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Sentinel Node Biopsy for Breast Cancer

Aspects and evolution

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Abstract

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Sentinel Node Biopsy (SNB) in clinical practice was pivotal to the shaping of modern diagnosis, staging and treatment of patients with breast cancer. The use of radioisotope (RI) and blue dye (BD) has led to high detection rates with low false negatives, but delivery-of-care limitations connected to these tracers as well as the need for methods addressing new clinical conundrums delineates the urge for new tracers with comparable performance, easier logistics and, ideally expanded implementations. Aim of the present thesis is to examine the outcomes of Superparamagnetic Iron Oxide (SPIO) nanoparticles, a new tracer based on magnetism for the detection of the sentinel nodes.

Paper I is a prospective multicentre trial comparing SPIO to RI+BD, with all tracers injected at the same patient. In 206 patients, SPIO had a similar detection rate (97.6 vs 97.1%, p=0.76) whereas concordance between methods was 98%. The study was completed by a meta-analysis of similar trials published until that point. The detection rates were comparable (fixed OR:1.10; 0.67,1.79, p=0.71), and so was concordance between tracers (fixed RD: 0.00; -0.01, 0.01, p=0.82). Discoloration was present after periareolar SPIO injection in 39% of patients, almost exclusively treated with breast conservation, which reduced to 8.6% after 15 months of follow-up.

Paper II was a pilot study of twelve patients with breast cancer and SNB performed where SPIO and the combination of RI+BD were injected, but SPIO was injected up to 15 days preoperatively, with total success in detection and complete concordance.

Paper III tested the performance of SPIO as a sole tracer in a pragmatic double-arm non-randomised trial comparing it to the combination of RI+BD. Detection was 95.7% for SPIO and 96.8% for RI (p = 0.59). The preoperative injection of SPIO (1-27 d) enhanced SPIO specific detection (95.7 vs 86%, p=0.002).

Paper IV is an interim analysis of a multicentre cohort study including patients with high-risk DCIS planned for breast conservation or any DCIS planned for mastectomy. SPIO was injected to "mark" the sentinel node but SNB was performed in a second operation only if invasive cancer was found at the first operation. In 151 included patients, this technique led to avoidance of 81.5% SNB, with a cost reduction of 14.1% for the entire cohort and 25.8% for the patients that did not have invasive cancer. The detection rate at reoperation was superior for SPIO and comparable with SNB detection at primary operation.

In conclusion, SPIO is a novel tracer for SNB in breast cancer with comparable performance, fit for performance in a global setting and with wider clinical implementations compared to RI +BD.

Keywords: Breast Cancer, Sentinel Node Biopsy, Superparamagnetic Iron Oxide

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To the patients we lose. It is for them we need to be better.

To young doctors in the making.
We have to make sure that they will
become better than we ever have.

Σην χάριν.

Στην ιερή μνήμη της Νότας, της Πηγής, της Στέλλας, της Ιωάννας.

Summum Sapientae, Doloris Summum

List of Papers

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

I Karakatsanis A, Christiansen PM, Fischer L, Hedin C, Pistioli L, Sund M, Rasmussen NR, Jørnsgård H, Tegnelius D, Eriksson S, Daskalakis K, Wärnberg F, Markopoulos CJ, Bergkvist L. (2016) The Nordic SentiMag trial: a comparison of super paramagnetic iron oxide (SPIO) nanoparticles versus Tc^{99} and patent blue in the detection of sentinel node (SN) in patients with breast cancer and a meta-analysis of earlier studies. *Breast Cancer Res Treat.*157(2):281-94.

II Karakatsanis A, Olofsson H, Stålberg P, Bergkvist L, Abdsaleh S, Wärnberg F. (2017) Simplifying logistics and avoiding the unnecessary in patients with breast cancer undergoing Sentinel Node Biopsy. A prospective feasibility trial of the preoperative injection of Super Paramagnetic Iron Oxide nanoparticles. *Scand J Surg.* doi: 10.1177/1457496917738867.

III Karakatsanis A, Daskalakis K, Stålberg P, Olofsson H, Andersson Y, Eriksson S, Bergkvist L, Wärnberg F. (2017) Superparamagnetic iron oxide nanoparticles as the sole method for sentinel node biopsy detection in patients with breast cancer. *Br J Surg.* 104(12):1675-1685.

IV A new technique to avoid sentinel node biopsy in patients with a preoperative diagnosis of ductal cancer in situ and its implementations on current practice. Manuscript, Submitted for publication.

Contents

Abbreviations

AJCC	American Joint Committee on Cancer
ALND	Axillary lymph node dissection
AUS	Axillary ultrasound
BCS	Breast-conserving surgery
CT	Computed tomography
DCIS	Ductal carcinoma in situ
DFS	Disease-free survival
DR	Detection rate
ER	Estrogen receptor
FNAC	Fine needle aspiration cytology
FNR	False-negative rate
HER2	Human epidermal growth factor receptor 2
HRT	Hormone replacement therapy
IBC	Invasive breast cancer
ICG	Indocyanine green
IHC	Immunohistochemical
ITC	Isolated tumour cells
LABC	Locally advanced breast cancer
LN	Lymph node
MRI	Magnetic resonance imaging
NACT	Neoadjuvant chemotherapy
NAT	Neoadjuvant therapy
PCR	Pathologic complete response
PgR	Progesterone Receptor
SEER	Surveillance, Epidemiology and End Results program
SN	Sentinel lymph node
SNB	Sentinel lymph node biopsy
SPIO	Superparamagnetic iron oxide
WBI	Whole breast irradiation
WHO	World Health Organization

1. Introduction

Breast cancer is one of the most common human malignancies. It is almost exclusively a female disease, since only 0.5-1% of new breast cancer cases involves males. In the course of recent years, advances in our understanding of the disease seem to challenge our traditional views and reshape clinical practice. Furthermore, breast cancer awareness initiatives have raised discussions and have given breast cancer the additional dimension of a social phenomenon. The aforementioned result in a constantly evolving landscape posing challenges alike for health providers and researchers.

2. Breast cancer. A brief overview

2.1. History

Tumours of the internal organs were essentially invisible to ancient medicine. This, however, was not the case with breast cancer, since it could be palpated and, later on in its clinical course, seen as fungating lesions as ulcerations developed. The oldest discovered evidence of breast cancer dates back 4200 years, to the Sixth Dynasty in Ancient Egypt. The Edwin Smith Papyrus describes eight cases of tumors or ulcers of the breast that were cauterized. The conclusion was emphatic: "There is no treatment." (1) For centuries, physicians described similar cases in their practices, with the same conclusion. From Hippocrates through to the 17th century, it was believed that breast cancer was caused by imbalances in the fundamental fluids that controlled the body; in particular, it was attributed to excess of black bile (2). Alternatively, patients often saw it as divine punishment. Later on, a wide variety of medical explanations would be proposed, such as lack of sexual activity, too much sexual activity, physical injuries, curdled breast milk, and various forms of lymphatic blockages, either internal or due to restrictive clothing. In the 19th century, the observation that breast cancer had the tendency “to run in the family” led the Scottish surgeon John Rodman to state that “fear of cancer caused cancer”, and that this anxiety was learned from the mother (3,4).

Although breast cancer was known in ancient times, it was uncommon until the 19th century, when improvements in sanitation and control of deadly infectious diseases resulted in dramatic increases in lifespan. Previously, most women had died too young to have developed breast cancer. Additionally, early and frequent childbearing and breastfeeding probably reduced the rate of breast cancer development in those women who did survive to Middle Age (4).

Mastectomy for breast cancer was performed at least as early as 548 A.D., when it was proposed by the court physician Aetius of Amida to the Byzantine Empress Theodora (5). It was not until doctors achieved greater understanding of the lymphatic system in the 17th century, that the spread of breast cancer to the lymph nodes in the axilla was described. The French surgeon Jean Louis Petit (1674–1750) performed total mastectomies which included removing the axillary lymph nodes, as he recognized that this reduced recurrence (6). Petit's work was built on by another French surgeon, Bernard Peyrilhe (1737–1804), who additionally removed the pectoral muscle underlying the breast, as he

judged that this greatly improved the prognosis (7). The Scottish surgeon Benjamin Bell (1749–1806) advocated removal of the entire breast, even when only a portion was affected (8). That work was carried on by William Stewart Halsted, whose name has been linked with the surgical management of breast cancer in the late 19th century. Halsted started performing radical mastectomies in 1882, helped greatly by advances in general surgical technology, such as aseptic technique and anesthesia. The Halsted mastectomy, known thereafter as radical mastectomy, involved removal of the breast, the regional lymph nodes in the axilla, and the underlying pectoralis major, whereas the procedure was often bilateral. The Halsted mastectomy was a morbid and amputating procedure which often led to long-term pain and disability, but was then seen as necessary in order to prevent the cancer from recurring. Before the advent of the Halsted radical mastectomy, 20-year survival rates were only 10%; Halsted's surgery raised that rate to 40%, without perioperative mortality. Following this doctrine, Owen Wangensteen described the technique of the extended or super-radical mastectomy, co-resecting the latissimus dorsi (9), whereas other surgeons included standard supraclavicular or internal mammary dissections, in order to minimize the risk for regional dissemination (10-12). Already by 1943 and based on the Halstedean concept, Haagensen et al. had described criteria of operability, describing those clinical features (13) that are today known to be associated with locally advanced breast cancer setting the bases for the Columbia Classification System that was one of the first breast cancer staging systems.

However, survival data from these maximalistic surgical approaches did not seem to justify their conduct. On the other hand, advances in the understanding of breast cancer together with the concept of disatant metastasis, led to perceiving cancer as a systemic illness as well as a localized one, and more sparing procedures were developed that proved equally effective provided that adjuvant treatment was given. After the conduct of the landmark 1977 study by the National Surgical Adjuvant Breast and Bowel Project (NSABP B-04), led by Bernard Fisher, radical mastectomies were largely abandoned, as study results showed that there was no statistical difference in survival or recurrence between radical mastectomies and less invasive surgeries (14).

2.2. Epidemiology

According to WHO and SEER data, the life-long risk for breast cancer in women is 12.4%, meaning that every eighth woman will be diagnosed with breast cancer at some point in her life. Further age adjusted data from SEER demonstrate that the number of new cases of female breast cancer was 125.0 per 100,000 women per year with median age 62 years, whereas the number of deaths was 21.5 per 100,000 women per year with a median age of 68 years.

In 2016, new breast cancer cases responded to 14.6% of all new cancer diagnoses, whereas breast cancer mortality accounted for 6.8% of all cancer-related deaths (15). Epidemiological data from Sweden (16) demonstrate similarities in prevalence, with 9444 new cases and 1431 deaths for the year 2015; in addition, it is the most common form of cancer (ca. 30%) among women. Internationally, there is a common observation that an increase in prevalence has been noted in the last 40 years which has been related with advances in diagnosis, life-style, parity pattern and hormonal replacement therapy. On the other hand, this is countered by a significant increase in overall survival with data demonstrating a 5-year overall survival higher than 90% in 2015 compared to approximately 60% in 1970, as shown by the Association of the Nordic Cancer Registries (NORDCAN) (17). This improvement is attributed internationally to timely diagnosis and advances in treatment.

2.3. A summary of histopathological and intrinsic biological features

2.3.1. Histopathology

Whereas malignancies may also arise from components of mesenchymal origin in the breast, which correspond to sarcomas, the standard when discussing breast cancer, is the malignancies arising from the epithelial component, that is, the carcinomas. Although discussed and referred to as a single disease, the constitute a diverse group of lesions that differ in microscopic appearance and biologic behavior.

Epithelial breast malignancies arise from the cells that line the breast lobules and the lactiferous ducts. Earlier, it was thought that there were more distinct features in the origin of these cells, but in later years, when the terminal ductal lobular unit (TDLU) was described, it has been shown that there is histologic continuity and that the heterogeneity of malignancies that may arise is associated predominantly with molecular characteristics (18).

Breast cancer has two important phases in its evolution: the in situ phase and the invasive phase. In situ, or pre-invasive carcinoma, is confined within the epithelial compartment. Invasive carcinoma, on the other hand, has breached the basement membrane, which is the barrier of the epithelium and infiltrates the connective tissue of the breast. Invasive carcinoma has *per se* the potential for metastasis, that is to establish secondary deposits at distant sites.

In situ carcinomas have traditionally been classified as either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS). The nomenclature relates neither to the sites of origin nor to the sites of the established diseases. DCIS is more often associated with invasive carcinoma of ductal type (IDC) and LCIS is more often associated with invasive carcinoma of lobular type

(ILC), but these associations are not exclusive. A rather more concrete difference between the two is the constant finding of the absence of E-cadherin expression in LCIS, a feature which is constantly present also in ILC (19). Recently, LCIS has been re-classified from being considered as a cancerous lesion to being considered as a risk factor for breast cancer, with an annual risk of 2 % (20). Therefore, aggressive surgical treatment is no longer advocated, and surveillance is recommended instead. It is currently registered as a "non-cancerous breast condition" by the American Cancer Society (21) and has been removed from the 8th edition of the TNM staging manual (22). It has also been suggested that it may be classified under the wider term lobular neoplasia (LN) instead (23). The only exception to this, is a pleomorphic variant (PLCIS), which should be surgically treated as DCIS (24). DCIS, on the other hand, is a heterogeneous clinicopathological entity which is considered a non-obligatory precursor to invasive breast cancer, accounting for approximately 85% of in situ breast carcinomas. If left untreated, it is estimated that up to 50% will upgrade to invasive cancer within 10 years (25). DCIS is generally treated similar to small, node negative invasive breast cancers. When breast conservation is performed, it has been shown that whole breast irradiation (WBI) reduces the risk of local recurrence (26,27).

IDC is the most common form of invasive breast carcinomas, accounting for approximately 75 to 80%, followed by ILC, at 15%. Apart from distinct morphological features, the expression of E-cadherin in IDC is a typical difference between IDC and ILC. Other, more uncommon invasive types are mucinous, tubular, comedo, inflammatory, medullary, and papillary carcinomas, and these together account for the remaining 10% of all cases (28).

2.3.2. Grading systems

Both in situ and invasive carcinomas are further sub-classified according to histopathological grade. Tumour grade is known to be a significant prognostic factor for breast cancer outcomes. Grading systems are commonly built on histopathological microscopic features. As far as DCIS is concerned, low- and intermediate- grade DCIS require cytologic, architectural and size criteria to be met, whereas high-grade DCIS requires only cytologic criteria. Features for low-grade DCIS (or grade 1) include round, regular to mildly irregular nuclei up to 2-3 times the size of a red blood cell (RBC) and complete absence of comedo necrosis whereas intermediate-grade DCIS (grade 2) is characterized by round, regular to mildly irregular nuclei up to 2-3 times the size of a RBC and substantial comedo necrosis. Finally, high-grade DCIS (grad 3) is characterized by pleomorphic nuclei more than 3 times the size of a RBC. Substantial comedo necrosis is also usually present, but not required for the diagnosis. Diagnostic criteria in clinical practice however, are clear for grades 1 and 3, with all intermediate cases classified as grade 2 (29). The grading by Holland is widely used in Europe and, apart from the present classification, it

considers cyto-nuclear differentiation and secondarily architectural differentiation, that is, cellular polarization (30). The Lagios system classifies DCIS as low grade (low nuclear grade and no necrosis), intermediate grade (intermediate nuclear grade and focal or absent necrosis), or high grade (high nuclear grade and extensive necrosis) (31). The different grading systems have been compared in intra-observer agreement studies with varying results (32,33). However, despite discrepancies, DCIS treatment outcomes are not dependent of the pathological features only, and other factors such as size, margin status, radiological features are important (34).

In invasive breast cancer, the most common and widely accepted grading system is the Nottingham score or, as otherwise known, the Elston-Ellis modification of the Scharf-Bloom-Richardson classification. It is a tri-variate score taking into consideration tubule formation, nuclear pleomorphism and mitotic activity, expressed as number of mitoses per high power field (HPF). Each variable is scored from a minimum of 1 to a maximum of 3. Total scores of 3-5 respond to grade 1, 6-7 to grade 2 and 8-9 to grade 3 (36,37).

2.3.3. Immunohistochemistry

Immunohistochemical (IHC) techniques provided the ground for further standardization and classification of breast tumours as well as therapeutic decision making. analysis of protein gene products.

2.3.3.1 Estrogen receptor (ER)

There are two isoforms of ER receptors, type α, which is the one used in clinical practice and type β, whose role in clinical routine is still a matter of ongoing research. Most human breast cancers (80-85%) are initially estrogen-dependent and undergo regression when deprived of their supporting hormone. The presence of significant amounts of ERα in breast cancer at the time of diagnosis is taken as an indication of hormone dependence (37). The primary ligand is 17-β-oestradiol which binds to the receptor and stimulates cell growth by transcription. Its impact on prognosis is favourable but can differ between treatment groups (38).

ER positivity is considered to reflect tumour sensitivity to endocrine therapy. The current recommendations from 2010 by the American Society of Clinical Oncology (ASCO) (39), including the 2017 St Gallen consensus statement (40), suggest that positivity >1% suffices to characterize a tumour as ER positive. However, it seems that tumours low in positivity are less responsive to endocrine treatment, as demonstrated in a retrospective study from the MD Anderson, based on 9639 women, where ER positive tumours with positivity <10% did not seem to benefit from endocrine therapy (41).

2.3.4.2 Progesterone receptor (PgR)

Despite that the role of progesterone receptors in breast cancer was unclear in the past, it is becoming increasingly clear that PgR-status is an independent prognostic factor in breast cancer. Two progesterone receptor targets, receptor activator of NfκB ligand and Wnt4, serve as downstream paracrine mediators of progesterone receptor-induced cell proliferation and stem cell activation, respectively (42). It has been found to be the major mitogen in human breast tissue thus playing a pivotal role in breast neoplasia (44) and has been also shown to affect the activity of the ER type α receptors, functioning as a molecular regulator to control ERα chromatin binding and transcriptional activity, which has important implications for prognosis and therapeutic interventions (45). PgR negativity has been demonstrated to be associated with poorer response to endocrine therapy and to poorer prognosis, which in some cases maybe comparable to hormone negative breast cancer (45,46).

2.3.4.3 HER-2neu (erbb-2)

The human epidermal growth factor (HER) subtype 2 is a tyrosine kinase receptor located on the cell surface. It is coded by the erbb-2 gene and is overexpressed in approximately 15% of all breast cancers. Biologically, tumours with HER-2 overexpression is associated with a more aggressive natural course, with higher recurrence rates and shorter overall survival, compared to their HER-2 negative counterparts. HER2-status is assessed with immunohistochemistry, and equivocal cases are further analysed with in situ hybridization. In this case, ratio of copies defines whether a tumour is amplified. Tumours with equivocal amplification are characterized after discussion on a multidisciplinary meeting (47).

2.3.4.4 Ki-67

Ki-67 is a monoclonal antibody directed against an antigen (Ki-67 protein) expressed only in proliferating cells. It is expressed as a percentage and responds to the of the fraction of proliferating tumour cells. Ki-67 values are reproducible and clinically useful, but only for clearly high or clearly low values. There is no standardized cut-off level for intermediate values. Therefore, it is advised that each pathology laboratory evaluate their median regularly, in order to allow for cut-off values (48-50).

2.3.4. Intrinsic subtypes

The heterogeneity of breast cancer prompted further investigation that led to extensive molecular tumour profiling. The hypothesis investigated, and proven right was that the phenotypic variability of breast tumours accompanied by differences in response to therapy, prognosis and survival, responded to different genomic characteristics. The ground-breaking research by Perou

et al. (51) managed to define "molecular portraits" with different profiles of gene expression. Subsequently, distinct gene signatures were found to be related with particular molecular characteristics, and subtypes were developed. More recent investigation by the Cancer Genome Atlas Network (52), led to a comprehensive definition of the subtypes. These were the ER positive/ luminal-like, the basal-like and the erbb-2 positive. The ER positive/luminal-like are a large subgroup of breast tumours, being the most heterogeneous in terms of gene expression, mutation spectrum, copy-number changes and patient outcomes. Basal-like tumours are also typically referred to as triple-negative breast cancers, since they are negative for ER, PgR and HER2. HER2 positive tumours, on the other hand, are characterised by HER2 DNA amplification and overexpression of the HER2-amplicon-associated genes. HER2 positive tumours present with two clinically distinct subtypes, each accounting for approximately 50%; one where the HER21 protein and HER2E mRNA subtypes overlap, where a strong signal of EGFR, pEGFR, HER2 and pHER2 is observed, and the other, where HER2 overexpression is observed in the ER+/luminal-like subtypes.

This led to the definition of the intrinsic subtypes, which are as follows:

- Luminal A: ER and PgR strongly positive, HER2 negative, low grade and low to intermediate Ki67
- Luminal: ER positive and PgR weak positive or negative, HER2 positive or negative, high grade and high Ki67
- Non Luminal HER2 positive: ER and PgR negative, HER2 positive, high grade and high Ki67
- Basal like or Triple-negative: ER, PgR and HER2 negative, high grade, high Ki67

In the discrimination between Luminal A and Luminal B intrinsic ER positive subtypes, it has also been described that clinical factors such as positive nodal status or clinical primary size should affect the characterization of a tumour as either Luminal A or Luminal B (53). This subtypes are in accord, not only with the biological behaviour of the tumour, but also with response to different types of systemic treatment. TNBC tend to recur earlier but, 5–8 years after diagnosis, their annual hazard of recurrence drops below the level of ER-positive tumours. Relapse of breast cancer may occur as late as >20 years after the initial diagnosis, particularly in patients with ER/PgR-positive disease (54).

Luminal A subtypes are the most responsive to endocrine treatment and least responsive to chemotherapy. Standard endocrine treatment consists of tamoxifen in premenopausal women or an aromatase inhibitor in postmenopausal women. As proliferation increases, so does effect of chemotherapy. Standard chemotherapy for breast cancer consists of a combination of anthracycline with a taxane. ER negative subtypes are chemo-sensitive, whereas

HER2 positivity means response towards monoclonal antibodies such as trastuzumab, pertuzumab, and lapatinib.

2.3.5. Gene signature arrays

Despite that intrinsic subtype classification guides the systematic treatment which is to be administered, there are always cases that are "between" subtypes, which is particularly true for ER positive, HER2 negative and node negative tumours. In such cases, commercially available molecular signatures for ER-positive breast cancer, such as Oncotype DX, EndoPredict, PAM50, Prosigna, and for all types of breast cancer (pN0–1), such as MammaPrint and Genomic Grade Index, may be used in conjunction with all clinicopathological factors, to help in treatment decision making.

The Oncotype DX, otherwise known as a the 21-gene signature panel, is the one most validated. The assay algorithm is based on the expression of 16 cancer-related and 5 reference genes, and provides a result (range: 0–100) representing estimated 10-year risk of distant recurrence with 95% confidence intervals (CIs). Based on their Recurrence Score results patients are classified into the low (<18), intermediate (18–30), or high (≥31) Recurrence Score group (55). It is a predictor of recurrence risk in ER positive, node-negative tumours as well as a predictor of response to chemotherapy, so as to allow for precision and avoidance of overtreatment. Results from the recent TailorXtrial depicted that adjuvant endocrine treatment alone worked as well as endocrine treatment and chemotherapy together not only for cases with low, but also with cases of intermediate risk, as it was predicted by the genomic score. After 9 years of follow-up, the rates of invasive disease-free survival were 83.3% for hormone therapy alone and 84.3% for hormone therapy and chemotherapy, and for overall survival, the rates were 93.9% and 93.8%, respectively (56).

The MammaPrint uses a microarray technology to assess the expression of 70 genes. Its development involved a cohort of 78 patients <55 years with ER+, HER2-negative or positive, and triple-negative early breast cancer who underwent surgery without systemic therapy and had long-term clinical follow-up. The genes assessed are associated with cell cycle, invasion, angiogenesis and metastasis. MammaPrint classifies patients into two groups: low risk and high risk.

Apart from original validation, the 70-gene signature was evaluated in a large prospective randomized trial. The "Microarray In Node-negative and 1–3 positive lymph node Disease may Avoid ChemoTherapy" (MINDACT) is a prospective trial which investigated whether adjuvant chemotherapy could be spared in patients who are low-risk by MammaPrint. Patients in MINDACT included those with ER+, HER2-negative, HER2+ or triple negative disease, and only those with discordant risk assessments (MammaPrint vs. Adju-

vant!Online) were randomized to chemotherapy vs no chemotherapy. The results demonstrated that, among women with early-stage breast cancer who were at high clinical risk and low genomic risk for recurrence, the receipt of no chemotherapy on the basis of the 70-gene signature led to a 5-year rate of survival without distant metastasis that was 1.5 percentage points lower than the rate with chemotherapy. The authors concluded that, given these findings, approximately 46% of women with breast cancer who are at high clinical risk might not require chemotherapy (57).

2.4. Staging

The primary breast tumour is staged according to size and direct invasion of surrounding structures. The common pattern of spread is via the lymphatic pathway, which in turn leads to metastases in the regional lymph nodes. The lymphatic vessels of the breast drain both medially to the internal mammary lymph nodes and laterally to the axillary lymph nodes. Even from the most medial aspect of the breast, both lymphoscintigraphy and analysis of lymph-node metastases suggest that the majority of lymphatic flow is toward the axillary lymph nodes so that the axilla is considered to be the most common regional nodal basin. Subsequently, the axillary lymph nodes were divided in three levels according to their anatomical relation to the pectoralis minor muscle. The common pattern of spread from the axilla involves further nodal basins, such as those of the sub-clavicular and supra-clavicular region and consequent haematogenous spread to distant sites. The most usual organs harbouring metastases from breast cancer are the liver, the pleura, the lung, the bones and the brain. However, knowledge of breast cancer biology is an evolving landscape, with significant impact on staging, treatment and prognosis. At present, the 8th edition of the AJCC TNM classification is the first to include information derived from immunohistochemistry and genomic signatures to classify tumours and possibly modify treatment recommendations, thus implying that tumour stage and prognosis is in direct association with intrinsic characteristics and biology.

At present, early breast cancer is characterized by the presence of lesions smaller than 5 cm and absence of clinically detectable axillary disease, with 2 cm being the cutoff between T1 and T2 lesions. T3 tumours are characterized by size larger than 5 cm but absence of invasion in surrounding structures. Finally, T4 tumours are by definition classified as locally advanced breast cancer and are characterized by direct invasion of the chest wall, the skin or both or by the entity known as inflammatory breast cancer. In cases of multifocal tumours, T-stage is defined by the size of the largest tumour rather than the total extent of the lesions and the prefix “m” is used to denote multifocality.

Clinical nodal status is defined by the presence of palpable nodes with the prefix “c” to indicate clinical impression. Subsequently, cN1 refers to mobile

palpable axillary nodes, cN2 to matted axillary nodes and cN3 to palpable axillary and parasternal nodes, or subclavicular nodes or supraclavicular nodes.

Finally, the absence of distant metastases is classified as M0, whereas clinical or radiological metastases is classified as M1. The detection of metastatic disease by molecular methods in absence of clinicoradiological signs of distant disease is characterized as cM0(i+) (22,55).

2.5 Treatment of the axilla. A paradigm shift

Nodal status in breast cancer has clinical significance. This is old and objective knowledge, a truth that has been there from the time of Halstead, despite the fact that it was viewed under a different prism then. Therefore, the first surgical approach to the treatment of breast cancer included the radical mastectomy, with removal of the entire target organ, the underlying pectoralis major muscle and all the anatomically relevant lymph nodes. The progression in our understanding of the disease and the advances in the field of clinical oncology and systemic therapy gradually reduced the need for *a priori* extended surgical procedures. The introduction of whole breast irradiation therapy (WBI) marked a significant cornerstone in breast surgery, allowing for breast conservation with comparable results to mastectomy in terms of local recurrence (59).

The paradigm shift of performing necessary-only surgery was also focused in the management of the axilla. It was seen that routine axillary dissection resulted in the retrieval of healthy lymph nodes in about 70% of cases, a percentage that has been stable despite the improvement in early diagnosis. At the same time, axillary lymph node dissection (ALND), as routinely performed (dissection in levels I and II), was shown to be accompanied by morbidity as high as 40% (60). Therefore, there was a clear need for a procedure providing staging information with less complications.

2.6 Sentinel Node Biopsy

Sentinel node biopsy (SNB) for breast cancer was introduced and validated in the middle of the 1990s (61,62) and became established as the golden standard for axillary staging in patients with breast cancer who present with a clinical and radiological negative axilla (63). The concept of sentinel node, as" the first lymph node in line draining the lymph from the primary tumour site" was introduced in surgical oncology with melanoma. The rationale was that, if the first node was not afflicted, no other nodes would. Once the oncological safety of the procedure was established (64), axillary sampling and blind axillary

dissections were practically removed from clinical practice, resulting in substantial decrease in associated surgical complications such as vascular and neural injuries, lymphedema, chronic pain and wound infections (65). Literally all studies have demonstrated the efficacy of SNB in the accurate determination of lymph node status and its superiority when related to ALND concerning procedure related complications.

2.6.1. Principles and technique

The golden standard for the mapping and identification in SNB has traditionally been the combination of radioactive colloid (Tc^{99}, hereafter RI) on a usual dose of 40-60 mBq with a blue dye (BD) for visual aid. The isotope is injected subareolarly or peritumourally a few hours before the operation or intraoperatively. The BD is injected intraoperatively and after the induction of anaesthesia, so that vital functions are secure, as its injection has been associated with anaphylactic shock in a frequency of 0.1% in the literature. The tracers reach the SN via the lymphatics. Thereafter, the SN site in the axilla is detected with the use of a hand-held gamma probe. Skin incision is performed and the SN is sought with the help of the gamma probe and the visual aid of the BD. The biopsy is considered completed when the probe signal in the axillary background is less than 10% of the SN signal without any remaining palpable LNs. The combination of RI and BD results in successful detection rates as high as 99% with a false negative rate of less than 5% (65,66). The procedure is illustrated in Figure 1 (67).

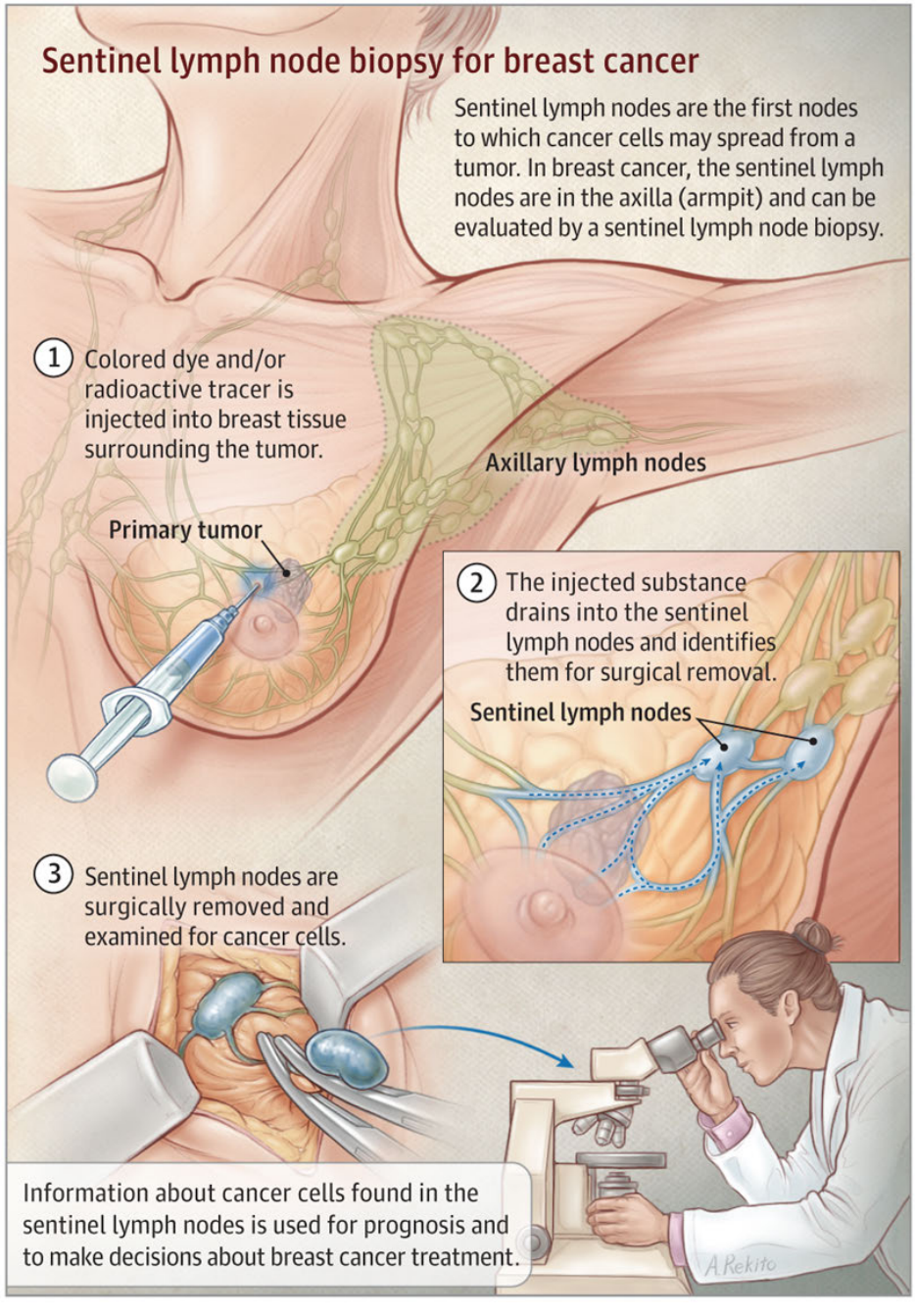


Fig.1 Sentinel node biopsy. Free for non-commercial reproduction from Heerdt AS. Lymphatic Mapping and Sentinel Lymph Node Biopsy for Breast Cancer. JAMA Oncol. 2018;4(3):431. doi:10.1001/jamaoncol.2017.4000.

There are, however, some drawbacks involved with the procedure. The use of RI demands a nuclear medicine department and complicated legislation and restrictions regulate the handling and disposal of radioactive material. The short half-life (6 hours) of the RI limits its usefulness and RI also confers possible hazards to the patient and staff. All this limit the access to the method. Interestingly, recent literature reports that only 60% of patients in developed countries have access to the procedure, with this figure dropping down to 5% in China and even lower in other parts of the world. The BD, on the other hand is allergenic, and has been associated with a few serious events (68). SNB is a part of the surgical routine for breast surgeons but, despite reduced morbidity when compared to more extended procedures, it is not an indolent one (69,70). A recent meta-analysis has demonstrated early and late postoperative morbidities such as restricted shoulder range of motion (up to 37.8% two years after surgery), pain (up to 56.6% one month after surgery), paraesthesia (up to 15.8%), axillary web syndrome (20%) and lymphedema (8.2% two years after surgery) among others (71). This means that, despite being less morbid compared to ALND, refinement of indications and technique will be beneficial for patient care and quality of life.

These facts stress the need for developing non-radioactive and non-allergenic tracers with comparable performance, but fewer side effects. In an optimal setting, it could provide novel clinical implementations, to establish minimally invasive or non-invasive methods to stage the axillary status

2.6.2 Superparamagnetic Iron Oxide Nanoparticles

A non-radioactive method for identifying the sentinel node using a superparamagnetic iron oxide (SPIO) tracer and a hand-held magnetometer has been developed. SPIO coated in biocompatible molecules have been used in the past as contrast agent for magnetic resonance imaging (MRI) for almost 20 years and has been proven to be non-toxic when injected intravenously. The use of SPIO in a clinical context as a tracer for SNB in breast cancer was first reported in 2013 (72). In the same way as for RI and BD the SPIO drains through the lymphatics and accumulates in the SN. A handheld probe is used to identify the SN, in the same way as the gamma probe is used for detection of RI containing lymph nodes.

The SPIO solution is dark brown, and the SNs are often colored, the brown-black appearance acts as a visual stain aiding intra-operative identification. Sienna+, a sterile aqueous suspension of SPIO coated with carboydextran, is the magnetic tracer that is intended and calibrated for use together with the SentiMag device. The carboxydextran coating prevents agglomeration while maintaining biocompatibility. The Z-averaged particle diameter, including the organic coating, is 60nm (<0.25 polydispersity). The diameter enables the SNs to selectively filter out the particles. After subcutaneous Sienna+ injection into the subareolar interstitial tissue, Sienna+ particles drain naturally to the lymph

nodes via the lymphatic system where they are physically filtered, trapped and concentrated. This allows them to be used as a lymph node marker, which can be identified by the SentiMag device. The magnetic probe (SentiMag) is patented and received a CE mark as medical device class IIa in December 2010. SPIOs exhibit superparamagnetic properties, characterized by a response to an external magnetic field while retaining no magnetic remnant in its absence. Additionally, the presence of SPIO in the tissue has not been shown to affect the accuracy of the pathologic examination or the conduct of immunohistochemistry in breast or nodal tissue (73).

3. Aims

3.1 Paper I

To compare the outcomes of SPIO with the combination of RI and BD in terms of detection rate, number of SN retrieved and concordance between methods. Secondarily, to perform a systematic review and meta-analysis of available studies.

3.2 Paper II

To investigate the feasibility of injecting SPIO in the preoperative period for a successful SNB.

3.3 Paper III

To investigate the performance of SPIO as a sole method for SNB.

3.4 Paper IV

To investigate the possibility of sparing unnecessary SNB procedures in patients with a preoperative diagnosis of DCIS with the help of SPIO.

4. Patients and Methods

4.1 Patient inclusion criteria

4.1.1. Papers I-III

Patients were eligible for recruitment if they were older than 18 years, diagnosed with invasive breast cancer or DCIS, with clinically negative axilla, and scheduled for a SNB. Exclusion criteria were hypersensitivity to dextran compounds, iron or Sienna+, intolerance to the isotope, iron overload disease, pregnancy, pacemaker or other implantable metallic devices close to the axilla, or mental condition rendering the patient incapable of giving informed consent to the study. All patients had to be available for postoperative follow-up. Appropriate candidates were identified from case presentations in the multidisciplinary rounds. In paper II, a healthy volunteer was also recruited.

4.1.2. Paper IV

Patients were eligible for inclusion if they were preoperatively diagnosed with DCIS any size and nuclear grade 3; nuclear grade 2 and preoperative size >20mm on imaging; mass effect on imaging or clinical examination; and finally, any grade or size DCIS planned for a mastectomy. Exclusion criteria were: intolerance or hypersensitivity to iron or dextran compounds; iron overload disease; pacemakers or other implantable devices in the chest-wall and pregnant or lactating patients. Direct reconstruction in cases of mastectomy was not considered an exclusion criterion.

4.2 Study design and setting

4.2.1. Paper I

Prospective, non-randomized double-arm comparative multicentric trial. Conducted in five Swedish and two Danish hospitals. The design of the systematic review and meta-analysis is described in the respective section.

4.2.2. Paper II

Prospective, comparative pilot study conducted in the Breast Unit of Uppsala University Hospital.

4.2.3. Paper III

Prospective, pragmatic non-randomized double-arm comparative trial. Conducted in two Swedish Hospitals.

4.2.4. Paper IV

Single-arm, multicentre prospective cohort study. Results presented in the paper stem from the interim analysis. In this phase of the study, the study was conducted in four Swedish hospitals.

4.3 Methods and considerations

4.3.1. Paper I

Landmark SNB trials were initially performed on a background of axillary clearance, allowing for results on the sensitivity, the accuracy and the false negative rate of the procedure. This has led to the establishment of RI and BD as tracers for SN with an anticipated FNR of 5-10%. The conduct of similar trials for SPIO would be ethically questionable, as ALND is to be avoided in the negative axilla. Therefore, it had to be assessed whether SPIO not only detects a SN, but, most importantly if it detects the same SN as the combination of RI and BD, which is expected to be the *true* SN, with the expected FNR mentioned above, Therefore, the methods would have to be tested simultaneously on the same patient.

Patients were injected with the radioactive tracer (^{99}mTc), usually 40-60 mBq, either the day of surgery or the day before. Injection site was chosen according to local standards. A vital blue dye (1-2 ml of Patent Blue V ®) was injected in standard fashion after the onset of anesthesia. Two ml of Sienna+ diluted with 3 ml saline was injected subareolarly either shortly before or after induction of anesthesia. The injection site was massaged for 5 minutes, and the operation was not to start until at least 20 minutes had elapsed. During operation, transcutaneous signal in the primary and the axilla was recorded, first with a handheld probe (SentiMag®, Endomagnetics Ltd, UK) to detect magnetic uptake and afterwards with the gamma probe. A short incision was made in the axilla over the area with the greatest uptake, and the sentinel node was sought for primarily using the SentiMag probe. Metal retractors and instruments were removed and plastic ones were used at that point. Thereafter

the finding was confirmed with the gamma probe and the SN(s) removed. All sentinel nodes were excised until the counts were lower than 10% of the highest count or a maximum of four nodes per patient were removed. Blue and/or brown nodes were also regarded as SNs. Magnetic and gamma- counts in the SNs were registered before the skin incision; in situ and ex vivo; and in the remaining axilla.

Patients were followed postoperatively and any discoloration was registered and measured repeatedly. The study flow is described in Figure 1.

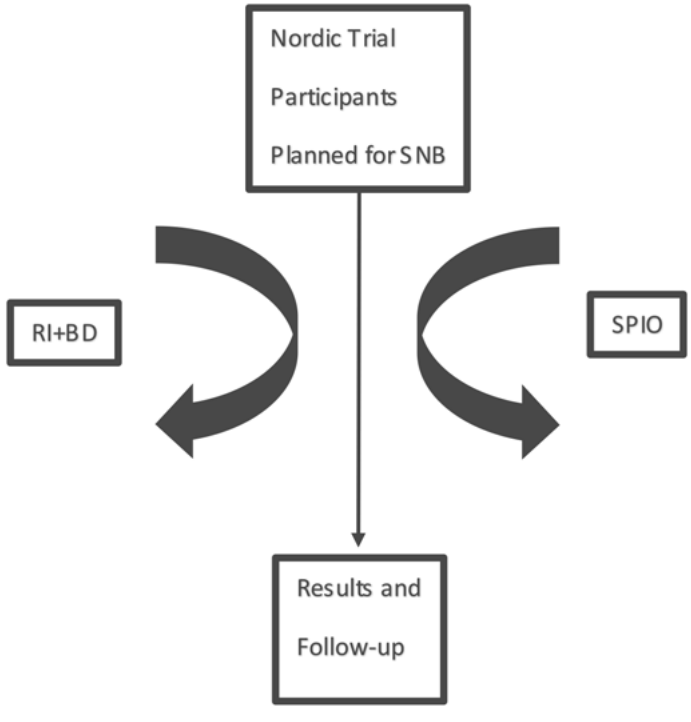


Fig.1 Schematic flowchart for the Nordic SentiMag trial.

4.3.2. Paper II

For this pilot study ("MagPilot"), two ml of SPIO (Sienna+®, Sysmex Europe GmBH, Hamburg, Germany) diluted with 3 ml local anesthetic (Xylocain®, 10mg/ml) was injected subareolarly during the preoperative visit in the outpatient clinic. The methods were otherwise identical to what has already been described for Paper I. The healthy volunteer injected with SPIO underwent follow-up in order to define how long there was transcutaneous ferromagnetic signal in the axilla.

The flow of the study is depicted in Figure 2.

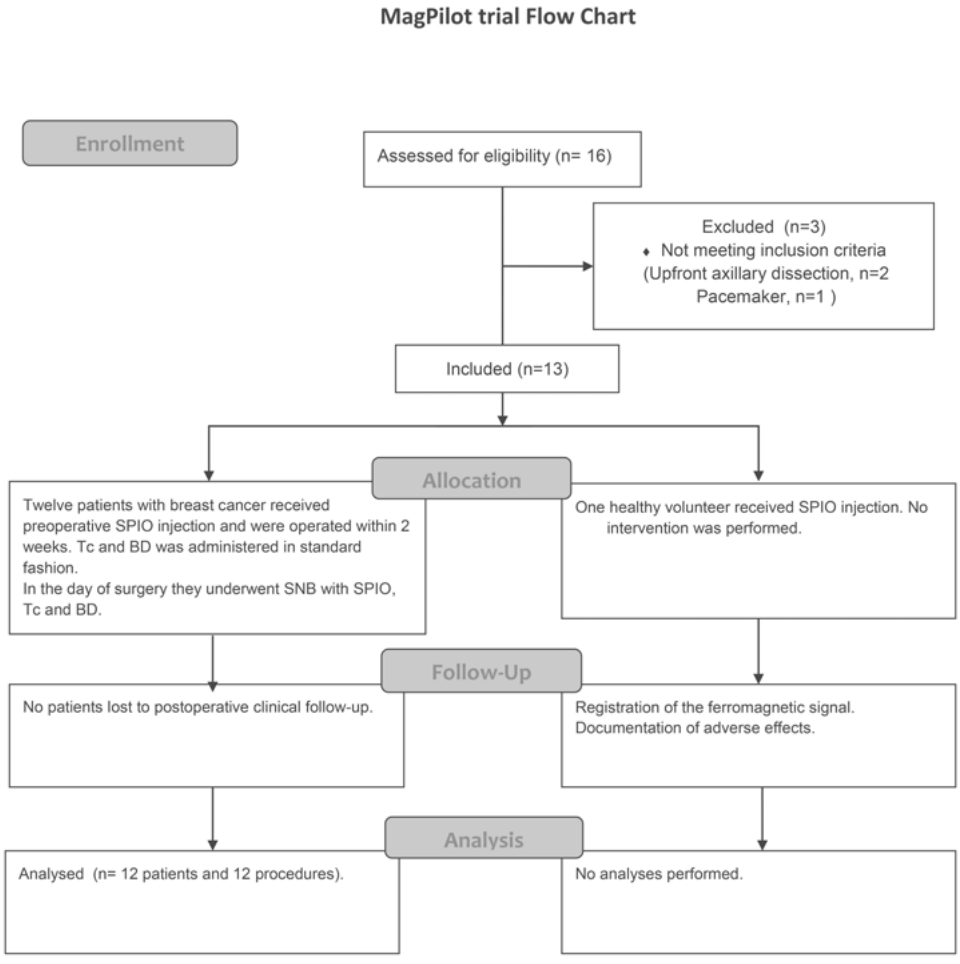


Fig.2 CONSORT Flowchart for MagPilot Study

4.3.3. Paper III

This pragmatic non-randomized trial investigating the performance of SPIO as a sole (*"μόνος"* in Greek, pronounced "monos") was conducted in two centres with comparable demographics and similar outcomes in SNB.

At Västmanlands County Hospital where SNB was performed with RI and BD, 40 or 60 mBq of RI was injected interstitially the morning before surgery, or the day before, -respectively-, at the Nuclear Medicine Department. At Uppsala University Hospital, SPIO (Sienna+®, 2ml blended with 3ml of local anaesthetic - Xylocain®, 10mg/ml) was injected interstitially either during the preoperative visit to the outpatient clinic (one to four weeks before the operation) or perioperatively, about one hour but, at least 20 minutes before the operation. Massage for five minutes at the injection site was performed only in perioperative administration. Patients planned to undergo a preoperative MRI were not injected prior to the MRI to avoid artefacts and were injected perioperatively, as described above. On the day of surgery, transcutaneous counts were registered with the respective probe (Neo 2000, Neoprobe 2100, Neoprobe Corp, Dublin OH, USA or Senti-Mag® Gen2, Endomagnetics Ltd, UK) after the induction of anaesthesia. In the SPIO arm, 1-2 ml of Patente Bleu® (Laboratoire Guerbet, Aulnay-Sous-Bois, France) were administered interstitially at the areolar border 10 minutes before skin incision, only if the transcutaneous signal was deemed inadequate by the operator. In the isotope arm, blue dye was injected routinely.

The study was designed according to the extension of the CONSORT statement for pragmatic trials19 and is summarized in the flow chart in Figure 3.

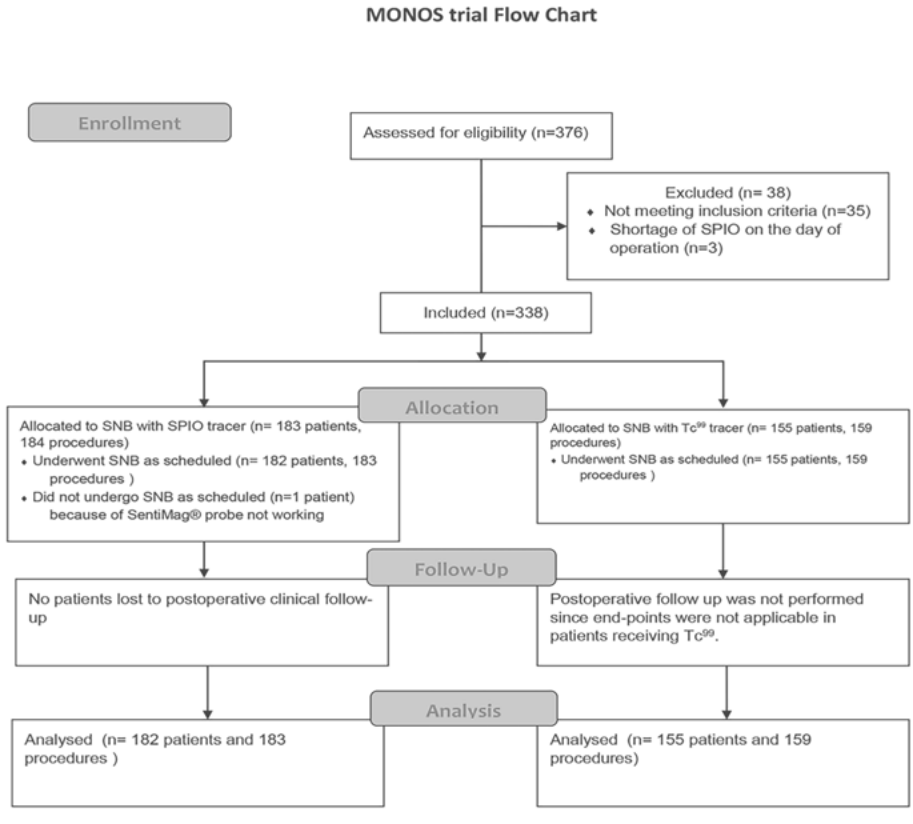


Fig. 3 CONSORT flowchart for MONOS trial

4.3.4. Paper IV

The concept of this study (SentiNot: "Senti" nel node biopsy in ductal cancer in situ; how to "Not" do it) is to investigate whether the feasibility of a successful SPIO guided SNB may help avoid SNB in patients with a preoperative diagnosis of DCIS by marking the sentinel node with SPIO at the first operation and remove it in a second operation, only if IBC is confirmed. On the day of surgery, patients were injected interstitially with 2 ml of SPIO (Sienna+®, Sysmex Europe GmBH, Hamburg, Germany) diluted with 3 ml of NaCl 0.9% or local anesthetic (Xylocain®, 10mg/ml) at least 20 minutes before the operation, followed by 5-minute massage, so as to allow for tracer migration. Breast procedure was performed as planned and in the end, the transcutaneous magnetic signal in the axilla detected by the SentiMag® probe (Endomagnetics, Cambridge, UK) was registered. If IBC was confirmed in final pathology, a SNB was performed in another session. Axillary mapping was performed with SPIO and radioisotope (Tc99) was used for comparison. Blue dye was

strongly recommended. In cases of breast conservation, injection site was defined by local routines. If a mastectomy had been performed, the radioisotope was injected intradermally, near the scar.

Transcutaneous magnetic- and isotope signals in the axilla, were detected and registered. SNB was conducted with the SentiMag® probe. After SN retrieval, the presence of radioisotope signal or BD was also registered; thereafter, the axilla was explored for radioactive and blue nodes, but not magnetic. Intraoperative frozen section was performed in order to avoid a third operation. If SNB failed, axillary dissection was performed according to surgeon's decision. In order to investigate the impact of the implementation of SentiNot policy on a national level, synchronous data on patients with a postoperative DCIS diagnosis were retrieved from the Swedish Cancer Registry. The Swedish Cancer Registry was founded in 1958 and the coverage for breast cancer is estimated to be 100% Appropriate potential candidates for the SentiNot study were identified, so as to assess how many SNB could have been spared.

Paper IV presents the results of a pre-specified interim efficacy analysis of the primary endpoint at 50% of recruitment.

4.4 Endpoints and statistical analyses

4.4.1. Paper I

The study assumes a 97% proportion detected by conventional SNB and SPIO SNB, a limit difference for equivalence of -4% and the expected difference between the proportions detected under both arms as 0%. This means that equivalence is accepted if the proportion detected under the SPIO arm is as low as 93%. Detection rate was additionally tested in a right-sided binominal test with the alternative hypothesis that the proportion of successful SNBs was greater than 93% for each tracer. Prospective sample size for a paired test with a 0.05 one-sided significance level and 80% power to reject the null hypothesis was 214 cases.

The primary end point of the study is the proportion of successful SNBs (DR per case) with either the standard (RI+BD/ or RI alone) or the magnetic technique (SPIO). Secondary end points included the proportion of SN detected (nodal DR) as well as the proportion of pathologically positive results (malignancy rate) per case and per node with either the standard or the magnetic technique. Moreover, the concordance and reverse concordance for successful detections (per patient and per node overall and in terms of malignancy) are compared. Concordance is defined as the number of both standard (RI+BD/ or RI alone) and SPIO positive patients or nodes, divided by the number of patients or nodes marked by the standard method (standard+ and SPIO+ /standard+). Reverse concordance is defined as the number of both

standard- and SPIO- positive patients or nodes, divided by the number of patients or nodes marked by the SPIO tracer (standard+ and SPIO+ ⁄SPIO+). Only tumor positive patients and nodes are included in the respective malignancy concordance rates. Additionally, the false negatives of the method and the overlap between the conventional and the magnetic technique has been addressed. Finally, skin discoloration is followed and registered. For all data, a 95 % confidence interval (95%CI) will be calculated. Means (95% CI) or medians (interquartile range, IQR) are presented as appropriate after Kolmogorov Smirnov and Shapiro Wilk tests of normality are performed. Rates are presented with 95% CI. A p-value of < 0.05 will indicate that the null hypothesis was rejected. Values will be calculated using SPSS (V 22.0. Armonk, NY: IBM Corp.).

A systematic review and meta-analysis of available literature will be undertaken according to Cochrane Database methodology for interventions. The reason that it is not performed as a meta-analysis of accuracy for diagnostic studies is that, in the absence of an axillary clearance background, the FNR of SPIO, on the one hand, and RI and BD on the other poses a severe restriction for results and conclusions (74). Study selection and data extraction will be performed independently by two authors. Endpoints from the data extraction and calculation included detection rates per case and per node, including malignancy, where available. Heterogeneity among studies will be assessed by means of the I2 statistic. Dichotomous data analyses will be performed by estimating the pooled odds ratio (OR), according to the Mantel Haenszel method. Concordance rates are also recalculated and presented, according to the current definition. Rate comparison was performed using the inverse variance method. All studies included in the meta-analysis will be evaluated according the MINORS revised criteria for prospective non-randomized trials (75) by an independent author so as to ensure objectivity. Analysis will be conducted using the RevMan5.3 software.

4.4.2. Paper II

Descriptive values are presented as medians with range and interquartile range, due to the small number of participants. Comparisons are performed with the Wilcoxon signed rank test. Correlation is performed with Spearman's ρ. Rates are given as percentages (%) and comparison between detection rates is performed with the Mc Nemar's test. Detection rates and comparisons are performed for tracer per tracer separately, but also between SPIO and the dual technique. Successful localization with the dual technique was considered as every case were SN was detected by the isotope, the blue dye or both. Concordance, as defined in paper I was also calculated. All analyses were performed with SPSS V 23.0 (Armonk, NY: IBM Corp). All tests were two-sided and a p-value lower than 0.05 was considered significant.

4.4.3. Paper III

MONOS was conceived as a prospective pragmatic trial, aiming to examine the feasibility of SPIO as the sole tracer of SNB in patients with early breast cancer. For that reason, a synchronous cohort of patients treated with the established standard dual technique (radioisotope and dye) had to serve as a control arm. Due to the nature of the intervention, blinding is not feasible for neither surgeons nor patients.

A standard DR of 97% for SNB is assumed from the literature. The consideration concerning sample size was the unforeseen difference in detection rates (R) as to those expected, so as to terminate the study prematurely. For that, a one-sided hypothesis had to be constructed so as to fill the condition that $R_{actual} - R_{expected} < \delta$, where δ set to 5%. Aiming for a 0.05 one-sided significance level and 80% power to reject the null hypothesis, the minimum sample size for each arm, is 127 cases.

Study primary endpoint is the detection rate for each method. Secondary endpoints ae the number of nodes retrieved, as well as the effect of injection timing and injection site in SNB DR. Moreover, a follow-up will be performed every three months in the patients who were enrolled in the SPIO arm in order to define the size and fading of skin staining in the postoperative period. The size of staining was measured in maximum dimensions and the intensity of the staining were registered. Moreover, the patients with a remaining skin staining were asked if they were affected by that (Yes or No). Regardless of how they felt, they were asked to evaluate if staining affected the aesthetic outcome by filling in a Likert item from 0 (not at all) to 5 (very much). The question is addressed at the last two follow up dates with a three months´ interval. Individual scores are documented, as well as the difference (Δ) between assessments per patient. Likert items are selected in the absence of a relevant validated questionnaire in available literature. Finally, the primary cost per case with the use of SPIO will be calculated and compared it to the use of RI and BD in the same health care setting. For that purpose, the costs of tracers per patients will be taken up, as well as the cost of the preoperative visit of the patient to the Nuclear Medicine Department.

Kolmogorov-Smirnov tests of normality are conducted where appropriate and descriptive values are presented accordingly as means (95% CI) or medians (iqr). Comparisons between arms are conducted with parametric tests where appropriate. Detection rates per case are calculated for each arm, taking into account the total detection rate (cases that were successful with either the tracer or the addition of patent blue or both) as well as the tracer specific detection rate (cases in which the sentinel node is detected with the tracer, regardless of the addition of patent blue). Separate calculations will be performed in the presence of malignancy. Comparisons between rates will be conducted using Fischer's exact test. The z-test is to be used for the comparison of proportions between independent populations (study arms). Likert item

data shall be treated with non-parametric procedures as indicated. Statistical analysis is performed using SPSS (V 23.0. Armonk, NY: IBM Corp.).

4.4.4. Paper IV

In order to approach an appropriate sample size with the study, one would require that only patients that upgrade to IBC are operated with a SNB. Data from the Uppsala Örebro regional breast cancer registry (2014) depict that 20% of patients with a preoperative diagnosis of DCIS will upgrade to IBC. In the same region, following guidelines results in that approximately 50% of patients with DCIS undergo SNB. A total of 246 with a preoperative diagnosis of DCIS is required to demonstrate that the true proportion that will upgrade to IBC is 20% accepting 5% uncertainty (corresponding to confidence limits of +/- 5%). Given that SNB is performed in an observed proportion of 50% of DCIS cases (Clopper Pearson 95% CI: 43.6%, 56.4%), the study aims that SNB will be performed only if IBC is diagnosed, that is, to an expected 20%. The sample size is adequate to demonstrate that an anticipated reduction in SNB by 60% is significant (z-statistic=11.763, $p<0.0001$).

An interim efficacy analysis of the primary endpoint using the O'Brien-Fleming procedure (76) was pre-specified at 50% of recruitment. The 2-sided p-value for the primary endpoint was subsequently set to 0.0054. For all other comparisons, a 2-sided p-value of 0.05 was considered significant. In order to investigate the impact of the implementation of SentiNot policy on a national level, synchronous data on patients with a postoperative DCIS diagnosis were retrieved from the Swedish Cancer Registry. Appropriate potential candidates for the SentiNot study were identified, so as to assess how many SNB could have been spared. Additionally, costs of inpatient and outpatient care were retrieved from the respective hospital registries and respond to actual healthcare expenses from 2015 to present day. Fixed estimates of costs for healthcare per year and per region were calculated by the pricing lists provided by the respective economic departments of the centres participating in the study, with a model provided by Uppsala-care.

Actual total cost per patient included admission on an outpatient or inpatient basis, operation and anesthesia per minute and SNB pathology, either standard or intraoperative frozen section. Results were reported according to the CHEERS statement (77). Descriptive values are presented as means with 95% confidence intervals (m, 95%CI) or medians with interquartile range (med, iqr) as appropriate. Continuous variables are compared with Student's t-test or non-parametric tests, depending on variable distribution. Dichotomous data were analyzed with Pearson's χ^2 test or Fisher's exact test and the McNemar's test is used for paired observations. All analyses of outcomes were performed per protocol. Statistical analyses were performed with SPSS v 24.0 (IBM, Armonk, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

4.5 Ethical considerations, ethics committee approval and trial registration

All the projects involved in the present thesis did not involve controversial or potentially harmful procedures for the patients. All studies were conducted according to the Helsinki declaration (78). All projects were approved by the ethics committee in Uppsala. Projects described in Papers II, III and IV were registered in a public trial registry. (https://doi.org/10.1186/ ISRCTN14097881 and https://doi.org/10.1186/ISRCTN18430240).

5. Results

5.1 Paper I

A total of 206 patients and procedures were included in the study. Mean age was 61.7 years and median BMI 25.4. Mean tumor size was 19.0 mm with values ranging from 1 to 150 mm. BD was used in 127 patients (61.7%). Patient and tumor characteristics are shown in Table 1.

The transcutaneous detection with Tc was successful in 202 of 206 patients (98.1%), and with Sienna+ in 189 (91.7%), resulting in a 6.4% difference ($p=0.0036$). Correlation analysis showed that the presence of the transcutaneous signal for both tracers was associated with BMI (Spearman's ρ for RI: -0.167, $p<0.05$ and Sienna+ -0.191, $p<0.01$). Age, tumor size, time between injection and previous surgery or type of surgery did not correlate with transcutaneous detection.

SN detection with the standard technique succeeded in 200 patients (97.1%) and 201 with Sienna+ (97.6%), displaying no difference ($p=0.76$). Both techniques were successful concomitantly in 196 cases (95.1%). Subsequently, per patient concordance was 98.0% and reverse concordance was 97.5%. Total failure for both techniques occurred in only one patient (0.48%), who had multiple macrometastases. Metastases were noted in 54 patients (26.2%). These were detected by both methods in 52 cases (96.3%), Tc in 53 (98.1) and Sienna+ in 52 (96.3%) resulting in concordance and reverse concordance per malignant case of 98.1% and 100% respectively. Per patient data of the Nordic study and studies included in the meta-analysis are presented in Tables 2 and 3.

Table 1. Patient characteristics

		n	%
Menopausal status			
	Premenopausal	30	14.6
	Postmenopausal	140	68.0
	Perimenopausal	6	2.9
	Not assessed	30	14.6
Type of surgery			
	Mastectomy	52	25.2
	Breast conserving surgery	154	74.8
pT			
	Tis	10	4.9
	T1	126	61.1
	T2	56	27.2
	T3	7	3.4
	Not assessed	7	3.4
pN			
	N0	152	73.8
	N1mi	20	9.7
	N1	27	13.1
	N2	6	2.9
	N3	1	0.5
	Not assessed	0	0.0
Grade			
	G1	37	18.0
	G2	74	35.9
	G3	32	15.5
	Not assessed	62	30.1
Ki67%			
	>15%	106	51.5
	<15%	60	29.1
	=15%	18	8.7
	Not assessed	22	10.7

A total of 403 SNs were retrieved. Out of these, 353 were detected by both techniques, (87.6%); 368 with the standard technique, (mean 1.79 and detection rate 91.3%); 376 with Sienna+, (mean 1.83 and detection rate 93.3%, p=0.34). The nodal concordance and reverse concordance were 95.9% and 93.9% respectively. Out of the total 403 nodes, 68 (16.9%) were malignant. RI detected 63 (92.6%) and Sienna+ 62 (91.2%), whereas both succeeded simultaneously in 60 nodes (88.2%). The concordance and reverse concordance for malignant nodes were 95.2% and 96.8%. Per node data of the Nordic study and studies included in the meta-analysis are presented in Tables 4 and 5.

Table 2. Per patient figures from the studies used in the meta-analysis. (nd: not defined, na: not applicable.)

Study (Ref.)	Total cases	Fail SLN	Successful standard technique	Successful SPIO technique	Both techniques successful	Malignant cases	Malignant cases detected by successful standard technique	Malignant cases detected by successful SPIO technique	Malignant cases detected by both techniques successfully	Malignant cases in which both techniques failed
Douek *et al*, 2014 (10)	160	3	152	151	146	39	nd	nd	nd	nd
Thill *et al*, 2014 (11)	150	2	146	147	145	34	31	33	31	2
Rubio *et al*, 2015 (12)	120	2	113	116	111	36	33	34	32	1
Pineiro *et al*, 2015 (13)	181	3	178	177	177	60	53	55	52	4
Ghilli *et al*, 2015 (14)	197	nd	195	193	187	57	56	55	54	1
Houpeau *et al*, 2016 (15)	108	2	103	105	102	46	44	45	43	1
Nordic study	*206*	*1*	*200*	*201*	*196*	*54*	*53*	*52*	*52*	*1*

Table 3: Rates per patient from the studies used in the meta-analysis. (*nd: not defined, na: not applicable. The denominator is always the total of patients per study. 95%CI: 95% confidence intervals using the Wilson procedure with a correction for continuity. Rate differences are given as |Standard-SPIO|. Fisher's exact test is performed and 2-tailed p-values are given. p-values<0.05 are considered significant.*)

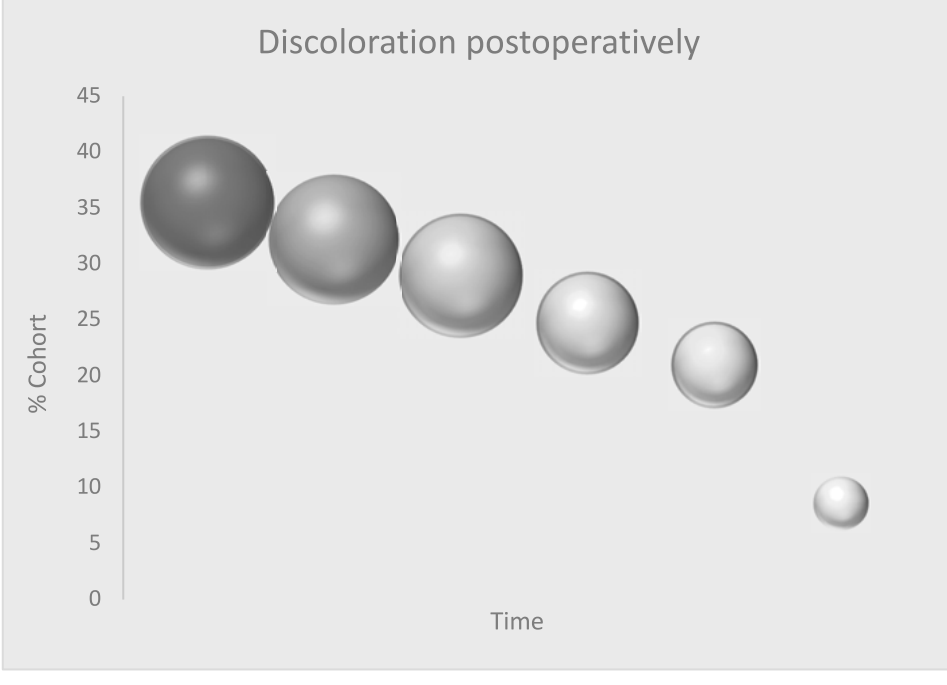
Study (Ref.)	Fail SLN rate (95%CI)	Standard technique detection rate (95%CI)	SPIO detection rate (95%CI)	Rate difference [p-value]	Malignancy rate (95%CI)	Malignant cases detected by successful standard technique	Malignant cases detected by successful SPIO technique	Rate difference for malignant cases [p-value]	Malignant cases detected by both techniques successfully	Malignant cases in which both techniques failed
Douek *et al*, 2014 (10)	1.9 (0.5, 5.82	95.0 (90.1, 97.66)	94.4 (89.3, 97.2)	0.6 [1]	24.4 (18.1,31.9)	na	na	na	na	na
Thill *et al*, 2014 (11)	1.3 (0.2, 5.2)	97.3 (92.9, 99.1)	98.0 (93.8, 99.5)	1.0 [1]	22.7 (16.4,30.4)	20.7 (14.7, 28.2)	22.0 (15.8, 29.6)	1.3 [0.89]	20.7 (14.7, 28.2)	1.3 (0.02, 5.2)
Rubio *et al*, 2015 (12)	1.7 (0.3,6.5)	94.2 (87.9, 97.4)	96.7 (91.2, 98.9)	2.5 [0.54]	30.0 (22.2, 39.2)	27.5 (19.9, 36.5)	28.3 (20.7, 37.4)	0.5 [1]	26.7 (19.2, 35.7)	0.8 (0.04, 5.2)
Pineiro *et al*, 2015 (13)	1.7 (0.4, 5.2)	98.3 (94.8, 99.6)	97.8 (94.1, 99.3)	0.5 [1]	33.2 (26.5, 40.6)	29.3 (22.9, 36.6)	30.4 (23.9, 37.7)	1.1 [0.9]	28.7 (22.4, 36)	2.2 (0.7, 5.9)
Ghilli *et al*, 2015 (14)	na	99.0 (95.8, 99.8)	98.0 (94.5, 99.4)	1.0 [0.69]	28.9 (22.8, 36)	28.4 (22.4, 35.4)	27.9 (21.9, 34.8)	0.5 [0.9]	27.4 (21.4, 34.3)	0.5 (0.03, 3.2)
Houpeau *et al*, 2016 (15)	1.9 (0.3, 7.2)	95.4 (89.5, 98.5)	97.2 (92.1, 99.4)	1.8 [0.72]	42.6 (33.2, 52.5)	40.7 (31.5, 50.6)	41.7 (32.4, 51.6)	1.0 [1]	39.8 (30.6, 49.7)	0.9 (0.05, 5)
Nordic study	*0.5 (0.03, 3.1)*	*97.1 (93.5, 98.8)*	*97.6 (94.11, 99.10)*	*0.5 [1]*	*26.2 (20.5, 32.9)*	*25.7 (20, 32.4)*	*25.2 (19.6, 31.8)*	*0.5 [1]*	*25.2 (19.6, 31.8)*	*0.5 (0.03,3.1)*

Discoloration

Follow up data were available in 186 of 206 patients (90.3%). The initial protocol stated a follow-up visit after 6 months, but since a marked discoloration was found in a considerable amount of women, the follow-up period was extended. Thus, median follow up was 310 days (IQR 182). Correlation analysis showed that the incidence of discolouring was strongly associated with breast conserving surgery (Kendall tau= -0.416, p<0.001), since 95.6% of patients

with discoloration had been treated with BCS. Age, BMI or the incidence of perioperative staining were not correlated ($p>0.5$).

Discoloration was present in 35.5% of patients postoperatively (0-3 months) and faded progressively in size and colour over time to 21% of patients after a year. Staining remained present in 8.6% 15 months after the operation, but much smaller and paler. Discoloration for the entire cohort and the BCS group are essentially the same (within 95%CI). Additionally, the curve demonstrated below in Figure 1 is identical for both. Finally, all the patients who had discolouration and were checked with the probe presented magnetic activity (positive prognostic value 100%); however, no correlation of the transcutaneous counts was found with size, intensity or duration of the skin discoloration.



	Months					
	0-3	4-6	7-9	10-12	12-15	15+
Staining Rate	35.5	32.3	29.0	24.7	21.0	8.6

Figure 1. Discoloration in the follow up cohort; size and colour of the spheres represent the median of the discoloured surface in cm^2 in the discoloured proportion of the cohort and the fading respectively.

Meta-analysis

Seven studies were included (see PRISMA flow below) with a total of 1118 cases performed and 2300 nodes retrieved.

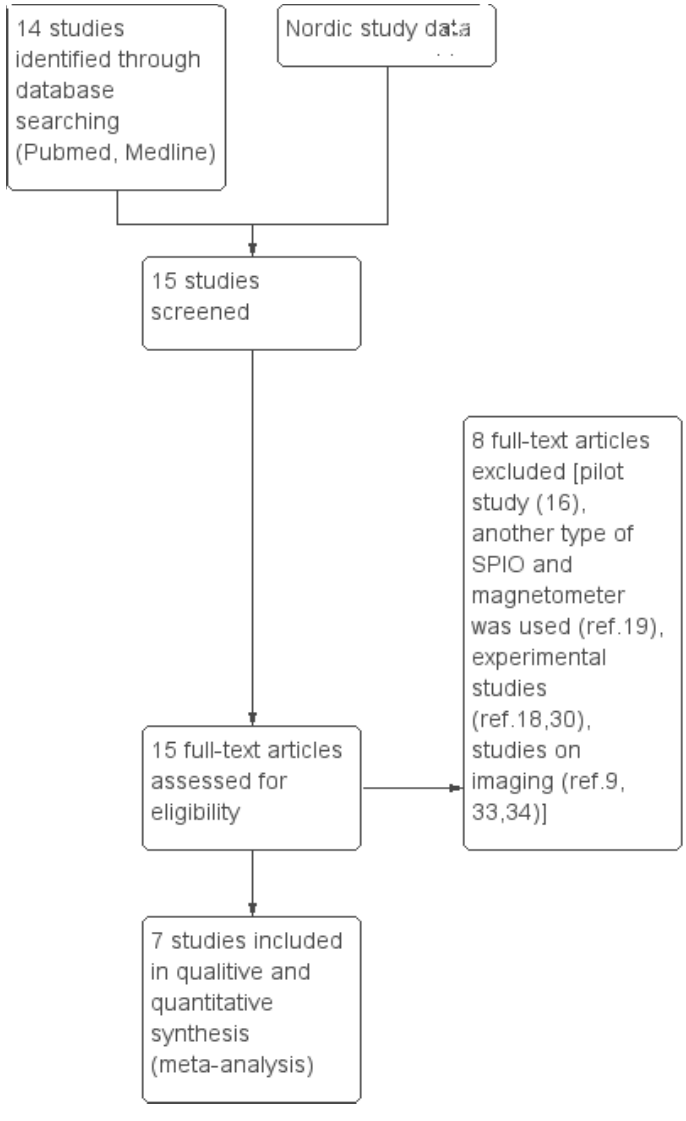


Figure 2. PRISMA flow chart for the systematic review. Numbers in parentheses refer to respective references from the published version of paper I.

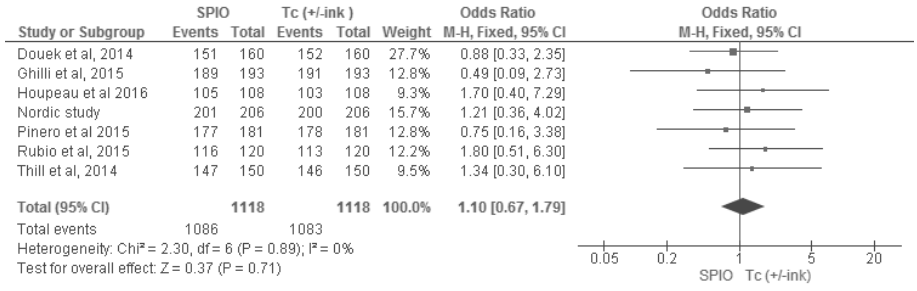
The included studies were all graded according to the MINORS criteria, as previously stated. The scoring is illustrated in Table 4.

Table 4. Rating of studies according MINORS criteria.

	Douek et al, 2014	Thill et al, 2014	Rubio et al, 2015	Pinero et al, 2015	Ghilli et al, 2015	Houpeau et al, 2016	Nordic study, 2016
A clearly stated aim	2	2	2	2	2	2	2
Inclusion of consecutive patients (no exclusion or details about the reasons for exclusion)	1	1	2	2	2	1	2
Prospective collection of data	2	2	2	2	2	2	2
Endpoints appropriate to the aim of the study	2	2	2	2	2	2	2
Unbiased assessment of the study endpoint (if not blind, it has to be explained)	2	2	2	2	2	2	2
Follow-up period appropriate to the aim of the study	2	2	2	2	2	2	2
Loss to follow up less than 5% (if important for primary endpoint)	2	2	2	2	2	2	2
Prospective calculation of the study size	2	1	2	1	2	2	2
An adequate control group	2	2	2	2	2	2	2
Contemporary groups	2	2	2	2	2	2	2
Baseline equivalence of groups	2	2	2	2	2	2	2
Adequate statistical analyses	2	2	2	2	2	2	2
Total	**23**	**22**	**24**	**23**	**24**	**23**	**24**

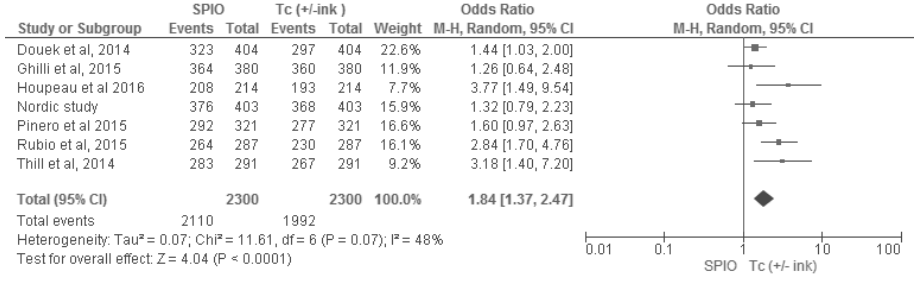
As far as results are concerned, no difference was observed in the detection rates per case in any of the studies (fixed OR:1.10; 0.67,1.79, p=0.71) between SPIO and conventional methods (Table 5).

Table 5. Detection rate per case



However, moderate heterogeneity was present (I^2=48%) among the studies as far as nodal detection rate was concerned (table 5). The random OR was 1.84 (1.37,2.47), resulting in significant difference in favor of SPIO (table 6).

Table 6. Detection rate per node



The detection rates in cases with a positive SNB were comparable for both methods (fixed OR:1.33; 0.63, 2.81, p=0.45) as well as detection rates per malignant nodes (fixed OR:1.55; 0.86, 2.79, p=0.14).

Concordance rates were recalculated for all included studies according to the definitions (standard+ and Sienna+/standard+) with 95% CI. No substantial differences were noted (Fig. 3).

Subsequent inverse variance analysis was conducted using "risk difference" defined as |Concordance-Reverse concordance|, depicting similar rates per case among studies (p=0.82) (table 7). The comparison of concordance rates per node however revealed heterogeneity among the studies (I^2=54%), as well as that more SNs are detected with SPIO (table 8). The same comparisons were conducted in the presence of malignancy, without any evidence that imply a difference per case (RD=0.00, 95% CI: -0.03, 0.02, p=0.73)) or per node (RD=-0.03, 05%CI: -0.06, 0.01, p=0.10).

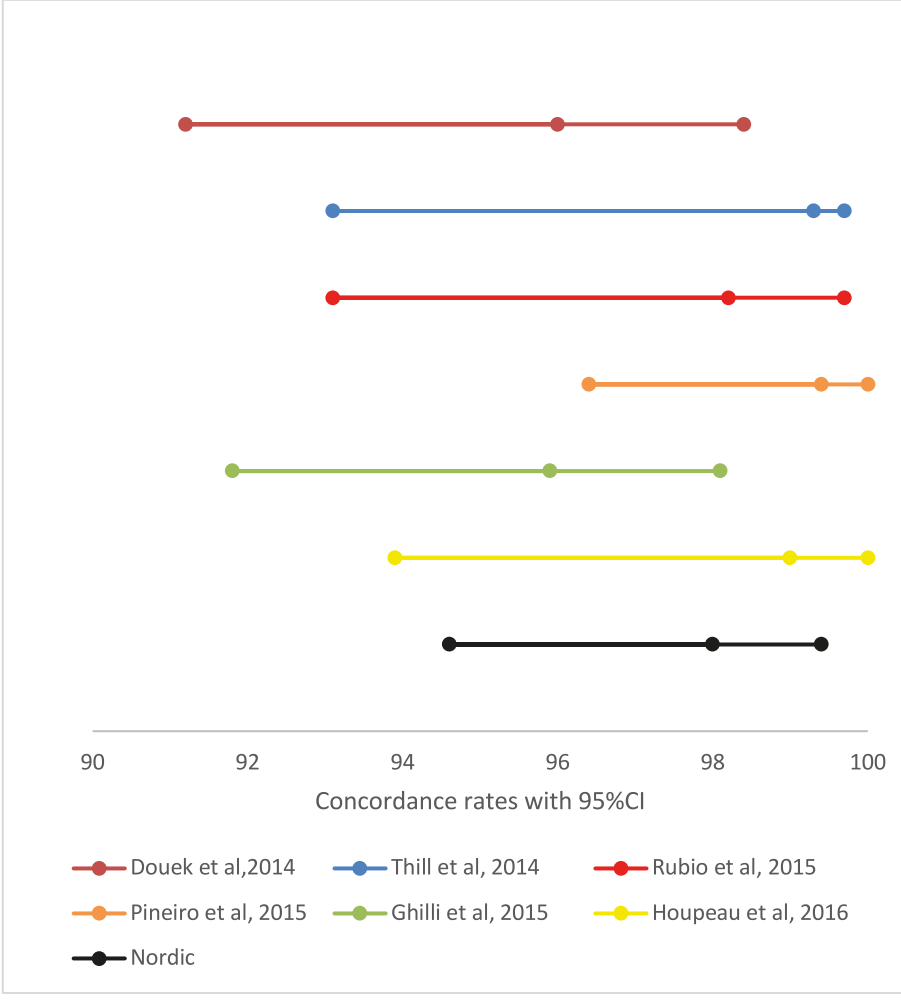


Figure 3. Forest plot depicting concordance rates for all included studies.

Table 7. Comparison of concordance versus reverse concordance rates per case.

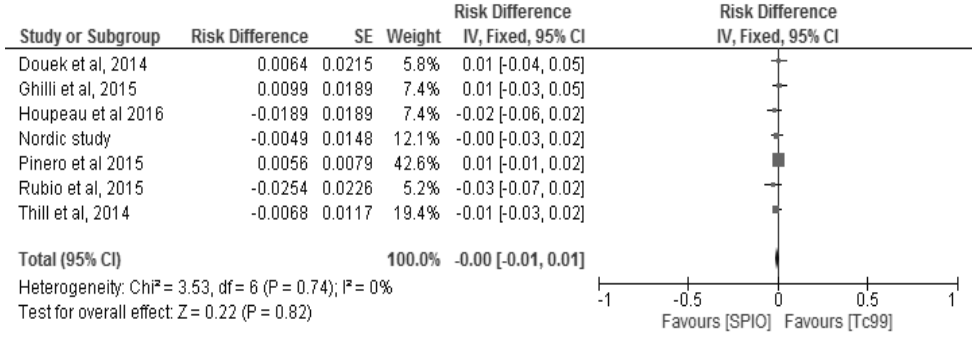
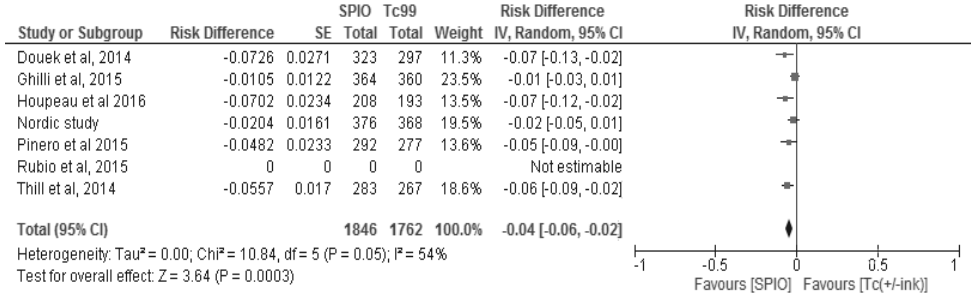


Table 8. Comparison of concordance versus reverse concordance rates per node.



5.2 Paper II

Twelve consecutive patients were included, operated from September 2014 to October 2014. Clinicopathological characteristics are presented in Table 1. SPIO was injected three to fifteen days before the operation (median eight days). Transcutaneous magnetic signal was present in all patients at operation and radioactivity was present in ten. In nine patients (75%), the SN was identified with all three methods. Blue dye was successful in nine cases (75%) and Tc99 in ten (83.3%). The dual technique was subsequently successful in ten cases (83.3%). SPIO was successful in all cases (100%). Therefore, no difference was noticed in detection rates between SPIO and isotope (p=0.500) or between SPIO and dye (p=0.250) and SPIO and the combined dual technique (p=0.500). Concordance between RI (and the dual technique) and SPIO per case was 100%. In these ten patients, there was complete nodal concordance for retrieved nodes (13/13, 100%), demonstrating that the exact same nodes were identified as sentinels by SPIO and RI. Nodal concordance between SPIO and the dual standard was 81.25% (13/16), because of three nodes that were colored but neither radioactive nor magnetic. A median of one sentinel (Table 1) was retrieved totally as well as per tracer. No differences were found

in the number of nodes retrieved with either SPIO or the dual technique (p=1.000). Metastases were found in three patients (25%). None of the three nodes detected only by the dye harbored metastasis.

The cases that blue dye and isotope failed were examined separately. One of the patients detected only with SPIO had undergone neoadjuvant therapy for an 85 mm gr 2 lobular cancer. The axillary sentinel node contained a macrometastasis and in the subsequent axillary clearance, nine out of 20 nodes contained macrometastases. The other patient where isotope and dye failed did not present any specific features. Finally, the patient in which only blue dye failed had previously been operated with a wide local resection because of DCIS.

In the present series, transcutaneous magnetic signal was a predictor of successful detection (positive prognostic value 100%).
In the first three patients with palpable lesions, the surgical specimen including the injection site of SPIO was sent for postoperative mammography. SPIO artefacts could not be detected on mammograms and no disturbance in the visualization of the breast lesions was noted. Then, in patient number four with a non-palpable lesion, SPIO was injected and pre-operative guide wire localization was performed. The SPIO was not detectable on mammograms before or after surgery and the lesion was clearly seen both pre-operatively and in the surgical specimen (Fig. 1a-c). An MRI was performed also after SPIO injection. SPIO induced artefacts were evident in imaging (Fig. 2d).

Table 1. Clinicopathological data.

Age (median, iqr)		70.5 yrs, (18)	
BMI (median, iqr)		24.7 kg/m^2 (6.3)	
Sex	Female	11	91.7%
	Male	1	8.3%
Primary Systemic Treatment	No	11	91.7%
	Yes	1	8.3%
Clinical tumour size (median, iqr)		30 mm (26)	
Multifocality	No	7	58.3%
	Yes	5	41.7%
Primary tumour size (median, range)		20 mm	(4,85)
Histological type	DCIS	1	8.3%
	IDC	8	66.7%
	ILC	2	16.7%
	Mixed type	1	8.3%
Nuclear grade	In situ, grade 3	1	8.3%
	1	2	16.7%
	2	5	41.7%
	3	4	33.3%

Receptor status	ER+HER2-	9	75.0%
	ER+HER2+	1	8.3%
	ER-HER2-	1	8.3%
	Not assessed (DCIS)	1	8.3%
T-stage	T_{is}	1	8.3%
	T_1	6	50.0%
	T_2	3	25.0%
	T_3	2	16.7%
Days between SPIO injection and operation (median, range)		8	(3,15)
Transcutaneous axillary counts at the day of the operation (median, range)		282 (50,1314)	
Ex vivo signal on SN (median, range)		4300	(200,9999)
SNs retrieved (median, range)		1	(1,3)

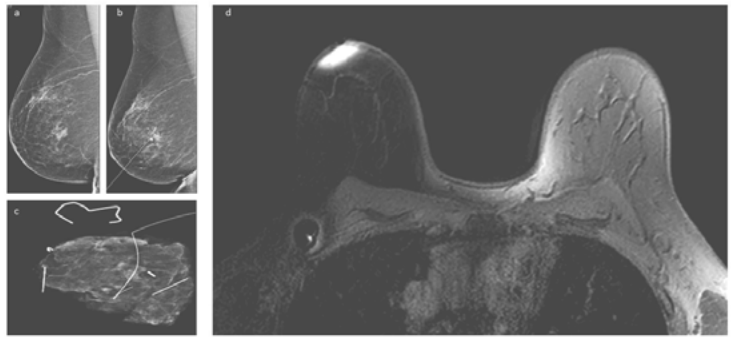


Figure 1. a. Preoperative mammography after SPIO injection. b. Placement of a localization guidewire without problems. c. Specimen mammogram. d. MRI after SPIO injection.

At histopathology, SPIO deposits were examined in relation to the breast lesion and in the SLN. There was an uptake of SPIO in macrophages subcutaneously at the injections site and in histiocytes in the SLN but there was no uptake in the primary tumors or in lymph node metastases (Figure 2a-b). On the contrary, the examination of frozen sections of SLNs was easier as the SPIO was not accumulated in metastatic cells. We could not see any disturbance of the following cytokeratin MNF (CKMNF) staining. The yellowish/brownish SPIO granules in the cytoplasm of the histiocytes were easily separated from the distinct CKMNF staining of the membranes and cytoplasm of tumor cells (Figure 3c).

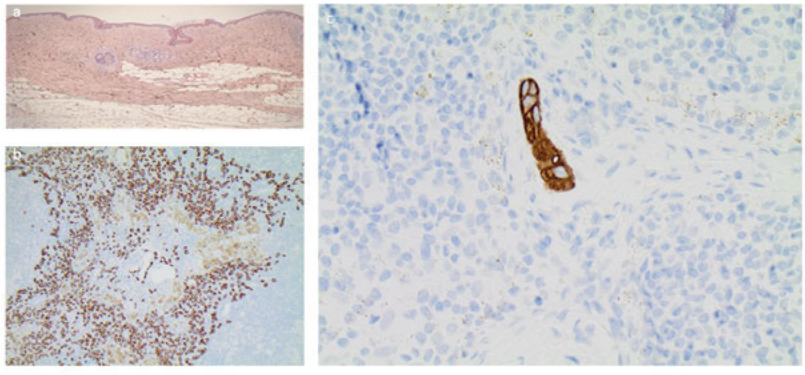


Figure 2. Histopathology in the tumor (a) and SLN (b) without any SPIO artefacts present. c. CytoKeratin MNF (CKMNF) staining of the membranes and cytoplasm is not distorted by the easily identified yellowish/brownish SPIO granules in the cytoplasm of the histiocytes.

The decline of the magnetic signal was followed in the volunteer injected with SPIO. The counts in the axilla increased with a peak three weeks after the injection and remained high for more than four weeks (Figure 4). Fifteen days after the injection, the volunteer passed a metal detector (Ceia02PN20) at Arlanda International airport without the detection of ferromagnetic signal.

Figure 4. Transcutaneous axillary ferromagnetic signal curve in a volunteer. No side effects were reported by the patients or the volunteer.

5.3 Paper III

A total of 343 SNBs in 338 consecutive patients with breast cancer were included. The 99mTc arm included 159 SNBs in 155 patients, whereas the SPIO arm included 184 procedures in 183 patients. Three patients were not included owing to lack of SPIO during 1 week. In the SPIO arm, BD was used in 92

procedures (50.0%). Characteristics of the study arms are summarized in Table 1. The only difference between groups was in BMI (P = 0.008).

- Detection rates and node retrieval

The DR was 95.7% in the SPIO arm and 96.8% in the RI arm (p = 0.586), and no difference was noted in tracer-only detection rates (93.4 vs 95.5%; p = 0.481). Multinomial logistic regression for BMI resulted in no difference, either in the overall (expB 0.989, 95% CI 0.869 to 1.126; p = 0.869) or the tracer-specific (expB 1.077, 0.990 to 1.172; p = 0.083) DR. No difference between arms was noted in patients with SN metastases (overall: 92.3 vs 89.8%, p= 1.000; tracer only: 92.3 vs 83.3%, p = 0.431). BD did not increase the detection rate (P = 0.491 for SPIO and P = 0.770 for RI).

In the SPIO arm, the nodal DR for the tracer was 93.5%. The mean number of sentinel nodes retrieved per procedure was 1.35 and the tracer-specific mean was 1.26. The nodal detection rate for RI-only was 90.3%, with a total mean of 1.89 nodes and a tracer-specific mean of 1.70. Comparison of means between the two groups verified that SPIO yielded fewer nodes, regardless of the use of ink (p< 0.001). However, no difference was demonstrated between nodal detection rates (p = 0.177). Previous surgery, age, BMI or presence of metastases were controlled as factors in the cases that SPIO or RI failed to detect the SNB and no association was found.

- Influence of timing of superparamagnetic iron oxide injection

In the SPIO group (183), a total of 108 patients (58.7%) had the nanoparticles injected a median of 16 (range 2–27 days) before surgery. BD was injected in 26 of these patients (24.1%).

Subgroups according to timing of injection were comparable, with the exception of incidence of BCS (P = 0.018) (Table 2). Data were available for 107 of the 108 patients who received SPIO before surgery, with a successful detection of SNB in 102 who had SPIO alone, and in 105 with the addition of BD, resulting in detection rates of 95.3 and 98.1% respectively. On the other hand, in the 76 patients who had a perioperative SPIO injection, SNB detection was successful in 65 who received SPIO alone and in 70 who had SPIO and BD; detection rates were thus 86 and 92% per cent respectively. There was a difference in DR for SPIO only between preoperative and perioperative administration (95.3 vs 86%; P = 0.031). In multinomial logistic regression analysis for timing of SPIO injection and type of surgery, the latter did not affect the detection rate (expB 1.383, 95 per cent c.i. 0.498 to 3.840; P = 0.534) and only preoperative injection was associated with increased tracer-specific detection (expB 3.255, 1.063 to 9.965; P = 0.039). The difference in sentinel node detection between preoperative and perioperative SPIO administration did not reach statistical significance when blue dye was added (98.1 vs 92%; p = 0.068). BD was used more frequently in patients who received a perioperative SPIO injection (66 patients; 88%; p < 0.002).

Preoperative SPIO injection also resulted in the harvesting of more SN. The mean number of SN retrieved when SPIO was used with or without BD was 1.21 for the perioperative injection group and 1.45 for the preoperative injection group (P = 0.029). The difference was more obvious for the number of sentinel nodes detected only by SPIO; preoperative injection was associated with the retrieval of 1.43 (1.28 to 1.58) nodes versus 1.03 (0.89 to 1.17) for perioperative administration (P < 0.001). All nodes retrieved in patients who received a preoperative injection were brown (Fig. 1), facilitating identification.

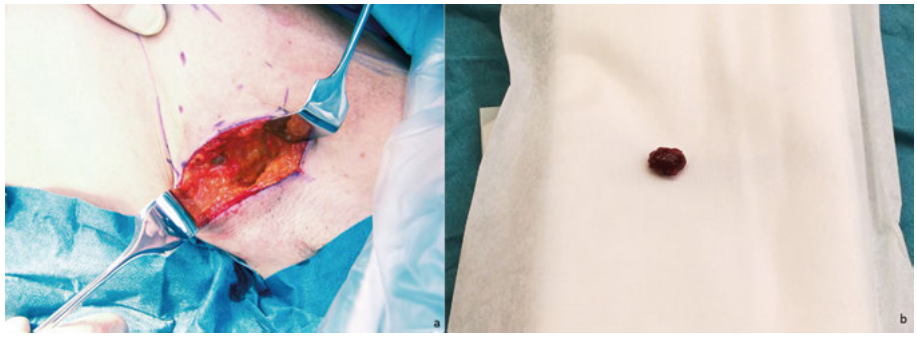


Fig. 1 a. In situ and b. ex vivo sentinel node in a patient injected with Sienna+®

Table 1. Characteristics of patients per study arm. Values in parentheses are percentage of patients unless indicated otherwise; values are *median (95 per cent c.i.) and †median (range). n.a., not applicable. §*z* test for independent samples, except ¶Student's *t* test, #Mann–Whitney *U* test and **Fisher's exact test.

	SPIO	RI	Total	*P*§
No. of patients	183	155	338	
No. of procedures	184	159	343	
Age (years)*	63.5 (61.8, 65.1)	65.0 (64.0, 68.0)	–	0.256¶
BMI (kg/m^2)*	25.8 (25.1, 26.4)	27.2 (26.4, 28.1)	–	0.008¶
Tumour size (mm)†	16 (2–80)	15 (2–83)	–	0.411#
Nuclear grade				0.233**
1	33	32	65	
2	80	79	159	
3	53	34	87	
Missing	18	14	32	
Histological type				0.935**
In situ	18	12	30	
IDC	132	114	246	
ILC	30	26	56	
Other	4	3	7	
Intrinsic subtype‡				0.093**
Luminal A	78	92	170	
Luminal B/HER2–	46	29	75	
Luminal B/HER2+	20	10	30	

HER2+	6	4	10	
Triple-negative	13	11	24	
Unknown	21	12	33	
Type of operation				
Mastectomy	57 (30.6)	52 (32.7)	108	0.670
BCS	126 (68.9)	105 (66.0)	231	0.579
SNB	1 (0.5)	2 (1.3)	3	0.481
Nodal metastasis (%)	21.9 (16.2, 28.7)	25.8 (19.3, 33.4)	–	0.390
Successful procedures				
Tracer only	171	152		
Tracer and dye	175	154		
Malignant	26	30		
Malignant detected by tracer only	24	25		
Malignant detected by tracer and dye	24	27		
Lymph nodes				
Total	247	300		
Tracer only	231	271		

Table 2. Characteristics of SPIO patients per injection timing.

	Timing of SPIO injection		
	Preoperative	Perioperative	*P*†
Median age (years)	65	68	0.116‡
Median BMI (kg/m^2)	25.2	25.6	0.416‡
Type of operation			0.018
BCS	81	46	
Mastectomy	27	30	
SN detected			0.068
Yes	105	70	
No	2	6	
No. of SNs*	1.45 (1.30, 1.59)	1.21 (1.05, 1.37)	0.029§
SPIO-specific SN detected			0.031
Yes	102	65	
No	5	11	
No. of SNs*	1.43 (1.28, 1.58)	1.03 (0.89, 1.17)	< 0.001§
Histological type			0.107
In situ	12	6	
IDC	79	53	
ILC	13	17	
Other	4	0	
T category			0.124
Tis	12	6	
T1a	4	1	
T1b	43	24	
T1c	19	18	
T2	23	24	
T3	4	3	
Intrinsic subtype			0.145
Luminal A	40	38	
Luminal B/HER2–	30	16	
Luminal B/HER2+	10	10	
HER2+	3	3	
Triple-negative	10	3	
In situ	15	6	
Metastasis in SN			0.316
Yes	15	15	
No	93	61	

- Follow-up, discoloration and patient satisfaction

Median follow-up in the SPIO group was 398 (i.q.r. 84) days. Some 39.9% of patients presented with skin staining that faded slowly in size and color over time. Albeit much smaller and paler, staining was still present in 35.9% after 15 months (Fig.2). BCS had been performed in 97%, representing a strong correlation ($P < 0.001$). Patients who received a deeper peritumoural injection of SPIO had less staining immediately after surgery, as well as less staining over time. In total, 58 of 73 patients who developed staining had received a periareolar injection, whereas only 15 had had a peritumoural injection ($p = 0.046$) (Table 3). No other characteristics or outcomes varied according to site of injection (periareolar versus peritumoural) in univariate analysis (Table 3).

All 65 patients with discoloration remaining after more than 10 months responded to the questionnaire at both time points. Only two patients in this subgroup (3%) complained that they were affected by the stain. Views regarding skin staining and cosmesis were mixed. The majority of patients considered staining a minor problem, if an issue at all (60% at the first assessment and 61% at the second). No substantial change in views was noted between the two time points ($p = 0.280$). The radar plot in Fig.3 depicts the answers of the interviewed patients on the two different time points.

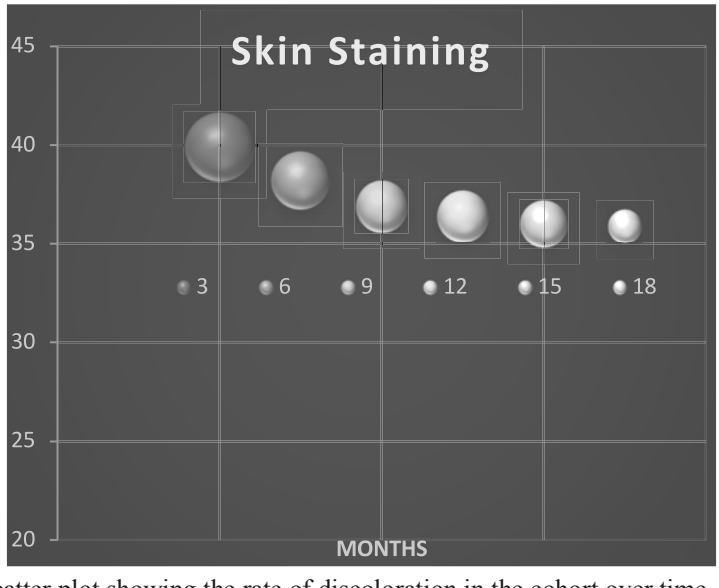


Fig. 2 Scatter plot showing the rate of discoloration in the cohort over time. Median stained areas are shown for discolored surfaces after exclusion of patients with no staining. The fading effect is given schematically with a preselected visual analogue.

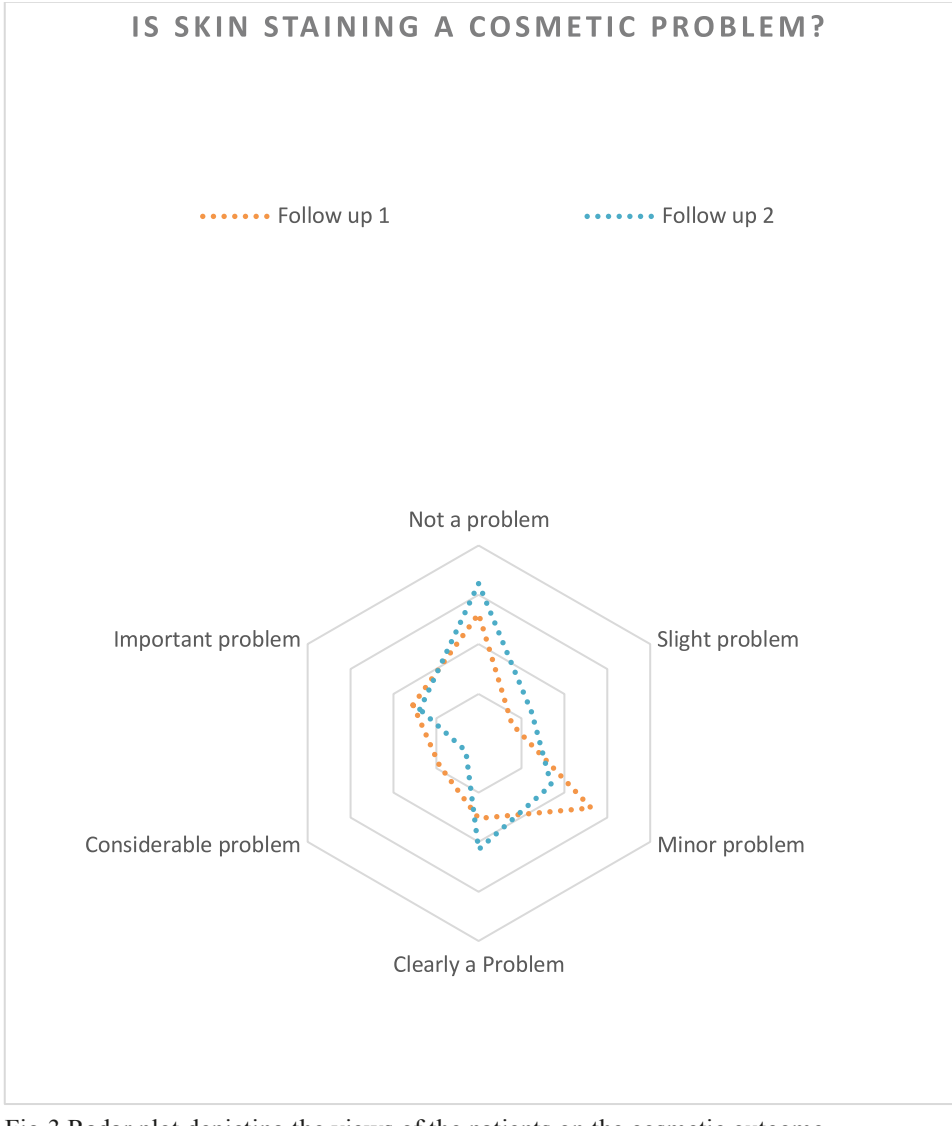


Fig.3 Radar plot depicting the views of the patients on the cosmetic outcome.

Table 3. Characteristics of SPIO patients per injection site.

	Site of SPIO injection		*P*†
	Periareolar	Peritumoural	
Median age (years)	65	66	0.265‡
Median BMI (kg/m^2)	25.2	25.5	0.901‡
SN detection			0.444
Yes	124	51	
No	7	1	
No. of SNs*	1.32 (1.20, 1.45)	1.42 (1.21, 1.64)	0.400§
SPIO-specific SN detected			0.563
Yes	118	49	
No	13	3	
No. of SNs*	1.21 (1.09, 1.34)	1.38 (1.16, 1.61)	0.158§
Histological type			0.665
In situ	15	3	
IDC	91	41	
ILC	22	8	
Other	3	1	
T category			0.124
Tis	15	3	
T1a	2	3	
T1b	49	18	
T1c	26	21	
T2	34	13	
T3	3	4	
Intrinsic subtype			0.295
Luminal A	58	20	
Luminal B/HER2–	29	17	
Luminal B/HER2+	16	4	
HER2+	5	1	
Triple-negative	6	7	
In situ	15	6	
Metastasis in SN			0.660
Yes	20	10	
No	111	43	
Skin staining			0.046
Yes	58	15	
No	72	38	

- Cost analysis

Logistics were simplified in the SPIO arm, as the preoperative visit to the department of nuclear medicine could be omitted. As far as the tracer and injection expenses per procedure were concerned, the average was €225 for the SPIO arm versus €252 for the RI arm, with SPIO being slightly cheaper by approximately €27.

Compared with perioperative administration, preoperative injection of SPIO saved an additional minimum of 20 min in the operating theatre, which is the time needed for SPIO to migrate to the axilla. With an average cost of €17.6 per min for the operating theatre in the Uppsala Örebro Region, (€352.7) was saved per procedure.

5.4 Paper IV

For the interim analysis, 151 patients (61.4%) had been recruited (Table 1). IBC was detected in 32. No differences in characteristics were demonstrated, when compared to the entire cohort (Table 2). In four, tumour size was <1 mm and no SNB was performed. The incidence of upgrade to invasive cancer (21.2%; 95%: CI 15.1, 28.7) was comparable to the 20% of the hypothesis, (p=0.607), as was the proportion that SN was performed (18.5%; 95% CI: 12.9, 25.6, p=0.258). The reduction in SNB was 81.5% (Chi square=174.726, p<0.0001) and an average of 4.4 SNBs were spared for each performed.

At reoperation (median 27 days, range 9-46), transcutaneous magnetic signal was present in all cases. Protocol violation led to exclusion of one, leaving 27 for analysis. The combination of SPIO and BD localized the SN in all cases (100.0%), whereas Tc and BD were successful in 18 cases (66.7%) resulting in a difference (Mc Nemar's, p=0.004). Similar were the differences in SPIO-only detection [(SPIO-only detected the SN in 24 cases (89.0%) and Tc-only in 16 (59.3%), p=0.039]. In univariate analysis, the factors related with a successful SPIO specific SNB biopsy on reoperation were type of surgery (mastectomy vs breast conservation 57.4 vs 100%, p=0.012), and surgeon's familiarity with the technique (100% vs 70%, p=0.041). However, none of these factors retained significance on multivariate logistic regression. Analysis of these cases revealed that, in the three cases SPIO "failed", it was the first attempt of the surgeon with the magnetic technique and in two cases, mastectomy had been performed. The nodes were specifically assessed afterwards by the pathologist and found to contain SPIO, just like the probe-detected nodes. The addition of BD eliminated this difference for type of surgery (100% vs 100%, p=1.000). Both tracers retrieved same number of SN (median 1, p=0.385). No adverse effects were noted. SPIO skin discoloration rate was 19.3% in the entire cohort, presented exclusively in BCS, with a mean stain of 3.4 cm^2. In patients who underwent SNB, SPIO discoloration was present in six (25.0%),

Table 1. Patient characteristics in the interim cohort.

SentiNot interim analysis cohort (n=151)			
Age (mean, 95% CI)		59.8 (58.0, 61.6)	
Radiologic DCIS size (mean, 95% ci)		39.6 (35.0, 44.1)	
Size cut-off	<40mm	89	58.9%
	≥40mm	62	41.1%
Nuclear Grade on Core Biopsy	2	41	27.1%
	3	100	66.2%
	Unknown	10	6.7%
Detection mode	Screening	134	88.7%
	Clinical	17	11.3%
Palpable lesion or radiologic mass effect	Yes	21	13.9%
	No	130	86.1%
Type of Breast Surgery	BCS	102	67.5%
	Mastectomy	49	32.5%
Transcutaneous axillary signal at the end of the operation	Yes	132	87.4%
	No	2	1.3%
	Na	17	11.3%
Pathologic DCIS size (mean, 95% CI)		40.8 (36.2, 45.3)	
Nuclear Grade on Specimen	2	27	17.9%
	3	123	81.5%
	Unknown	1	0.7%
Invasive cancer in specimen	Yes	32	21.3%
	No	119	78.7%
Invasive cancer size (mm) (mean, 95%CI)		8.9 (3.1, 14.7)	
SPIO induced skin staining	Yes	29	19.3%
	No	121	80.7%
SN as a second operation	Yes	28	18.5%
	No	123	81.5%

whereas BD staining in eleven (54.2%, Mc Nemar's p=0.092). SentiNot policy resulted in substantial cost containment of surgical care, with a mean reduction of 812 USD (95% CI: 543, 1081) per patient, corresponding to a reduction of 14.1% (4953 vs 5765 USD, p<0.001) for the entire cohort. When addressing patients that would have been treated with SNB without having IBC, the mean reduction was 1477 USD (95% CI: 1422, 1533), resulting in a 25.8% reduction (4242 vs 5719, p<0.001).

During the same period, 1688 patients in Sweden were treated for a preoperative diagnosis of DCIS but had pure DCIS confirmed by specimen pathology. Clinicopathological features were similar to the SentiNot cohort (Table 3). Totally, 1005 (59.5%) underwent axillary evaluation. As shown in Table 4, predictive factors in multivariate analysis were younger age (58.9 vs 61.2 years, p<0.001), larger DCIS size (36.7 vs 22.0 mm, p=0.005), and mastectomy (axillary evaluation in 85.4% of mastectomies, p<0.001). Regarding nuclear grade, less axillary surgery associated with grade 1 (29.5% vs 70.5%,

p<0.001) and grade 2 (50.9% vs 49.1%, p<0.001); grade 3 did not retain significance as a predictive factor (77.5% vs 22.5%, p=0.083).

Thirteen patients (1.3% of those sampled and 0.8% of the entire cohort) had metastases. Of the 24 patients that underwent ALND, only three were due to metastasis and the rest were due to SNB failure. Application of the SentiNot inclusion criteria would reduce SNB from 59.5% to 13.3% (McNemar's p<0.001), considering that the remaining cases would not have been considered for SNB (DCIS grade I or grade II and <20mm, treated with breast conservation). However, all cases could have been treated within the SentiNot concept.

Table 2: Preoperative descriptive values of patients with invasive breast cancer. *In the last column, p-values from the indicated tests are provided with respect to whether these parameters are different from the cohort or the non-invasive cancer group. a: Student's t-test. b: Fisher's exact test.*

SentiNot patients who upgraded to IBC (n=32)				
Age (mean, 95% ci) (median ,iqr)		59.8 (57.7, 61.9)		0.955 [a]
Radiologic DCIS size (mean, 95% ci) (median, iqr)		47.8 (35.9, 59.6)		0.103 [a]
Nuclear Grade on Core Biopsy	2	10	31.3%	0.461[b]
	3	19	59.4%	
	Unknown	3	9.4%	
Detection mode	Screening	28	87.5%	0.760[b]
	Clinical	4	12.5%	
Palpable lesion or radiologic mass effect	Yes	7	21.9%	0.158[b]
	No	25	78.1%	
Type of Breast Surgery	BCS	22	68.8%	1.000[b]
	Mastectomy	10	31.3%	
SPIO Stain	BCS	9	100%	0.336[b]
	Mastectomy	0	0%	
Transcutaneous axillary signal at the end of the operation	Yes	32	100%	na

Table 3. Characteristics of patients in Sweden diagnosed with pure DCIS postoperatively from 2015 to 2017.

Patients with postoperative DCIS in Sweden (2015-2017). N=1688			
Age (mean, 95% ci) (median ,iqr)		60.0 (59.4, 60.6)	
DCIS size (mean, 95% ci) (median, iqr)		30.8 (29.5, 32.1)	
Detection mode	Screening	1226	72.6%
	Clinical	460	27.3%
	Unknown	2	0.1%
Type of Breast Surgery	BCS	1094	64.9%
	Mastectomy	567	33.5%
	Missing	27	1.6%
Nuclear Grade	1	122	1.2%
	2	535	31.7%
	3	612	36.3%
	Unknown	419	24.9%
Axillary surgery	None	669	39.6%
	SNB	981	58.1%
	ALND	24	1.4%
	Missing	28	1.7%

Table 4. Characteristics of patients in Sweden diagnosed with pure DCIS postoperatively from 2015 to 2017 as to whether axillary surgery was undertaken. Continuous variables are presented as means with 95% CI and categorical variables as observations (%). a: Student's t-test, b: Chi-square, c: for mastectomy.

N=1688		Axillary Surgery		Univariate analysis	Multivariate analysis	
		Yes	No	p-value	Odds ratio, (95% CI)	p-value
Age (y)		58.9 (58.2, 59.6)	61.2 (60.3, 62.1)	<0.001[a]	0.986 (0.976, 0.996)	<0.001
DCIS Size (mm)		36.7 (35.0, 38.5)	22.0 (20.4, 23.5)	<0.001 [a]	1.013 (1.006, 1.019)	0.005
Nuclear Grade	1	36 (29.5%)	86 (70.5%)	<0.001 [b]	0.244 (0.137, 0.433)	<0.001
	2	272 (50.8%)	263 (49.2%)		0.480 (0.318, 0.724)	<0.001
	3	474 (77.5%)	138 (22.5%)		1.444 (0.953, 2.186)	0.083
	Unknown	94 (52.5%)	85 (47.5%)			
	Missing	97 (63.4%)	56 (36.6%)			
Type of Breast Surgery	BCS	521 (47.6%)	573 (52.4%)	<0.001 [b]	4.389[c] (3.242, 5.942)	<0.001
	Mastectomy	484 (85.4%)	83 (14.6%)			

6. Discussion

The role of SNB in the clinical decision making process for patients with breast cancer is pivotal. Ever since the first landmark SNB trials were conducted, the combination of RI and BD was quickly established for the standard of care in clinical practice with a standardized technique, detection rated as high as 99% and acceptable FNR (65). However, the drawbacks related with difficulty to access, manipulate and dispose the RI, as well as the allergenic properties of the BD prompted the investigation for new tracers. But, which properties should be sought in a novel tracer? It has become increasingly clear that increasing implementations for SNB in clinical practice are mandating for a technique with high accuracy, low learning curve, ease of execution and unhindered access.

As far as accuracy in SN DR is concerned, the studies conducted within the present thesis have succeeded in presenting comparable results for SPIO with the dual combination of RI and BD. The non-inferiority trial design was selected because the Nordic trial (Paper I) and the MONOS trial (Paper III) with the following rationale: the conduct of trials designed for superiority or statistical equality would result in a very large sample size required in order to reject the null hypothesis, since the margin for improvement of the DR from a rough approximate of 96-97% is rather narrow. From a pragmatic point of view, the clinical value of such a result would be relevant, but would not probably suffice to accumulate all the amount of evidence to motivate change of practice. The DR of 97.6% for SPIO achieved in the Nordic trial and 95.7% in MONOS are not only statistically comparable to the RI and BD combination, but probably represent a very satisfactory tracer performance in everyday clinical practice. This fact was confirmed in the meta-analysis performed and, interestingly, another systematic review conducted in the same material, reproduced a weighted pooled DR of 97.1%, which is also representative, albeit methodologically more crude (79).

The issue of obtaining direct information on a tracer-specific FNR was addressed within the Nordic trial and the meta-analysis by addressing concordance per patient and per node. The reason for selecting this method was that, despite that a rough FNR for the combination of RI and BD is expected in the literature, largely depending on the number of SNs retrieved, it is not possible to define what the FNR would be for each given patient. On the other hand, performing background axillary clearance in order to define the true FNR for each tracer is not ethically or scientifically acceptable. Therefore, considering

the synchronous administration of SPIO as an "intervention" to the "control arm" of RI and BD allowed for the comparison of DR between methods and concordance allowed for an estimate of whether the tracers detected, not only as many, but also the same nodes. With concordance rates as high as 100% and a pooled weighted average of 98.1%, the results were deemed satisfactory. In the meta-analysis conducted within the project, this was explained by depicting the "Risk Difference" as an effect size, by calculating the difference in each study between concordant patients/nodes in relation to the Tc and BD and "reverse" concordance, that is concordant patients/nodes in relation to the SPIO positive ones. The hypothesis would be that, a high risk difference with a p-value that would denote significance would imply that one of the two methods detects either more or less patients/nodes and that a high concordance rate as to one method only may not reflect the truth. The fact that "risk differences" were near zero demonstrates that the methods are indeed highly concordant and it may safely be concluded that a comparable FNR for the SPIO to that of the combination of RI and BD may be assumed.

Data from the meta-analysis demonstrated a higher number of nodes retrieved with SPIO than with RI. This, however was not a finding that was not confirmed in the Nordic trial. Moreover, in the MONOS, less nodes were removed with the SPIO method but with similar detection rates per patient and per node. This implies that some of the previous reports might have been affected by the simultaneous use of both methods, making it difficult to disentangle detection by either method and to define possible overlapping. Additionally, it might suggest that SPIOs tend to accumulate in higher concentrations on the first node/-s in line, a hypothesis enhanced by the intraoperative observation that, in cases where more than one nodes were retrieved, they were all coloured, in anatomical proximity to each other and most often in the expected location (80). No residual signal in the axilla was found after their removal. The clinical importance of this fact remains to be elucidated; it seems however reasonable to assume that a biopsy which avoids extensive axillary dissection takes less time to perform and is accompanied by lower morbidity (81). On the other side, it is of interest to see if the removal of less nodes is related with more frequent regional recurrences, since the FNR has been shown to be higher with the removal of one node only (82).

SPIO induced skin discoloration was extensively described in papers I and III. Out of the previously published studies (83-90), discoloration in the form of a brown-greyish tattoo had been briefly mentioned in some publications. Rubio et al (85), reported discoloration in 19%, which faded progressively after 6 months, comparable to the effect produced by blue dye. Piñero et al (86) report the pigmentation, whereas Ghilli et al (87), report an incidence of 40% at six months which is transient in 91% of pigmented cases. Similar observations regarding staining were made with the use of another type of SPIO, ferucarbotran, in which a skin stain that lasted 2 months and resolved spontaneously thereafter was described (90). The absence of data motivated follow-

up and quantification of this effect. Postoperatively, it was present at 35.5% of patients and faded slowly over time. In 8.6%, a pale discoloration was present after 15 months.

The discoloration found after Sienna+ injections implies that the substance remains for a long time in the breast. In the Nordic trial, this was confirmed since magnetic counts were present in the tissue up to 515 days after the operation. Therefore, injection of Sienna+ could be administered prior to the operation, thus facilitating planning and logistics. Moreover, it implies that a deeper peritumoral injection with subsequent excision of that area, such as that proposed by Ghilli et al (87), may result in smaller or no discoloration.

The feasibility of a preoperative SPIO injection was subsequently examined in paper II. In this pilot study, the feasibility of preoperative SPIO injection is demonstrated in a variety of patients. An obvious advantage is that the intervention is simple and does not require specialized personnel or the presence of a physician, since the injection can be administered by the breast nurse in the outpatient clinic. In this study, successful SNB was performed up to fifteen days after SPIO injection. However, considering the positive prognostic value of the transcutaneous signal for a successful SNB, since it is present, it may be assumed that a SNB should also be feasible within this interval. Apart from the obvious simplification of logistics, this suggests flexibility in the timing of the operation. This hypothesis constituted the basis for the project described in Paper IV.

After the conduct of the pilot study described in paper II, a novel finding of considerable importance for the SPIO described in paper III is the feasibility of injecting the tracer in the preoperative setting with superior results to that of the perioperative injection. Compared to the isotope, logistics are simplified, since there is no need for extra preoperative visits at the Nuclear Medicine Department. Compared to SPIO perioperative injection, more SNs are identified, higher tracer-specific detection rates are achieved and no extra intraoperative time or massage on the injection site is required for the tracer to migrate in the axilla, meaning shorter operating time. Furthermore, the need for blue dye is limited, and the staining of the nodes functions as an optical aid. In contrast to the Nordic trial, in the current data set there was no correlation between transcutaneous detection rates and BMI or interval between SPIO injection and operation. In routine, this means that clear cut off values for transcutaneous counts so as to predict if blue dye is necessary or a predictive model for the ideal time interval between SPIO injection and operation could not be deduced. Reviewing the results, it was seen that, whereas blue dye was injected in 93 patients, it proved necessary only in four. Additionally, data overview showed that it was administered more often in the beginning of the trial, and its use decreased as the operators felt more comfortable with the method.

The feasibility of preoperative injection led to an interesting clinical implementation, concerning SNB in patients with a preoperative diagnosis of DCIS

which is described in Paper IV. A shown in the results section, the practice is not clear and the criteria for upgrade result in an over treatment of patients with DCIS in almost 60% of cases on a national level, when one examines Swedish data. The preoperative marking of the SN for a biopsy, only in cases of upgrade to IDC resulted in a significant reduction in the conduct of SNB with important gains, not only by morbidity that was spared but also by sparing resources involved in delivery of surgical care. The DR at reoperation was satisfactory in cases of mastectomy, thus challenging the view that SNB should be conducted at the time of mastectomy whereas in cases of BCS, the problems of a lower DR or the question of finding the "correct" SN have been addressed. This implies that the marking of the SNB may have broader clinical applications, even in the conduct of risk-reducing mastectomies, where certain centers have the practice of performing a SNB with the rationale that it cannot be performed at another session.

The conduct of studies on novel techniques always poses the challenges of feasibility, applicability, methodology and lack of reference values, which dictate the context of limitations. In all the projects included in the present thesis, a logical stepwise approach from one to another was pivotal for the deduction of safe conclusions, and in that way mandated the type of trials to be conducted. The Nordic trial, example given, included patients that were their own controls, as this was the most efficient way to assess concordance between SPIO and RI. The pilot study described in paper II included consecutive patients being their own controls, in order to assess, not only DR, but also concordance between methods in the context of a preoperative injection. Lack of randomisation may be considered a limitation of paper III; however, the investigators took a pragmatic approach to determine whether the magnetic technique is functional under normal conditions. The isotope and dye method has been the standard for many years, with identification rates up to 99.5%. A very large number of participants would be required to demonstrate the superiority of another method. According to a prospective sample size calculation with results from the Nordic trial, with DR of 97.1 and 97.6% for RI and SPIO respectively, a total sample of 32 390 patients would be required, for 80% power and $P < 0.05$, to demonstrate that SPIO is superior as a method of detection. In this context, an explanatory RCT would be useful and methodologically appropriate, but rather difficult to carry out because of the large sample needed. However, the purpose of the MONOS study was not to demonstrate statistical superiority. On the contrary, the advantage of a pragmatic approach is that allows documentation of the feasibility of a new method in the appropriate context that ensures high quality in a trial, such as a low-risk, simple intervention and cluster-level application in a healthcare system with comprehensive electronic records and condition-specific registries (91,92). All of these were present for the MONOS trial. Another issue is whether randomisation would actually affect issues such as discoloration, patient satisfaction or

costs. It is clear, however, that it would have been methodologically more appropriate.

A consideration regarding Paper IV was that the study hypothesis was logically expected to be proved. This factor, together with the anticipated slow recruitment, due to the inclusion criteria, mandated an interim analysis. The conduct of interim analyses within clinical trials is challenging. In order to avoid overestimating the effect size, the O'Brien-Fleming procedure was selected (93). Despite reaching the primary endpoint, the interim analysis was pre-specified mainly as means to evaluate the study concept, rather than to terminate prematurely. The results presented in Paper IV are very encouraging and seem to allow for tailored treatment in DCIS, enabling for necessary-only interventions, with favorable effects on health economy.

7. Future perspectives

As stated earlier, the quest for a "better" novel tracer in SNB needs to aim for more than statistical superiority. The use of SPIO as a tracer for SNB seems to have promising clinical implementations, that are currently accruing data in ongoing studies.

The SentiNot concept, described in Paper IV, is an ongoing and recruiting study, and a protocol amendment in inclusion criteria is expect to broaden inclusion and allow for robust clinical conclusions on the use of SPIO in this setting.

On ongoing multicenter dose-and-timing optimization study, approved by the Swedish Medical Products agency is hoping to allow for definitive results on the dose and time-frame of SPIO injection for SNB (94).

Marking of the SLN with isotope does not give a precise preoperative localization since lymphoscintigraphy techniques have poor spatial resolution. This makes preoperative biopsies impossible with technetium as a marker. Superparamagnetic iron oxide (SPIO) can identify SLN in axillary MRI in patients with breast cancer and recently the feasibility of a SPIO injection as a contrast material in MRI sentinel lymphography and as a tracer for SLN biopsy using an integrated method with CT was demonstrated, with excellent three dimensional imaging quality. An additional advantage of SPIO already reported is that it remains in the tissue for up to four weeks, making it ideal to trace and investigate on the SLN preoperatively. Moreover, current data have shown that an ultrasound (US) guided biopsy of lymph nodes with suspect metastases will be successful up to 93% of cases, demonstrating sensitivity of 65% and specificity of 100%. These data suggest that preoperative MRI in the axilla after the injection of Sienna+ could be used as an adjunct to a minimally invasive staging procedure, but also enable targeted biopsies.

The ongoing "Mag" netic tracer for an enhanced "U"ltra "S"ound (MagUS) pilot study aims to evaluate the efficacy of MRI in the preoperative evaluation of the SLN status as well as the efficacy of the implementation of MRI guided SLNB through a core needle biopsy facilitated through an US (95).

Moreover, skin staining is the most common side effect of SPIO injection. It has been shown to be related to the prolonged residence of the substance in the tissue, a remark that is enhanced by the facts that stained tissue has magnetic signal and that it is almost exclusively observed in breast conserving surgery (BCS). This is a matter of interest for patients who need to be followed postoperatively with MRI. Despite the fact that the indications are few, the

long lasting staining may pose a restriction since MRI will be contaminated by SPIO artefacts. The aim of the PostMagMRI study is to address the compatibility of postoperative MRI in patients that have undergone SNB with SPIO mapping, where the association between the presence of ferromagnetic signal and skin staining will be associated to the presence of SPIO specific artefacts on MRI (96).

Finally, the introduction of a new magnetic seed (Magseed, Endomagnetics, Cambridge UK) for the localization of non-palpable tumours has gained interest. Currently, a peritumoural injection of SPIO is used for SNB by our unit. In the MONOS study, a deeper peritumoural injection demonstrated comparable SN detection rates to a subareolar injection earlier used, with the advantage of less skin staining. For the present combined technique, the hypothesis was that SPIO injected dorsally to a lesion would amplify the transcutaneous magnetic signal in tumours located deep in the breast. Intraoperatively, the surgeon could be guided by the maximum focal signal provided by the Magseed® placed ventrally to the lesion. This focal signal was easily distinguished from the background signal of the SPIO. The hypothesis is also that injecting the SPIO close to the lesion will result in a surgical removal of the majority of the SPIO in the breast with less skin staining and reduced risk of MRI artefacts postoperatively (which has to be proved). A pilot study has recently been completed and a randomised control trial is currently accruing data within our group (97).

8. Conclusions

SPIO seems to be a promising method with novel applications for the evaluation of the SN in patients with breast cancer. Favorable characteristics include applicability in a global setting, no need for extra resources and an isotope free method with comparable detection rates. Further studies will elucidate the importance of this novel method.

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