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

ABDI-FATAH HERSI

ACTA
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UPSALIENSIS
UPPSALA
2021

ISSN 1651-6206
ISBN 978-91-513-1316-0
URN urn:nbn:se:uu:diva-455673

Dissertation presented at Uppsala University to be publicly examined in Psykiatriaulan, Västmanlands Sjukhus Västerås, 721 89, Västerås, Wednesday, 1 December 2021 at 09:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Professor Thorsten Kühn (Department of Gynecology & Obstetrics, Klinikum Esslingen, Esslingen, Germany).

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

Abdi-Fatah Hersi, Centre for Clinical Research, County of Västmanland, Västmanlands sjukhus Västerås, Uppsala University, SE-72189 Västerås, Sweden.

ISSN 1651-6206
ISBN 978-91-513-1316-0
URN urn:nbn:se:uu:diva-455673 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-455673)

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 end-point 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 end-point 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.

Acknowledgements

I would like to express my sincere gratitude to everyone who has supported me during my academic and clinical journey. A special thanks to the following individuals:

My principal supervisor, associate professor **Staffan Eriksson**: You believed in me even when I doubted. Your selflessness in helping and promoting others have always been admirable. I will be forever grateful and in your debt for everything you have shown me. Thank you for your guidance, mentorship and most importantly for your friendship.

My co-supervisor, Professor **Fredrik Wärnberg**: Thank you for your constructive ideas and for your support.

My co-supervisor, **Andreas Karakatsanis**: This thesis would have never come together without your hard work and ability to carry through the projects. Thank you for everything you have taught me.

Centre for Clinical Research – Region Västmanland: Thank you for all the support and grants. A special thanks to Professor **Kent Nilsson, Maria Dell Uva Karlsson, Maria Pettersson,** and **Mariana Ehn**.

To associate professor **Abbas Chabok**, my clinical supervisor and friend. You were the one who first told me that I should be a surgeon, you were the first to tell me to pursue research and most importantly you have never doubted my capabilities even when I gave you reason to. Thank you and your beautiful family for giving me a home away from home.

I would like to thank all the colleagues at the Department of Surgery at Västmanlands Hospital for their support and understanding. I would like to especially thank **Kevin Afshari, Kennet Lindahl, Claes Rudberg, Mats Andersson, Karl Andreasson, Yvette Andersson, Rafal "Gigi" Gorczynski and Piotr Tracz**.

To everybody at the Breast Unit at Västmanlands Sjukhus, thank you so much for your support and help in creating this body of work. Special thanks

to **Eva Alm** for always keeping us on track, and thank you **Ulrica, Karina, Marica, Elisabeth, Kathy.** To professor **Leif Bergkvist**, thank you for building a great unit with excellent research.

Head(s) of the Department of Surgery Västmanlands Hospital: Thank you **Anders Nilsson** for believing in me and for giving me the opportunity to become a surgeon. Thank you, **Eva Strand,** for always being kind and understanding. To the current head of the department **Hailemarim Mahteme,** thank you for being an inspiration and for always pushing me to stand tall.

To **Anders Hellberg**, thank you for your mentorship and for showing me what it really means to be a doctor first and a surgeon second.

To my current Heads of Department, Professor **Anna Elander**, **Peter Apelgren** and all my colleagues at the **Department of Reconstructive Plastic Surgery, Sahlgrenska University Hospital**: Thank you for welcoming me with open arms and making me feel at home.

To all my co-authors, thank you for your help and contribution.

To my elementary school teachers **Jörgen Ovesen and Milena Cvjetkovic,** thank you for believing in me and showing me the joy of academic success. Your hard work and understanding have not been forgotten.

Special thanks to my friend and research colleague **Allan "Memo" Jazrawi**. Thank you for all the laughs and for making me feel lazy whenever I was in your company.

To **Siw Svensson and Sonja Rosengren**, thank you for teaching me how to behave in an operating theater and thank you for making my first stumbling steps into the surgical world easier.

To my brothers **Murad, Deldar, Sabri, David, and Hussein**, thank you for standing by my side and for sharing the ups and downs of life with me. Who would have thought we would come this far! And to my dear friends **Erik, Victor, Romario and Oscar** thank you for your understanding and support.

To **Benjamin Hasselquist**, thank you my dear friend for all the insightful discussions during the years. I remember when we used to be up at 4am drinking disgusting energy drinks and discussing our future in between quizzing each other. Look at us now! To **Axel Möller**, thank you for all the great advice along the way my friend.

To my beloved family: I could have never reached my goals without you. I remember where we came from, I remember how we used to dream about the small thing's others take for granted, I remember all the sacrifices my older siblings had to make for me to have the privilege to focus on my studies. Nothing makes me happier than seeing pride and joy in your eyes. To my younger siblings, you are the inspiration and daily motivation for me to work harder, I am so proud of the women/men you have become. My ambitions have kept me away from you for too long, I truly hope you know I did it all for you!

To my older brother **Abdisamed**, thank you for being my role model and thank you for always being two steps ahead of me. To my older sister **Deeqa**, none of us would have made it this far without your sacrifices.

To my dear mother **Iman "Chico" Hassan**: You came to this country not knowing the language nor the culture. During the years I have watch you struggle each day, sacrifice after sacrifice with the sole purpose of giving your children better circumstances and better opportunities. Words cannot describe the gratitude I feel Hooyo maacan. It is said that paradise lies beneath your mothers' feet, I have never doubted this. I hope you can smile now Hooyo; I hope you can be happy now Hooyo. I truly thank you for everything!

And to all the patients who participated in these studies, thank you.

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Editor: The Dean of the Faculty of Medicine

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