
If magnetic signal > 20	1.11	0.8	1.18	<0.001 *
Nodal detection rate, malignancy (%)				
If magnetic signal > 0	93.8	79.5	100	0.005 #
If magnetic signal > 20	93.8	79.5	100	0.005 #
Nodal SPIO-RI concordance (%)				
If magnetic signal > 0	92.3	87.6	97.1	<0.001 #
If magnetic signal > 20	100	87.2	96.8	<0.001 #
Nodal SPIO-RI concordance, malignancy (%)				
If magnetic signal > 0	96.3	79.4	100	0.009 #
If magnetic signal > 20	100	74.4	100	<0.001 #

*: ANOVA, #: Pearson's χ^2 test.

Table 4. Cross-tabulation—Sentinel lymph node detection—in total numbers/cohort.

Nordic Trial Cohort									
Magnetic signal > 0					**Magnetic signal > 20**				
		Radioisotope					Radioisotope		
		Yes	No	Total			Yes	No	Total
SPIO	Yes	368	8	376	SPIO	Yes	323	48	371
	No	6	22	26		No	27	4	31
	Total	372	30	402		Total	350	52	402
SentiDose 1.5 mL cohort									
Magnetic signal > 0					**Magnetic signal > 20**				
		Radioisotope					Radioisotope		
		Yes	No	Total			Yes	No	Total
SPIO	Yes	298	0	298	SPIO	Yes	275	26	301
	No	6	47	53		No	38	12	50
	Total	304	47	351		Total	313	38	351
SentiDose 1.0 mL cohort									
Magnetic signal > 0					**Magnetic signal > 20**				
		Radioisotope					Radioisotope		
		Yes	No	Total			Yes	No	Total
SPIO	Yes	300	59	359	SPIO	Yes	299	61	360
	No	9	3	12		No	10	1	11
	Total	309	62	371		Total	309	62	371

4. Discussion

In the largest patient dataset to date, lowering SPIO volume to 1.0–1.5 mL did not affect SLN detection. The SLN detection rate per patient was at least 96.7%, constantly comparable to RI ± BD and unaffected by SPIO dose, timeframe and injection site. Moreover, different doses, injection timeframes and sites resulted in equally high SPIO-RI concordance rates.

These findings are consistent with recent results by Alvarado et al. [19] and Rubio et al. [20]. In these studies, however, SPIO was administered intraoperatively and injected in the subareolar area. The present results provide more evidence that, not only can a smaller dose be equally efficient, but also that an extended injection timeframe in the preoperative period might enhance the detection rate and SLN retrieval. It seems that preoperative injection allows for higher SPIO concentration in the SLN, which was demonstrated in the present study by the fact that there were no "low-signal" SLNs in this patient group and that brown coloring of the SLN was more intense. In addition, not only were there more SLNs retrieved, but the nodal detection rate was also higher, indicating that preoperative SPIO injection allows for accumulation in the SLNs, whereas SPIO in the lymphatics, which may produce a "magnetic background", is washed away to the circulation. Whilst the mean number of SLNs retrieved in patients injected preoperatively was 2.2, SPIO-RI nodal concordance was as high as 97%, demonstrating that there is no risk that the magnetic tracer would yield an unnecessary increase in the mean number of SLNs excised.

In previous studies from our group, results have shown that a preoperative injection of SPIO can be extended to more than 30 days before surgery with equally high SLN detection rates [14,17,24], but this had not been tested with a reduced SPIO dose. It is now clearer that timeframe is probably more important than the dose itself. In this context, SPIO is a highly effective tracer because it yields very high detection rates, but at the same time provides

flexibility and ease of administration, as it can be injected both intraoperatively and also at the outpatient clinic, sparing intraoperative time and resources and facilitating logistics.

Skin staining after SPIO injection is a concern, although several reports have shown that most patients do not consider it a problem [14,17,20]. In the SentiMagIC study [19], skin discoloration after a 2.0 mL subareolar injection was reported in 15.6% of patients. However, the proportion of BCS and the time for follow-up were not specified.

In the SUNRISE study by Rubio et al. [20], using subareolar injections in patients who underwent BCT resulted in staining varying from 59% in patients who received 1.0 mL to 83.3% in patients who received 2.0 mL. In the present results, a deeper, peritumoral injection seems to be associated with less skin staining, consistent with previous findings [14,17], implying that excision of the SPIO-stained injection site reduces the skin staining rate. A peritumoral injection and a smaller SPIO dose might also address the concern that has been reported for postoperative magnetic resonance imaging (MRI) artifacts [25], as the bulk of SPIO is excised with the tumor. Currently, our group is accruing data to specifically address this issue within the prospective POSTMAG MRI trial [26]. Despite that flexibility in injection site in the 1.0 mL cohort may have not allowed for the formation of two patient cohorts with distinctive characteristics, the study protocol allowed flexibility in the second cohort regarding the injection site, as manufacturer instructions during the study period stated that periareolar injection can be applied intraoperatively, regardless of dose, but peritumoral might require a longer time. At the same time, analysis of other data from our group published elsewhere [17] were in favor of a deeper injection, achieving comparable detection rates and resulting in less skin staining. Those previous conclusions are confirmed in the present results.

The study design did not include patient randomization, which is the standard robust methodological approach [27]. However, given the fact that study participants stemmed from the same reference population and that no differences in baseline patient demographics or tumor data could be demonstrated, implementing randomization would have been highly challenging for logistics in the multicenter setting without necessarily adding much more to the study results [28]. The technique of one-stage IPD meta-analysis was utilized, so as to improve the quality of data and expand the type of analyses that may be performed, thus producing more reliable results than the comparison with aggregate or historical data [29]. In the particular dataset, the homogeneity of study populations and protocols between the Nordic trial and the SentiDose suggests low risk of ecological bias, and within- and across-studies information do not differ substantially [30]. This resulted in a large patient dataset, highly representative of the relevant background population of breast cancer patients. Additionally, the study was performed in diverse clinical settings, including both university and regional hospitals, and breast cancer units that use SPIO routinely or not. This fact reflects a pragmatic value to the applicability of the study results, as they reflect routine practice rather than highly selected cases of patients. On the other hand, less exclusion criteria might have added more to study pragmatism, but that would have, in turn, created more patient subgroups and deviated from the primary aim of the trial, which was to investigate the performance of lower SPIO doses.

In a large patient dataset, it is now shown that a reduction down to half of the stipulated dose is highly effective and that a deeper preoperative injection yields more SLNs while retaining a high SPIO-RI concordance rate and resulting in less skin staining, when injected peritumorally. The use of SPIO in other clinical situations, such as SLN identification and dissection in malignant melanoma [31], prostate cancer [32–34], penile cancer [35] and uterine cancer [36], has been investigated, with interesting implementations.

Regarding breast cancer, the present results build on a substantial body of evidence that renders SPIO a very effective SLN tracer, that should not be considered an alternative to the RI anymore, as the comparable performance, ease of access and flexibility in delivery of care are important properties for clinical routine and implementation in the global setting. In this context, long-term follow-up and more studies to address specific clinical situations is paramount, in order to reach a robust and clinically relevant conclusion.

5. Conclusions

Magtrace® in lower doses (1.5 mL, 1.0 mL) is noninferior for SLN detection in patients with breast cancer compared with Sienna+® and highly concordant with the dual technique. Apart from perioperative administration, it was shown that preoperative peritumoral injection of 1.0 mL not only facilitated logistics but also increased detection rate and nodal yield, with high concordance with the dual technique with the additional advantage of less skin staining. Magnetic-guided SLN detection not only has the potential to omit isotope-based axillary mapping but preoperative administration allows for novel implementations to meet tailored needs of breast cancer patients.

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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.

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Paper IV

A Randomised Clinical Trial comparing Magseed® with Guide Wire localization in nonpalpable breast cancer scheduled for Magtrace® assisted sentinel lymph node biopsy: The MagTotal RCT

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Abstract

Guidewire localization is widely regarded as the gold standard method of localizing non-palpable breast tumors even though it has drawbacks. Magnetic seed (magseed®) localization is a safe and feasible alternative for localizing and excising non-palpable breast tumors. The combination of magnetic seed localization together with superparamagnetic iron oxide nanoparticles (SPIO) for breast cancer scheduled for breast-conserving surgery (BCS) together with sentinel lymph node biopsy (SLNB) have also been reported. In this multicentre randomized pilot study, we aimed to compare localization with either Magseed® or guidewire in breast cancer patients scheduled for BCS + SLNB between September 2018 and May 2021. All patients received SPIO peritumoral and preoperatively for identification of the sentinel lymph nodes (SLN). If randomized to magseed® localization (n = 91) the patient received it by the radiologist up until 30 days before surgery and if allocated to guidewire localization (n = 116) the patient received it on the day of the surgery. All patients were injected with SPIO, ultrasound guided by radiologist if allocated to magnetic seed or by the surgeon if allocated to guidewire, up until 30 days before surgery. Primary endpoint was reoperation rate due to positive margins. In 207 patients (n = 91 in magseed and n = 116 in guidewire) there was no significant difference in reoperation rate (3.3% in magseed vs 7% in guidewire group, p = 0.354). Furthermore, there was no significant difference in SLN detection rate (97.8% vs 100%, p = 0.187) and both groups had comparable mean number of SLNs retrieved (2.52 vs 2.62 nodes, p = 0.763). Magnetic seed localization together with SPIO for SLNB is a viable and safe alternative to guidewire localization.

Introduction

Non-palpable breast tumours are a surgical challenge that is steadily increasing in incidence, approximately half of all breast cancer diagnosed in Sweden and more than 1/3 globally are non-palpable at diagnosis (1-3). The most common method of localizing a non-palpable breast tumour is by guidewire localization (GWL) which has some drawbacks such as wire displacement/migration, patient discomfort, interference with pathological assessment of specimen and limitation in incision placement (4-6). Also, placement of the guidewire (GW) is usually performed on the same day as the surgery. However, in recent years there has been an upswing in the development of newer more patient friendly methods of non-palpable breast tumour localization such as radio guided occult lesion localization (ROLL), radioactive seed localisation (RSL), cryo-assisted localization, intralesional tracer administration and intraoperative ultrasound amongst others (7-14).

Magseed® is a paramagnetic 5 mm steel seed (Endomagnetics Ltd, Cambridge, UK) that can be inserted preoperatively by ultrasound or stereotactic guidance for non-palpable breast lesions. Intraoperative localization will be performed by the same handheld magnetic probe system (SentiMag®, Endomagnetics Ltd, Cambridge, UK) used for sentinel lymph node biopsy (SLNB) when applying superparamagnetic iron oxide nanoparticles (SPIO) as a tracer for sentinel lymph node (SLN). SPIO has been proven non-inferior to radioisotope (RI) + blue dye (BD) for SLNB (15-19). The feasibility and safety of magnetic seed localization as well as the complete magnetic surgical approach combining the magnetic seed for tumour localization and magtrace® for SLNB has also been previously reported (20-26).

Our aim was to compare magnetic seed with guidewire localization in a randomised clinical trial in patients with non-palpable breast tumours scheduled for breast-conserving surgery (BCS) + SLNB with regards to re-operation ratio of the breast due to positive margins. All SLNBs were performed primarily by using SPIO (magtrace®) as tracer.

Method

In this prospective RCT, patients were recruited at three Swedish hospitals between September 2018 and May 2021. Inclusion criteria were ductal breast carcinoma in situ (DCIS), or invasive breast cancer (T_{1-3}) requiring localization and scheduled for SLNB. Patients with iron overload diseases, hypersensitivity to iron/dextran compounds, pacemaker or other implantable metal devices were excluded. Pregnant and/or lactating patients were also excluded. All patients provided oral and written consent. The study was approved by the regional ethics committee at Uppsala University (dnr 2017/508) and registered in a prospective database (ISRCTN11914537).

Procedure

The patients were included and randomised to a localization method at first visit to the outpatient clinic, a magnetic seed or guidewire. Patients that were randomised to magnetic seed localization received it by the radiologist 1-30 days preoperatively, guided by ultrasound or mammography, at the same time as SPIO (magtrace® 1-2 ml) was injected. The magnetic seed was inserted ventral to the tumour and SPIO was injected dorsal to or in the periphery of the tumour in cases of microcalcifications, deeper seated lesion or cancer with diffuse growth patterns. Our hypothesis was that SPIO would create a magnetic rim surrounding the lesion that would be excised in the specimen but without affecting the focal signal of the ventrally placed magnetic seed. If randomised to guidewire, the guidewire was inserted on the same day or the day before surgery and patients received SPIO 1-30 days preoperatively injected by the surgeon. Blue dye (Patent V Blue®) was used at the surgeon's discretion.

The SentiMag® handheld magnetic probe was used during surgery to locate and excise SLNs in all patients. All SLNs detected by the SentiMag® probe, brown and/or blue colour were excised. The conventional 10% cut-off of the SLN with the highest signal was applied. In patients allocated to magnetic seed localization the same handheld magnetic probe was used for tumour localization and excision. The resection of the magnetic seed marked breast tumour was guided by the maximum signal on the SentiMag® probe which we know from the manufacturer corresponds to five millimetres from the seed, as such residual tissue with remaining magnetic signal was not routinely excised. Transcutaneous signal with SentiMag® in the breast and axilla was registered. After the excision of the primary tumour, a specimen count as well as a background count in the breast was performed. The presence and size of postoperative skin staining was also registered. All breast specimens went through

intraoperative mammography to confirm successful localization. SPIO signal counts for each excised SLN and for the specimen marked with the magnetic seed were recorded *in vivo* as well as *ex vivo*. In patients randomized to guidewire localization the resection of the breast tumour was according to routine practice.

Sample size, data collection and statistical analysis

Reoperation rate due to positive margins after excision reported in the literature varies widely (5% - 25%) when using guidewire assisted excision (27, 28). In a previously published pilot study of 32 patients who underwent a total magnetic surgical approach when performing BCS + SLNB, no patient underwent reoperation (20). We aimed to include 200 patients for this randomized pilot study. The size of a larger study with adequate power is depending on results from this pilot. E.g., a non-inferiority study with an estimated difference of no more than 5% would need >2,000 patients (29).

Randomization was performed by using a block size of ten and an allocation ratio of 1:1 using a random number generator software. The block randomization was generated by the principal investigator and allocation to either guidewire or magnetic clip was carried out by the surgeon who enrolled the patient at the respective clinic. Clinical and descriptive patient data, tumor characteristics, tracer specific data, SLN specific data, pre/postoperative histological data, data related to the surgery, intraoperative magnetic signals, specimen weight and data specific to the localization method allocated to the patient were collected. Possible adverse events and postoperative remaining staining were recorded.

The primary endpoint was breast re-operation rate due to positive margins. Secondary endpoints were SLN detection rate and average number of SLN retrieved per patient. Quality of life (QoL) was also assessed by patient-reported outcome questionnaire (BreastQ) and will be reported elsewhere after one year follow-up.

Comparison of numeric variables were performed by unpaired student t-test or Mann-Whitney U test depending on the assumption of normal distribution whereas categorical variables were analysed by means of Fisher's exact test. Univariate logistic regression analysis was performed for clinically significant variables, but no multivariate regression analysis was performed due to few events in the explanatory variable affecting the accuracy

of the model (30). The manuscript was prepared according to the "Consolidated Standards of Reporting Trials" (CONSORT) statement (31)

Quality of life survey – BreastQ

All patients received a QoL survey at inclusion (baseline), at the postoperative visit, six months and finally 12 months postoperative. The QoL survey chosen was BreastQ, a standardized validated patient-reported outcome survey specific for breast surgery (32, 33). The QoL survey results will be reported elsewhere.

Results

In total, 207 patients were recruited and randomized to either magseed® (n = 91) or guidewire (n = 116) localization. All patients received and tolerated SPIO injection well for the SLNB and no SPIO related adverse effects were reported in the groups. Furthermore, no localization method related complications occurred within the two groups. Patient and tumor characteristics are described in Table 1. There were no significant differences between the groups with regards to age, body mass index (BMI), tumor size, tumor type, tumor biology, or the excised specimen weight.

Table 1. Patient & tumor characteristics

Variable	Outcome	Magnetic seed	Guidewire	p-value
Patients, n=207		91	116	
Age (years)	Mean (SD)	63 (10)	62 (10)	0.815
BMI	Mean (SD)	27 (5)	27 (5.1)	0.838
Maximum radiologic size (mm)	Mean (SD)	13.14 (7.2)	12.74 (6.7)	0.690
Screening detected	No	10 (11%)	11 (9.6%)	0.818
	Yes	81 (89%)	104 (90.4%)	
Palpable at diagnosis	No	86 (94.5%)	115 (99.1%)	0.089
	Yes	5 (5.5%)	1 (0.9%)	
Histology	Ductal	76 (84.4%)	96 (82.8%)	0.330
	Lobular	7 (7.8%)	15 (12.9%)	
	DCIS	2 (2.2%)	3 (2.6%)	
	Other	5 (5.6%)	2 (1.7%)	

Estrogen receptor status	Negative	3 (3.5%)	10 (8.9%)	0.155
	Positive	83 (96.5%)	102 (91.1%)	
HER-2 status	Neg	81 (96.4%)	108 (96.4%)	1.000
	Pos	3 (3.6%)	4 (3.6%)	
Ki67 (%)	Mean (SD)	21 (14)	19 (14)	0.266
Specimen size (gram)	Mean (SD)	44.93 (28.3)	53.53 (82.1)	0.305
Interval from SPIO injection to surgery (days)	Mean (SD)	7 (5.1)	10 (8.3)	<0.001
Magnetic background count in breast (SentiMag® signal)	Mean (SD)	6340 (3866)	6673 (3938)	0.586
Discoloration breast injection site	No	84 (93.3%)	109 (94.8%)	0.768
	Yes	6 (6.7%)	6 (5.2%)	

Fisher's Exact test for testing categorical variables, unpaired t-test used for numerical variables

The reoperation rate due to positive margins was 3.3 % in the magnetic seed group and 7 % in the guidewire group (p = 0.35). The SLN detection rate was 97.8 % in the magnetic seed group and 100 % in the guidewire group (p = 0.19). The mean (SD) number of SLN retrieved was 2.52 (1.7) in the magnetic seed cohort versus 2,62 (1.8) in the guidewire cohort (p = 0.76).

Table 2. Primary & secondary endpoints

Variable	Outcome	Seed	Wire	P-value
Reoperation	No	87 (96.7%)	107 (93%)	
	Yes	3 (3.3%)	8 (7%)	0.35
Successful Sentinel lymph node biopsy	No	2 (2.2%)	0 (0%)	
	Yes	87 (97.8%)	116 (100%)	0.19
Number of sentinel lymph nodes excised	Mean (SD)	2.52 (1.7)	2.62 (1.8)	0.76

P-values for number of SN excised based on Mann-Whitney U-test, all other based on Fisher's Exact test

Discussion

In this randomized clinical trial comparing magnetic seed and guidewire assisted localization in non-palpable breast cancer there were no significant difference between the groups with regards to re-operation rate due to positive margins nor the SLN detection rate using SPIO as single tracer. This is concurrent with our previous findings regarding the feasibility and safety

of the total magnetic approach using both magseed® for tumor localization and magtrace® for SLN detection (20)

Guidewire localization has long been the gold standard method of localizing non-palpable breast tumors even though it has some clear drawbacks (5, 6). Magnetic seed localization on the other hand enables a more flexible scheduling of surgery, and the incision placement can be planned better enabling a better aesthetic outcome (20-22). Furthermore, one could easily argue that the magnetic seed is a more patient friendly method which makes it a compelling alternative to the guidewire (25). On the other hand, our initial hypothesis regarding the magnetic background count due to peritumoral SPIO injection (20), being reduced secondary to the specimen excision seems flawed. We noticed a comparable magnetic background signal in both groups after the specimen was excised. This poses two potential problems; first is the fact that the learning curve for the total magnetic technique could be harder due to the differentiation the surgeon must make between the background signal in the breast and the focal higher signal that comes from the magnetic seed. Second, with the remaining magnetic background signal one could draw the conclusion that postoperative artefacts on magnetic resonance imaging (MRI) would not decrease. The first problem is specific for the total magnetic technique while the second problem will be present in both groups since the reason is the accumulation of SPIO in the breast tissue at injection site. A possible technical solution to the first problem would be development of a handheld magnetometer that could differentiate between the signals given by the magnetic seed and SPIO. Regarding the second issue, our group is currently studying the effect of possible MRI artefacts in patients who have previously underwent breast cancer surgery using both peritumoral and subareolar SPIO injections (34).

All SLNs excised within the current trial were localized primarily by SPIO using the SentiMag® device. The SLN detection rate were comparable between the groups and adequately high as compared with the gold standard dual technique (17). All SPIO injections were administered preoperatively and peritumoral, we know from previous studies that a preoperative injection yields a higher average number of SLNs excised (16-18) E.g., in the SentiDose trial the average number of SLNs in the cohort which received the lower (1.0 ml) preoperative SPIO injection were 2.2, the current study showed an average number of 2.52 and 2.62 SLNs (16). The comparable average number of SLNs excised in these cohorts

strengthens the reasoning that a preoperative injection is what allows the SPIO to accumulate in the SLNs.

Our findings are consistent with those published by Micha et al. (25) and Zacharioudakis et al. (24) in their respective nonrandomized cohort studies comparing magnetic seed with guidewire localization. In their results, they found no significant differences regarding re-operation rate, Micha et al. reported 17% re-operation frequency in magseed® vs 16% in the guidewire cohort ($p = 0.40$) and Zacharioudakis 16% in magseed® cohort vs 14% in the guidewire cohort ($p = 0.69$). Micha et al. also reported a comparable median preoperative tumor size (13 mm vs 15 mm $p = 0.22$) between the cohorts. However, both studies reported a higher total frequency of re-excision than in the present results (3.3% vs 7% $p = 0.35$). This could be indicative of different approaches in the UK compared with Sweden where the volume resected usually is higher enabling the re-excision rate to be lower (35), as Micha et al reported their re-excision rate was lower than the 22% re-excision frequency reported in the UK NHS Breast Screening program (25).

Interestingly, median specimen weight (gram) between the groups in the report published by Micha et al (25) were significantly different (21 g vs 27 g $p = 0.006$) while there was a difference but not a significant one in the results published by Zacharioudakis et al (24) (magseed® 39.6 g vs. guidewire 44.5 g $p = 0.206$) and the present results (45 g vs 54 g $p = 0.305$).

Non-palpable breast cancer is a challenging clinical reality which requires inter-disciplinary cooperation between surgeons and radiologist. The surgeon must balance the volume excised and the subsequent defect with achieving adequate oncologic resection margins. Guidewire has and still is the most frequently used method for localization but in the recent years the development of additional localization methods has widened the feasible alternatives at the surgeon's disposal (5, 6, 8, 10, 11, 36). When reviewing the literature regarding localization methods for non-palpable breast cancer it is apparent that there is little to no differences between them regarding re-excision rates due to positive margins.
On the contrary, the differences between them seems to lie in other variables such as the amount of tissue excised, the logistics, patient and doctor experiences, cost-effectiveness, surgery time and accessibility.

With more localization methods developed and clinically tested comes the ability for adjusting and individualizing the surgery for each patient according to their physical properties, tumor size and tumor location. The current results shows that magseed® in combination with SPIO for the sentinel lymph node biopsy is a feasible and oncological safe alternative to guidewire in non-palpable breast cancer planned for SLNB.

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