

# **Superparamagnetic iron oxide for sentinel lymph node biopsy**

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UNIVERSITY OF GOTHENBURG

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*To my beloved mother*

Power is given only to those who dare to  
lower themselves and pick it up.  
Only one thing matters, one thing:  
to be able to dare!

- Fyodor Dostoevsky



# Superparamagnetic iron oxide for sentinel lymph node biopsy

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## ABSTRACT

Superparamagnetic iron oxide nanoparticles (SPIO), used as a tracer for sentinel lymph node (SLN) detection, offer several logistical benefits over the standard method using radioactive technetium-99m and blue dye. The aim of this thesis was to explore SPIO as a tracer for SLN biopsy in patients with cutaneous melanoma or breast cancer. In *Paper I*, we demonstrated that SLN detection with an ultra-low dose of 0.02-0.5 mL SPIO injected intradermally in patients with melanoma was feasible, and we also showed that SPIO-enhanced MRI could have potential as a non-invasive modality to predict SLN status in vivo. In *Paper II*, an ultra-low dose of 0.1 mL of SPIO was investigated in patients with breast cancer and demonstrated to be feasible. In *Paper III*, MRI artefacts and skin discoloration caused by SPIO were evaluated. The MRI artefacts were small with minimal skin discoloration not affecting patient-reported outcomes. In *Paper IV*, individual patient data were evaluated for optimal SPIO dose, injection site, and timeframe as well as quantifying the iron content in the retrieved lymph nodes. An intra-tumoral injection and SPIO administrated less than 2 hours before surgery appeared to be less optimal for SLN detection.

In conclusion, SLN detection with an ultra-low of SPIO was found to be feasible in patients with melanoma or breast cancer, not compromising MRI results and with only small skin discoloration. These results lay a foundation for larger trials that may ultimately change future clinical practice for patients undergoing SLN biopsy.

**Keywords:** melanoma, breast cancer, sentinel lymph node biopsy, superparamagnetic iron oxide nanoparticles

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# SAMMANFATTNING PÅ SVENSKA

Förekomsten av tumörspridning till regionala lymfkörtlar är viktig för att bestämma prognos och behandlingsstrategi vid flera olika tumörtyper. Portvaktsskörteln är den första lymfkörteln som tar emot lymfa från ett tumörområde. Idag utförs rutinmässigt kirurgisk borttagning av portvaktsskörteln hos patienter med exempelvis malignt melanom och bröstcancer. För närvarande används ofta en dubbelteknik vid identifiering av portvaktsskörteln, ett radioaktivt spårämne kopplat till ett bärarprotein och en blå färg. Dessa båda spårämnen injiceras i anslutning till tumören och dräneras via lymfan och fastnar i portvaktsskörteln. Genom att använda en handhållen radioaktivitetsmätare guidas kirurgen till portvaktsskörteln, där den blå färgen dessutom gör lymfkörteln synlig för kirurgen. Nuvarande dubbelteknik har dock sina begränsningar. Tillgängligheten av radioaktivt technetium-99m är begränsad och den korta halveringstiden skapar en komplex logistik för både patient och sjukvård. Den blå färgen kan i sällsynta fall även framkalla allergiska reaktioner. En ny teknik är att i stället för ett radioaktivt spårämne använda ett järninnehållande spårämne, super-paramagnetisk järnoxid (SPIO), som gör portvaktsskörteln magnetisk och som visats ha likvärdiga resultat jämfört med tidigare dubbelteknik. Nackdelar med att använda SPIO vid bröstcancer är att den kan framkalla hudmissfärgning vid injektionsstället samt lämna artefakter på uppföljande magnetresonanstomografi (MR) undersökningar. Den rekommenderade dosen av SPIO vid bröstcancer är 1.0-2.0 mL som injiceras i bröstet, skulle man kunna använda en lägre dos av SPIO skulle teoretiskt risken för hudmissfärgning och MR-artefakter kunna minskas. Huvudsyftet med denna avhandling är att utvärdera om en ultra-låg dos SPIO (0.1mL) har samma diagnostiska säkerhet, jämfört med den befintliga dubbeltekniken, vid identifiering av SLN hos patienter med malignt melanom och bröstcancer, samt om detta kan leda till minskade hudmissfärgningar och MR-artefakter och om MR undersökning efter SPIO injektion skulle kunna identifiera portvaktsskörtel metastaser redan innan operation.

*Delarbete I* är en genomförbarhetsstudie (n=15), där den befintliga rutinen med injektion av radioaktivt spårämne ( $Tc^{99m}$ ) följt av lymfscintigrafi samt injektion av blå färg jämförs med en låg dos SPIO (0.02-0.5 mL) för detektion av portvaktsskörtel hos patienter med malignt melanom, något som tidigare inte gjorts. Patienterna genomgick dessutom MR undersökning före och efter SPIO injektion för att utvärdera huruvida SPIO förstärkt MR kan predicera tumörspridningen till portvaktsskörtel. Identifiering av portvaktsskörtel med

SPIO som spårämne var möjligt hos alla patienter, och studien öppnar även upp för möjligheten att förutsäga tumörspridning till portvaktskörtel med hjälp av en SPIO förstärkt MR undersökning även om konfirmerande studier är nödvändiga.

**Delarbete II** är också en genomförbarhetsstudie (n=50), som utvärderade om den befintliga rutinen med injektion av radioaktivt spårämne ( $Tc^{99m}$ ) och blå färg är jämförbar med en ultra-låg dos av (0.1mL) SPIO som spårämne för detektion av portvaktskörtel hos patienter med bröstcancer. Resultaten visade att portvaktskörteln kunde detekteras med en ultra-låg dos av SPIO hos alla patienter, även här behöver dock en större konfirmerande studie genomföras för att etablera denna metod i klinisk rutin, vilket för närvarande är pågående.

**Delarbete III** är en uppföljningsstudie av delarbete II (n=50). Här undersöktes om en ultra-låg dos av SPIO kunde minska MR-artefakter och hudmissfärgningar vid 6 och 12 månader efter bröstbevarande kirurgi. Resultaten visade att SPIO relaterade MR artefakterna var små och i regel inte påverkade bröstdiagnostiken, vi kunde även visa att den ultra-låga dosen endast orsakade små hudmissfärgningar vilket inte påverkade patienternas upplevelse.

**Delarbete IV** är en retrospektiv internationell meta-analys av individuella patientdata inkluderande fem studier från tre sjukhus i Sverige och tre studier från ett sjukhus i Nederländerna. Data analyserades för att undersöka om variationer i SPIO-dos, injektionsställe och tidsintervall mellan injektion och kirurgi kan påverka detektionen av portvaktskörteln hos patienter med bröstcancer. Ytterligare analyser utfördes för att kvantifiera mängden järn i varje extraherad SLN. Dosen 1.0mL hade en högre detektionsgrad av portvaktskörteln jämfört med övriga doser (<0.5mL, 1.5mL och 2.0mL). Den lägsta detektionsgraden noterades hos patienter som fick SPIO injicerad i tumören. När SPIO injicerades mer än två timmar före operation, observerades en bättre detektionsgrad jämfört med övriga tidpunkter. Det fanns ingen statistisk skillnad i den upptagna mängden järn när portvaktskörtlar utan metastaser jämfördes med portvaktskörtlar med metastaser.

# LIST OF PAPERS

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

- I. Mirzaei N\*, Katsarelias D\*, Zaar P, Jalnefjord O, Johansson I, Leonhardt H, Wärnberg F, Olofsson Bagge R.

*Sentinel lymph node localization and staging with a low-dose of superparamagnetic iron oxide (SPIO) enhanced MRI and magnetometer in patients with cutaneous melanoma of the extremity - The MAGMEN feasibility study.*

Eur J Surg Oncol. 2022 Feb;48(2):326-332. \*Joint first authors.

- II. Mirzaei N, Wärnberg F, Zaar P, Leonhardt H, Olofsson Bagge R.

*Ultra-Low Dose of Superparamagnetic Iron Oxide Nanoparticles for Sentinel Lymph Node Detection in Patients with Breast Cancer.*

Ann Surg Oncol. 2023 Sep;30(9):5685-5689.

- III. Mirzaei N, Wärnberg F, Zaar P, Diniz MO, Karakatsanis A, Leonhardt H, Olofsson Bagge R.

*The effect of an ultra-low dose of superparamagnetic iron oxide (SPIO) injected intra-dermally on postoperative skin discoloration and breast MRI artefacts in patients with breast cancer undergoing sentinel lymph node biopsy.*

Manuscript.

- IV. Mirzaei N\*, Christenhusz A\*, Wärnberg F, Olofsson Bagge R, Karakatsanis A, Staffan Eriksson, Job van der Palen, Simanowski J, Salamzadeh S, Dassen A, Alic L.

*Magnetic Sentinel Lymph Node Biopsy in Early Breast Cancer Patients: An Individual Patient Data Meta-Analysis of Tracer Uptake.*

Manuscript. \*Joint first authors.

# CONTENTS

1.	INTRODUCTION.....	1
1.1	The era of axillary management.....	2
1.2	Lymph node assessment.....	3
1.2.1	Clinical assessment.....	3
1.2.2	Ultrasound.....	3
1.2.3	Lymphoscintigraphy.....	4
1.3	Sentinel lymph node biopsy technique.....	4
1.4	Accuracy of sentinel lymph node biopsy.....	5
1.5	Sentinel lymph node biopsy in melanoma.....	6
1.6	Sentinel lymph node biopsy in breast cancer.....	8
1.7	Histopathological staging of sentinel lymph nodes.....	8
1.8	Morbidity after axillary surgery.....	10
1.9	Superparamagnetic iron oxide nanoparticles.....	11
2.	AIM.....	12
3.	PATIENTS AND METHODS.....	13
3.1	Overview.....	13
3.2	Paper I.....	13
3.2.1	Study design.....	13
3.2.2	Selection and patient inclusion.....	13
3.2.3	Data collection.....	14
3.2.4	Endpoint.....	14
3.3	Paper II.....	14
3.3.1	Study design.....	14
3.3.2	Selection and patient inclusion.....	15
3.3.3	Data collection.....	15
3.3.4	Endpoint.....	15
3.4	Paper III.....	15

3.4.1	Study design .....	15
3.4.2	Selection and patient inclusion.....	15
3.4.3	Data collection.....	16
3.4.4	Endpoint .....	16
3.5	Paper IV.....	16
3.5.1	Study design .....	16
3.5.2	Selection and patient inclusion.....	17
3.5.3	Data collection.....	17
3.5.4	Endpoint .....	17
3.6	Statistical analysis .....	17
3.7	Methodological considerations.....	19
4.	SUMMARY OF RESULTS .....	20
4.1	Paper I.....	20
4.2	Paper II .....	20
4.3	Paper III.....	20
4.4	Paper IV.....	21
5.	DISCUSSION .....	23
6.	ETHICAL CONSIDERATIONS .....	27
7.	CONCLUSIONS .....	28
8.	FUTURE PERSPECTIVES .....	29
	ACKNOWLEDGEMENT.....	30
	REFERENCES.....	32

# ABBREVIATIONS

ACOSOG	American College of Surgeons Oncology Group
ALND	Axillary lymph node dissection
BD	Blue dye
CLND	Completion lymph node dissection
ELND	Elective lymph node dissection
FNR	False negative rate
FPR	False positive rate
FGT	Fibroglandular tissue
H&E	Hematoxylin and eosin
HER2	Human epidermal growth factor receptor 2
MRI	Magnetic resonance imaging
NSABP	National Surgical Adjuvant Breast and Bowel Project
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy
SDIEQ	Skin Discoloration Impact Evaluation Questionnaire
SPIO	Superparamagnetic iron oxide nanoparticles
Tc <sup>99m</sup>	Technetium-99m

# 1. INTRODUCTION

Sentinel lymph node biopsy (SLNB) is an important method for staging the axilla for defining prognosis and making treatment recommendations in patients with melanoma and breast cancer (1, 2). Identification of the sentinel lymph node (SLN) often involves a dual technique using the radioactive tracer technetium-99m ( $Tc^{99m}$ ) associated with a carrier protein, and a blue dye (BD) (3, 4). The availability of  $Tc^{99m}$  is limited, and many hospitals worldwide lack a nuclear medicine department, which is required for access to  $Tc^{99m}$ . Handling radioactive materials entails complex logistical challenges for both patients and healthcare facilities using nuclear medicine resources (5). Use of  $Tc^{99m}$  may also be limited by its 6-hour half-life. In rare cases, BD can cause severe allergic reactions and is therefore recommended to be injected after induction of anesthesia (6, 7). BD has also been associated with genotoxicity causing DNA strand breaks in vitro (8).

Therefore, alternative tracers, such as superparamagnetic iron oxide nanoparticles (SPIO), have been tested to overcome these limitations. The implementation of SPIO has demonstrated comparable results to the dual technique (9-13). Several studies have investigated different doses, injection techniques, and time intervals between injection and surgery to refine SPIO use (11, 13-16). On the basis of previous research, the recommended SPIO dose is 1.0–2.0 mL for identifying SLNs in patients with breast cancer (15, 17). Interestingly, SPIO act as a contrast agent within lymph nodes, thus potentially allowing for magnetic resonance imaging (MRI) to detect the presence of metastasis (18, 19).

The use of SPIO has two main drawbacks: (i) local breast MRI artefacts after breast conserving surgery and (ii) skin discoloration at the injection site, similarly to that observed with BD (14-16). Both these drawbacks might be overcome by an injection method allowing for a lower dose of SPIO, thus enabling SPIO to become the global standard of care independently of access to nuclear medicine. Therefore, this thesis focuses primary on evaluating SLN detection by using SPIO in patients with breast cancer and cutaneous melanoma.

## 1.1 The era of axillary management

Surgical assessment of the axilla is valuable for staging and decision-making regarding adjuvant therapy in patients with cutaneous melanoma and breast cancer (20-22). Jean Louis Petit (1674–1750) believed that the enlarged lymph nodes in the axilla gave rise to breast cancer and should be removed (23). James Syme (1799–1870) suggested removing all axillary glands in patients with breast cancer as a remedy for the disease (23). Halsted (1852–1922) emphasized the importance of thorough en bloc removal of the axillary lymph nodes together with the breast tumor, believing that lymph nodes served as a natural defense against the dissemination of cancer cells (24). Herbert Snow (1847–1930) stated that treatment of melanoma should routinely include elective lymph node dissection (ELND) to establish regional disease control. However, the radical axillary dissections recommended by Halsted and the ELNDs recommended by Snow came under scrutiny in investigations of long-term survival benefits in patients with or without nodal metastatic disease. Multiple randomized trials have shown no benefit with immediate lymph node dissection (LND) compared to a delayed LND concerning survival (25-27).

In 1977, the SLN concept was introduced by Canabas in patients with penile cancer (28). However, Donald L. Morton, an American surgical oncologist, is recognized as the pioneer in identifying SLNs in patients with melanoma (2). The randomized Multicentre Selective Lymphadenectomy Trial (MSLT-I trial), the National Surgical Adjuvant Breast and Bowel Project trial B-32 (NSABP-B32) and the Milan trial have demonstrated that SLNB has comparable reliability and achieves equivalent overall survival to those of lymph node dissection in patients with melanoma and breast cancer (22, 29, 30).

Subsequently, SLNB has been implemented in both melanoma and breast cancer surgery, thereby distinguishing node negative patients from those with nodal involvement who require an additional completion lymph node dissection (CLND). However, also the relevance of CLND has been questioned and changed. The MSLT-II trial, a prospective randomized trial was designed to investigate if CLND improved melanoma specific survival for patients with positive SLN. No significant difference in melanoma specific survival was reported after 9.8 years follow up (31). Further, the American College of Surgeons Oncology Group Z0011(ACOSOG Z0011) trial randomized patients with less than three positive SLNs undergoing breast conserving surgery and subsequent whole breast radiotherapy to either ALND or no further surgery. Also this study reported no statistically significant differences in loco-regional recurrences between patients undergoing SLNB versus ALND at a median of 6.3 years of follow up (32). This is further

supported by the recent results from the randomized SENOMAC trial, that demonstrated non-inferiority of omitting ALND in patients with breast cancer having one or two positive SLNs (33).

However, many countries worldwide have struggled to establish the use of SLNB, partly because of a lack of resources, e.g., technetium. Hence, an easily accessible tracer is greatly needed. The magnetic tracer SPIO could potentially fill this gap.

## 1.2 Lymph node assessment

### 1.2.1 Clinical assessment

Clinical assessment of lymph node stations can face challenges in distinguishing between malignant and benign lymph nodes (34, 35). However, palpation enables the evaluation of lymph node size and shape, thus aiding in assessment of their texture, tenderness, and mobility. Identifying whether lymph nodes are enlarged or fixed can facilitate differentiation between benign and malign conditions and can guide further investigation and management decisions.

### 1.2.2 Ultrasound

Ultrasound is a non-invasive technique that aids in predicting metastatic involvement in lymph nodes according to morphological criteria such as size, shape, margins, cortical thickness, and hilar compression or displacement (36, 37). If the cortical thickness exceeds 2.5–3 mm, or loss of hyperechoic hilum is observed, malignancy should be suspected (38). In such cases, a diagnostic procedure such as ultrasound-guided fine needle aspiration or core needle biopsy is performed.

In patients with cutaneous melanoma, assessing the axillary or inguinal lymph nodes with ultrasound is not commonly performed unless suspected signs of malignancy, such as enlarged and fixed lymph nodes, are observed. In such cases, ultrasound is a valuable tool for predicting lymph node involvement and guiding biopsy if necessary. However, in patients with breast cancer, evaluation of axillary lymph nodes is an essential part of the evaluation, particularly when malignancy is suspected. Therefore, the Swedish guidelines recommend that ultrasound evaluation of the axilla is performed in patients with early breast cancer (39).

### 1.2.3 Lymphoscintigraphy

Lymphoscintigraphy is a diagnostic imaging technique used to visualize lymphatic drainage from tumors. In melanoma, SLN mapping with lymphoscintigraphy is essential for the prediction of lymphatic drainage patterns, thus enhancing SLN identification (40, 41). The lymphatic drainage from tumors may diverge toward separate parallel afferent lymphatic pathways leading to one or more SLNs in most commonly the axillary or inguinal lymphatic basins (42).

Therefore, a combination of lymphoscintigraphic mapping, radioactive tracer and intraoperative BD is used to ensure accurate SLN detection (43-45), as well as to aid in identifying the basins at risk of metastatic disease and determining whether the lymphatic drainage from the tumor has passed the “sentinel” lymph node in an orderly manner, or reached the second and third echelon nodes (42).

In breast cancer, unlike melanoma, the lymphatic drainage patterns are relatively predictable, and the primary tumor drainage is to the axillary lymph nodes. A study by McMasters et al., in the early 2000s, and a multicenter randomized trial, have confirmed that lymphoscintigraphy does not contribute to the SLN detection rate in patients with breast cancer yet is time-consuming (46, 47). Therefore, a frequently applied approach for identifying SLNs during breast cancer surgery is to use Tc<sup>99m</sup> in combination with BD, but without lymphoscintigraphy.

## 1.3 Sentinel lymph node biopsy technique

In the beginning of the SLNB era, intraoperative intradermal injection of BD was used as a mapping method to detect SLNs. During surgery, a skin flap was raised to reveal the blue-stained lymphatic duct, which was then dissected through the fatty subcutaneous tissue all the way to the lymphatic basin, thus enabling the identification and removal of blue-stained lymph nodes (2). To ensure that SLNB was as reliable as ALND, the refinement of the SLN procedure involved routine lymphoscintigraphies for all patients, mapping the lymphatic pathway to the SLN before surgery (48-51). The confirmation of the “true” SLNs was then decided through guidance from both the axillary lymphoscintigraphic mapping and the blue SLN coloration during surgery. However, the use of preoperative lymphoscintigraphy required refinement and was time consuming, and substantial surgical experience was necessary to raise

the skin flaps and dissect along the blue stained lymphatic ducts to find the “true” SLNs.

Krag and colleagues further developed the SLN technique by using a handheld gamma probe (3). The gamma probe, a scintillation probe for measuring accumulated radioactivity, was designed to detect the gamma radiation emitted by Tc<sup>99m</sup> and guide surgeons to the precise locations of SLNs (43, 52). Reintgen and Cox integrated the lymphatic mapping in both patients with melanoma and patients with breast cancer (53, 54). The introduction of the handheld gamma probe made the surgery easier, removing the need to dissect along the lymphatic ducts, and provided further conformation supporting accurate detection of the “true” SLN (55). Improvements in the technique and the assurance of finding the “true” SLNs led to changes in clinical practice and decreased unnecessary surgery and removal of non-SLNs.

For both melanoma and breast cancer, a rule of thumb is to remove and consider all lymph nodes containing at least 10% of the radioactive counts measured in the SLN with the highest counts retrieved as SLNs. Additionally, all blue stained and enlarged suspicious lymph nodes are removed (56).

Currently, alternative tracers are available, and compared with the dual technique, SPIO have been found to be non-inferior in multiple studies (9-12, 57-59), as has indocyanine green (60-62).

## 1.4 Accuracy of sentinel lymph node biopsy

Before the SLNB technique became fully trusted, and its comparability to ALND was established, several randomized trials were conducted to compare its accuracy with that of an ALND (21, 63, 64). In investigating a method’s accuracy, various aspects must be considered. The method should consistently yield correct results under similar conditions and should provide precise measurements or outcomes with minimal variability. Furthermore, the method should be generalizable and reproducible, have minimal bias and error, and have high sensitivity and specificity.

The accuracy of SLNB is important to gain correct prognostic information determining the status of the nodal basin. The procedure was developed to identify the subset of patients likely to benefit from the removal of occult metastases by undergoing a subsequent CLND. Morton et al. suggested that as many as 80% of the ELNDs was unnecessary and could be avoided by the

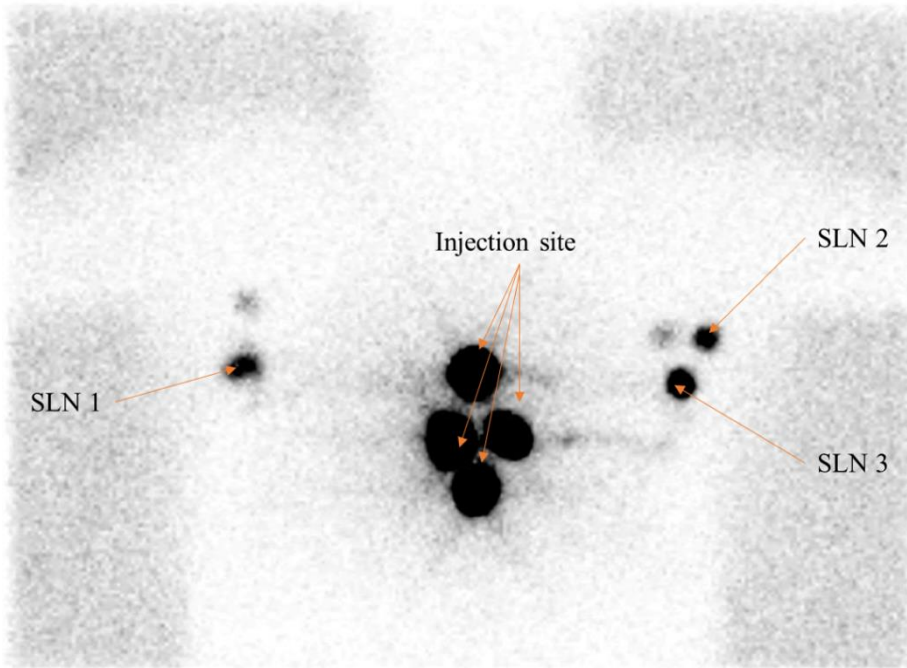
introduction of SLNB, thus potentially resulting in a similar decrease in morbidity (65). However, in a prospective multicenter study including 2,451 patients with melanoma undergoing SLNB, a false negative rate of 10.8% was reported (66). Subsequent melanoma studies have reported an approximately 3–9% risk of nodal recurrence in patients with a negative SLNs (67-69), and the false negative rate for SLNB in patients with breast cancer is in a range between 5-7% (70). The false negative rate and accuracy of a procedure, in this case SLNB, are influenced by a various of factors, including a correctly performed lymphoscintigraphy, the surgeon's experience with the procedure, a correct histopathological evaluation of the removed nodes, as well as patient-associated factors, such as advanced age and body mass index (66, 71).

## 1.5 Sentinel lymph node biopsy in melanoma

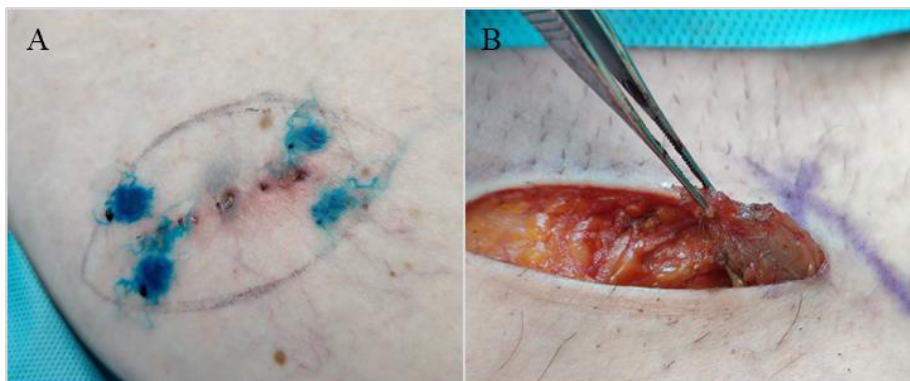
Melanoma is the most fatal form of skin cancer and is increasing in incidence worldwide; the highest reported rate is in Australia with 42 and 31 per 100,000 person-years for men and women, respectively (72). A combination of clinical and pathologic factors determines the stage and prognosis of melanoma, including Breslow tumor thickness, tumor ulceration, lymph node metastases, and distant metastasis (73-75). SLN status is an important prognostic factor for patients with primary cutaneous melanoma (76, 77). For patients with thin melanomas, Breslow thickness  $\leq 1$  mm, the likelihood of a positive SLN is approximately 5% (78, 79). Patients with intermediate thickness melanomas, 1–4 mm, have a risk of almost 15-20% (80). Finally, patients with thick melanomas,  $\geq 4$  mm, have an approximately 32–39% risk of having a positive SLN (81, 82). Current Swedish national guidelines recommend SLNB in patients with a Breslow thickness  $>1$  mm (83). For patients with a positive SLN, previously a CLND was mandated. However, two randomized studies have shown that CLND do not increase melanoma-specific survival, and the current recommendation is therefor to follow patients with ultrasounds every 4-6 months instead (31, 64).

Currently, the common clinical procedure for patients with cutaneous melanoma is to inject Tc<sup>99m</sup> intradermally at the four borders of the previous excision scar, followed by a dynamic lymphoscintigraphy, which provides pictures illustrating the lymphatic drainage to the appropriate SLN basin. This procedure is performed at a nuclear medicine department. The lymphoscintigraphy images are then reviewed to identify the lymphatic pathway to the lymphatic basin/basins, most commonly in the axilla or the

inguinal groin (Figure 1). Intraoperatively, an intradermal injection of BD is administered (Figure 2A). Before incision, the SLN is located transcutaneously with a hand-held gamma probe.



**Figure 1.** Lymphatic drainage to SLNs in both axilla from a primary cutaneous melanoma on the central back.



**Figure 2A:** BD injected intradermally at the four borders of the previous excision scar in a patient with melanoma. **B:** Blue and brown SLN in the right inguinal basin, (photograph taken by Nushin Mirzaei, with the patient's consent).

## 1.6 Sentinel lymph node biopsy in breast cancer

Breast cancer is the most frequently diagnosed cancer and the primary cause of cancer-associated deaths, among women. In Australia/New Zealand and northwestern Europe, breast cancer incidence rates exceed 90 per 100,000, whereas in Eastern and Central Africa, the incidence is below 30 per 100,000 (84). Breast cancer prognosis depends on a combination of clinical and pathological factors, including patients' age, tumor stage, tumor type, margin status, and lymphovascular involvement. Furthermore, biological factors, such as estrogen and progesterone receptor status, human epidermal growth factor receptor 2 (HER2) expression, and the level of Ki67, also influence prognosis and treatment response. Also SLN status has an important prognostic value in early-stage breast cancer (85, 86).

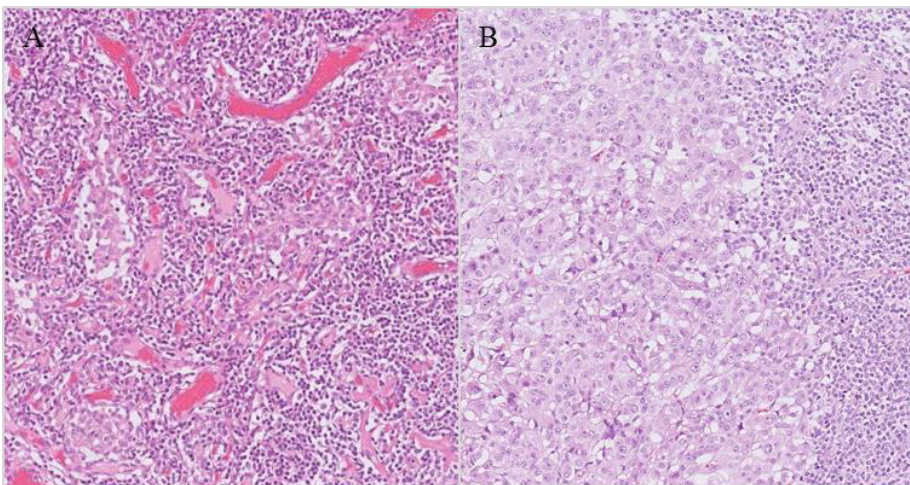
Historically, radical lymph node dissection was performed in all patients with breast cancer (87), but was associated with chronic arm morbidity and impaired quality of life (88, 89). The Milan trial and the NSABP-B23 trial were groundbreaking in establishing SLNB in breast cancer (22, 85). SLNB is mainly used for staging breast cancer in patients with clinically and ultrasonographically node-negative axilla (90). SLN status gives important information when recommending adjuvant treatments. For patients with less than three positive SLN, the ACOSOG Z0011 and SENOMAC trial has shown that a ALND is not mandated (21, 33). Furthermore, the AMAROS and the OTOASAR trials have demonstrated that adjuvant radiotherapy to the axilla, instead of a ALND, yields comparable recurrence rates with reduced morbidity (91, 92). Currently, the Swedish guidelines recommend SLNB for all patients with invasive breast cancer (39).

## 1.7 Histopathological staging of sentinel lymph nodes

The histopathological analysis of SLNs in breast cancer involves serial sectioning, wherein thin sections are cut, stained with hematoxylin and eosin (H&E), and examined under a microscope. This method enables pathologists to assess the microscopic structures of tissues for diagnostic purposes. This process generates numerous small tissue volumes for analysis, and therefore is both time-consuming and expensive. The histopathological assessment of

SLNs in melanoma differs from that in breast cancer. In melanoma, also immunohistochemical staining for melanoma-associated antigens such as S100, MART-1, and HMB45 is included (93). The immunohistochemical staining for these specific proteins is primarily used to confirm the presence of melanoma cells in SLNs and assess their molecular characteristics (94).

In breast cancer, macro-metastases are defined as metastasis larger than 2.0 mm, and this can typically be identified with a single H&E section (Figure 3). If macro-metastases are detected, no further sectioning is necessary. However, if no macro-metastases are found, additional thinner sections are examined to ensure thorough examination of the presence of metastases. Micro-metastases are defined as metastases ranging from 0.2 to 2.0 mm in size, whereas isolated tumor cells are metastases smaller than 0.2 mm in size (95). These distinctions are crucial for accurate diagnosis and treatment planning. Several studies have reported no statistical difference in axillary recurrence when ALND was not performed in patients with micro-metastasis (96-98). The first results of the Swedish prospective multicenter trial SENOMIC reported low axillary recurrence and an estimated 3-year event-free survival rate of 96.2% when ALND was not performed in patients with micro-metastases (99). The Swedish guidelines do not recommend ALND for patients with SLN involvement containing isolated tumor cells or micro-metastasis (39).



**Figure 3.** Histological sections of SLNs with or without macro-metastases in a patient with breast cancer. **A:** SLN without metastases (H&E staining). **B:** SLN with metastatic breast cancer (H&E staining).

Unlike breast cancer, in melanoma is a macro-metastasis defined as a clinically detected lymph node, whereas a micro-metastasis is defined as a lymph node metastasis detected through a SLNB. If the lymph node contains even one cancer cell, it is regarded as a positive SLN.

## 1.8 Morbidity after axillary surgery

Lymph node dissection is associated with long-term morbidity, including numbness, pain, lymphedema, weakness, and impaired range of motion and short-term morbidity, such as infection and wound healing (65, 100).

Although the era of SLNB began in patients with melanoma, short- and long-term morbidity after axillary surgery was studied predominantly in patients with breast cancer. The ALMANAC trial demonstrated better quality of life in patients undergoing SLNB compared to ALND (level I–III or sampling of four nodes) after 18-months of follow-up (101). The prolonged duration of arm swelling, and numbness significantly affected the patients' quality of life, whereas movement limitations and pain decreased over time (101). NSABP B-32 evaluated arm morbidity in clinically node negative patients with breast cancer randomized to SLNB or level I–III ALND. After a 3-year follow-up the study reported more pain induced shoulder abduction, larger arm volume, and more problems with numbness with ALND compared to SLNB (102).

Although SLNB decreases the risk of complications to a greater extent than ALND, the procedure is not free from complications. The preliminary results from the SOUND study, which randomized patients with breast cancer without clinically nor ultrasound suspected lymph node metastases to either SLNB or observation, has indicated significantly lower arm disability in the observation group (103). The commonly described SLNB morbidities are discomfort, shoulder dysfunction, and sensory changes. Other frequent complications include seroma, hematoma, and wound infection. These complications are observed at comparable frequencies between patients with melanoma and breast cancer (104-106). The ACOSOG Z0010 clinical trial reported 1.0% wound infections, 1.4% axillary hematomas, and 7.1% axillary seromas as 30-day postoperative morbidities associated with SLNB (106). Moreover, significantly higher wound infection and seroma rates were observed in patients with more than five SLNs removed. In the same study, 6 months after surgery, 10.4% of the younger patients (40–49 years old) reported having axillary paresthesia (106). Furthermore, BMI and age were associated with the

incidence of lymphedema, which was reported to be 4–7% after SLNB (105, 107).

## 1.9 Superparamagnetic iron oxide nanoparticles

SPIO are a type of ferromagnetic material known for its magnetism. Due to their nanoscale size (approximately 60 nm) and magnetic properties, they have garnered substantial interest in the biomedical field. SPIO have been used as an intravenous contrast agent for MRI (18). Superparamagnetic nanoparticles can become magnetic in the presence of a magnetic field and then demagnetize when the field is removed. SPIO can be used together with a hand-held magnetic probe as a tracer for SLNB. The nanoparticles are coated with carboxydextran molecules, which facilitate stabilization and migration of the particles to SLNs, where they become trapped.

SPIO-enhanced MRI has shown promising results, with an accuracy of 95% for diagnosis of SLN metastases in a small trial (19). When SPIO accumulate in a benign node, the particles induce a decrease in signal intensity. Thus, malignant nodes exhibit focal high signal intensity (19). A non-invasive diagnostic tool such as SPIO-enhanced MRI could potentially play an important role in SLN detection in the future, thus decreasing unnecessary SLNB procedures, diminishing physical and psychological morbidity in patients, and lowering treatment costs.

However, SPIO can induce MRI artefacts manifested as signal void areas around the injection site. In patients with melanoma, the injection site is excised in a wide local excision procedure, but for patients with breast cancer, depending on the injection site and the depth, SPIO can obscure adjacent structures and compromise the overall image quality. Therefore, it is essential to acknowledge these MRI limitations to avoid potential diagnostic challenges if breast MRI is necessary. Nonetheless, the issue of MRI artefacts due to SPIO can be addressed by excising the tissue containing the nanoparticles.

## 2. AIMS

The primary objective of this thesis was to assess the role of SPIO as a tracer for detecting SLNs in patients diagnosed with cutaneous melanoma and breast cancer. The specific aims included the following:

- I To evaluate if it is feasible to use an ultra-low dose of SPIO injected intra-dermally to identify SLNs compared to the standard method of using Tc<sup>99m</sup> and BD, and to evaluate whether SPIO-enhanced MRI can predict SLN status in patients with cutaneous melanoma.
- II To evaluate if it is feasible to use an ultra-low dose of SPIO injected intra-dermally to identify SLNs in patients with breast cancer compared to the standard method using Tc<sup>99m</sup> and BD.
- III To evaluate if an ultra-low dose of SPIO injected intra-dermally in patients with breast cancer creates SPIO related breast MRI artefacts affecting diagnostic interpretation after breast conserving surgery, and if SPIO causes skin discoloration and if this has any potential impact on quality of life.
- IV To evaluate SLN detection comparing SPIO and Tc<sup>99m</sup> and BD, and to examine the influence of various SPIO doses, injection sites, and timing on SLN detection and the amount of iron nanoparticles in the retrieved lymph nodes.

## 3. PATIENTS AND METHODS

### 3.1 Overview

*Table 1. Papers included in this thesis and their respective study design*

PAPER	DESIGN	POPULATION	ENDPOINT
<b>I</b>	Prospective feasibility study	Cutaneous melanoma n = 15	SLN detection
<b>II</b>	Prospective feasibility study	Breast cancer n = 50	SLN detection
<b>III</b>	Prospective feasibility study	Breast cancer n = 38	MRI artefacts Skin discoloration
<b>IV</b>	Retrospective multicenter individual patient meta-analysis	Breast cancer n = 908	SLN detection SLN iron content

Abbreviations: SLN, sentinel lymph node; MRI, magnetic resonance imaging

### 3.2 Paper I

#### 3.2.1 Study design

A prospective single center feasibility study evaluating SPIO as a tracer for detecting SLN in patients with cutaneous melanoma. The study additionally evaluated the potential of SPIO-enhanced MRI as a tool for determining SLN status.

#### 3.2.2 Selection and patient inclusion

Between January 2018 and September 2020, all patients with histologically confirmed cutaneous melanoma on the extremities who were deemed suitable for wide local excision and SLN biopsy were included. Staging was based on the American Joint Committee on Cancer 8th edition cancer staging manual.

### 3.2.3 Data collection

The day before surgery, a baseline MRI of the nodal basin was conducted and was followed by administration of SPIO. Four hours later, a second MRI (SPIO-enhanced MRI) was performed to visualize magnetic SLNs and to assess the feasibility of predicting SLN status. Subsequently, in accordance with clinical routine,  $Tc^{99m}$  was administered, followed by a lymphoscintigraphy. Patients were divided into three cohorts according to the net volume of SPIO administered.

After the onset of anesthesia, BD was administered according to clinical routine. During surgery, all procedures started with the magnetic technique, and the radioactive technique was only used to verify the removed nodes and to verify that no radioactive nodes had not been removed. Intraoperative BD served as a complementary method for SLN detection. All SLNs exhibiting magnetic and/or radioactive counts and/or blue staining were retrieved and sent for histopathological analysis. After surgery, two radiologists unaware of the histopathological outcomes regarding the presence of tumor cells in SLNs evaluated the MRI to determine SLN status.

### 3.2.4 Endpoint

The primary endpoint was SLN detection rate, defined as number of SLN detected by SPIO compared to  $Tc^{99m} \pm BD$ . The secondary endpoint was to determine the false negative rate and false positive rate for sentinel lymph node staging using SPIO-MRI.

## 3.3 Paper II

### 3.3.1 Study design

A prospective, single-arm, dose-escalation study evaluating SPIO as a tracer for detecting SLN in women with breast cancer was conducted. A dose escalation protocol was established for SPIO at 0.1 mL, 0.25 mL, and 0.5 mL, with each dose administered to a minimum of five patients. If four or more SLN procedures were successful, further dose escalation was not pursued.

### 3.3.2 Selection and patient inclusion

Between November 2021 and January 2023, women with histologically confirmed, clinically lymph node negative breast cancer planned to undergo elective breast-conserving surgery and SLN biopsy were included.

### 3.3.3 Data collection

Intradermal injection of SPIO was administered within 7 days before the day of surgery. In accordance with the clinical protocol, Tc<sup>99m</sup> was administered on either the day before surgery or the day of surgery. During the surgical procedure, after the onset of anesthesia, BD was administered according to clinical routine. During surgery, the magnetic technique was used as the initial approach, and the dual method, including preoperative administration of Tc<sup>99m</sup> and optional intraoperative BD, was only used to verify the removed nodes and to verify that no radioactive nodes had not been removed. The magnetic and radioactive counts in the axilla and breast were recorded before incision and after surgery. All SLNs with magnetic and/or radioactive counts and/or blue staining were retrieved and sent for histopathological analysis.

### 3.3.4 Endpoint

The primary endpoint was sentinel lymph node detection, defined as the number of SLN detected by SPIO compared to Tc<sup>99m</sup> ±BD.

## 3.4 Paper III

### 3.4.1 Study design

This paper presents a pre-planned analysis of the secondary endpoints in the trial presented in paper II, evaluating SPIO related skin staining or breast MRI artefacts after breast conserving surgery.

### 3.4.2 Selection and patient inclusion

Between November 2021 to January 2023, with follow-up until January 2024, women with histologically confirmed, clinically node-negative breast cancer undergoing elective breast conserving surgery and SLN biopsy were included.

### 3.4.3 Data collection

Skin discoloration was prospectively followed up by photography and measurements at four timepoints: baseline after SPIO injection, and 1, 6, and 12 months after surgery. The SPIO artefacts in the operated breast were evaluated by MRI at two timepoints: 6 and 12 months after surgery.

The assessment of SPIO-associated MRI artefacts was conducted using a non-validated, pre-defined, six-level ordinal scale as follows: grade 0 = no artefacts, grade 1 = artefact visible in the subcutaneous tissue, good diagnostic quality; grade 2 = artefact <5 mm in the fibroglandular tissue (FGT), good diagnostic quality; grade 3 = artefact 5–10 mm in FGT, readable with minimally impaired clinical assessment; grade 4 = artefact 11–30 mm in FGT, readable with slight impaired clinical assessment; grade 5 = artefact >30 mm in FGT, impaired clinical assessment. This grading system was used since no validated scale existed for assessing MRI artefacts associated with SPIO.

The non-validated and modified Skin Discoloration Impact Evaluation Questionnaire (SDIEQ) was used to examine patient reported outcomes. The questionnaire uses a four-level ordinal scale as follows: 0 = not at all, 1 = a little, 2 = a lot, and 3 = very much.

### 3.4.4 Endpoint

The primary endpoint was the presence of SPIO MRI artefacts at 6 and 12 months after surgery. The secondary endpoint was the presence of SPIO-related skin discoloration at one month, six months, and 12 months after surgery, as well as patient reported outcomes using SDIEQ.

## 3.5 Paper IV

### 3.5.1 Study design

A retrospective, multicenter, individual patient level meta-analysis including data from clinical trials conducted in the Netherlands (three trials) and Sweden (five trials). Individual patient data were collected to evaluate how different SPIO doses, injection sites, and timeframes affected SLN detection. Additionally, the iron content in the retrieved SLNs was evaluated.

### 3.5.2 Selection and patient inclusion

Data from the individual patients enrolled in were collected. In each trial, a combination of an SPIO tracer and a magnetic detector, alongside a gamma probe in cases using  $Tc^{99m}$ , was used for the detection of SLNs in patients with breast cancer scheduled for breast conserving surgery and SLN biopsy.

### 3.5.3 Data collection

The data were collected according to the following criteria: injection dose (<0.5 mL, 1 mL, 1.5 mL, and 2 mL), injection site (intra-tumoral, peri-tumoral, subareolar, and peri-areolar), and time interval between injection and surgery (0–2 hours, >2–36 hours, >36–168 hours, and >168 hours). Additionally, the iron content in the retrieved SLNs was quantified according to a predefined look-up table.

### 3.5.4 Endpoint

The primary endpoint was to evaluate SLN detection comparing SPIO with  $Tc^{99m} \pm BD$ . The secondary endpoint was to quantify the amount of iron present in the retrieved SLNs.

## 3.6 Statistical analysis

The primary aim of this thesis was to assess SLN detection by using a low dose of SPIO as a tracer. Accordingly, the data are presented predominantly descriptively, including reporting the mean, standard deviation, median, and interquartile range. In this way, patient characteristics and the distribution of the data are delineated to identify any inconsistencies, such as outliers or missing data. When the data were normally distributed, the mean and standard deviation are presented, whereas for non-normally distributed data, the median and interquartile range are presented.

In **paper I**, the proportion of SLNs incorrectly identified as negative, i.e., non-metastatic by MRI, was calculated by dividing the number of false negative SLNs by the sum of true positive and false negative SLNs (false negative rate), whereas the proportion of SLNs incorrectly identified as positive, i.e., metastatic according to MRI, was calculated by dividing the false positive SLNs by the sum of false positive and true positive SLNs (false positive rate).

The false negative rate is highly relevant when a negative outcome from a diagnostic test could result in a missed diagnosis or insufficient treatment. Overall, the false negative rate provides valuable understanding of the reliability and performance of diagnostic tests, screening tools, and detection systems. The correlation among the three SPIO dose cohorts and the artefacts within the SLNs was calculated using the Spearman rank-order correlation coefficient, given that the data were ordinal and continuous. The chi-square test was used for comparing differences between categorical variables, i.e., the proportion of SLNs detected by lymphoscintigraphy versus MRI.

In **paper II**, the agreement between the magnetic and radioactive techniques was determined by calculation of the concordance and reverse concordance. Concordance was estimated by division of the number of SLNs positive for both SPIO and  $Tc^{99m} \pm BD$  by the total number of SPIO-positive SLNs. Reverse concordance was defined as the number of SLNs positive for both  $Tc^{99m} \pm BD$  and SPIO divided by the total number of  $Tc^{99m} \pm BD$  -positive SLNs.

In **paper III**, the significance of differences in the area ( $cm^2$ ) of skin discoloration, across various timepoints was determined with the non-parametric Friedman test. This choice was made due to the non-normal distribution of the data and the need to test multiple measurements of skin discoloration at different timepoints. The probability value of the association between the observed frequency of patients with skin discoloration within the categorical timepoints was determined with Fisher's exact test. The independent groups of patients with skin discoloration either removed or not removed were compared with the non-parametric Mann-Whitney U test, to determine whether residual SPIO counts at the injection site after surgery depended on whether the skin discoloration was removed. The frequency of MRI artefacts is presented in percentages.

In **paper IV**, the association between the observed frequency distribution of the categorical variables of SPIO doses, injection site, and injection timeframe were determined with the chi-square test. The independent group of SLNs with or without metastasis were compared with the Mann-Whitney U test. The purpose of this analysis was to determine whether the quantified iron content in SLNs was dependent on the existence SLN metastases.

## 3.7 Methodological considerations

In feasibility studies, several methodological considerations must be considered to ensure accuracy, reliability, and utility. The endpoints and scope of the feasibility study must be clearly defined to ensure that all relevant aspects are adequately addressed. Furthermore, before the study is conducted, a thorough literature review is necessary to gain understanding of existing knowledge, knowledge gaps in similar studies, potential challenges, and best practices associated with the project's feasibility. The main aim of a feasibility study is to assess the practicality, viability, and potential success of a proposed project, venture, or intervention, to determine whether the project is technically, economically, legally, and operationally feasible.

A well-formulated null hypothesis is an important first step allowing researchers to design and structure the study according to the research endpoint and available resources. To simplify the assessment of the study endpoint, an adequate sample size representative of the study population is chosen for performing analyses, thus indicating whether the proposed project is feasible and meets the criteria for proceeding to the next stage of implementation. Furthermore, an appropriate valid and reliable data collection method should be established, while ensuring that ethical considerations are met.

Planning for follow up is crucial as dropout can impact the results. Numerous challenging factors must be considered, including time and resource allocation, scheduling appointments, and coordination among the participants to ensure patient compliance. Overall, the demanding nature of follow-up procedures underscores the importance of a patient-centered approach, effective communication, and coordinated care delivery to optimize patient outcomes and experiences.

Documentation is critical to provide transparency in the context of any uncertainties, constraints, or limitations that might affect the findings of the feasibility study.

In data analysis, several aspects should be considered to ensure the accuracy, reliability, and utility of the findings; e.g., the distribution of the data for each variable of interest. The data distribution determines which analytical method is appropriate for comparison of relevant variables or factors, as well as for the correlation analysis. Potential confounding factors should also be considered. The interpretation of the results should consider any strengths or limitations in the discussion of the implications of the findings for future studies.

## 4. SUMMARY OF RESULTS

### 4.1 Paper I

SLN detection with SPIO was demonstrated to be feasible in all three cohorts of SPIO doses in patients with cutaneous melanoma, with a detection rate of 100%. Notably, the lowest SPIO dose was associated with fewer MRI artefacts in the axilla, thus simplifying the evaluation of SLNs in the axilla for prediction of potential status. However, one patient had a false positive prediction, another patient had a false negative prediction, and two patients had true positive predictions, resulting in a FNR of 50% and a FPR of 13%. No adverse effects were reported.

### 4.2 Paper II

A SLN was detected in the initial four patients receiving an ultra-low dose of 0.1 mL SPIO. Due to this successful outcome, any dose escalation was not required according to protocol, and all subsequent patients also received a dose of 0.1 mL SPIO. Detection of SLNs with a dose of 0.1 mL SPIO was demonstrated to be feasible in all 50 patients undergoing breast conserving surgery. In total, 98 SLNs were harvested, 90 SLNs were detected by SPIO. Among these, 80 SLNs were also found to be Tc<sup>99m</sup>±BD positive. Of the 88 SLNs detected by Tc<sup>99m</sup>±BD, 80 SLNs were also found to be SPIO positive resulting in a concordance of 89% and a reverse concordance of 91%.

### 4.3 Paper III

The ultra-low dose intradermal injection of SPIO was not associated with grade 5 MRI artefacts impairing diagnostic assessment of the glandular tissue of the breast. Table 2 illustrates the frequency of MRI artefacts at the 6 and 12 month MRI follow-up. The area of skin discoloration due to the intradermal injection of 0.1 mL SPIO was measured at baseline, and 1, 6, and 12 months after surgery. The median areas were as follows: 0.20 cm<sup>2</sup> (IQR 0.20–0.57), 0.72 cm<sup>2</sup> (IQR 0.20–1.61), 0.75 cm<sup>2</sup> (IQR 0.14–1.77), and 0.79 cm<sup>2</sup> (IQR 0.14–1.41), with  $p = 0.36$ . No cosmetic concerns associated with the skin discoloration were reported at 12 months' follow up.

**Table 2.** Frequency of MRI artefacts after 6- and 12-month follow-up in T1- and T2-weighted MRI images.

Grade	6 month MRI n = 37		12 month MRI n = 37	
	T1 weighted	T2 weighted	T1 weighted	T2 weighted
0	1/37 (2.7%)	1/37 (2.7%)	1/37 (2.7%)	2/37 (5.4%)
1	20/37 (54.1%)	22/37 (59.5%)	19/37 (51.4%)	20/37 (54.1%)
2	7/37 (18.9%)	9/37 (24.3%)	7/37 (18.9%)	9/37 (24.3%)
3	4/37 (10.8%)	3/37 (8.1%)	6/37 (16.2%)	5/37 (13.5%)
4	5/37 (13.5%)	2/37 (5.4%)	4/37 (10.8%)	1/37 (2.7%)
5	0/37 (0.0%)	0/37 (0.0%)	0/37 (0.0%)	0/37 (0.0%)

Grade: 0 = no artefacts; grade 1 = artefact visible in the subcutaneous tissue, good diagnostic quality; grade 2 = artefact <5 mm in FGT, good diagnostic quality; grade 3 = artefacts >5–10 mm in FGT, readable with minimally impaired clinical assessment; grade 4 = artefact >11–30 mm in FGT, readable with slight impaired clinical assessment; grade 5 = artefact >30 mm in FGT, impaired clinical assessment.

Abbreviations: MRI, magnetic resonance imaging; T1, longitudinal relaxation time; T2, transverse relaxation time

## 4.4 Paper IV

The pooled analysis of individual patient data from eight clinical studies demonstrated high patient-based and SLN-based detection with SPIO compared with Tc<sup>99m</sup> ± BD (96.9% vs 94.3%,  $p = 0.01$  and 92.9% vs 88.7%,  $p < 0.001$ ) (Table 3). The dose of 1.0 mL SPIO was preferable in both patient-based and SLN-based detection. The intra-tumoral injection site was associated with the lowest SLNs detection, and SPIO administered more than 2 hours before surgery was associated with better patient-based SLN detection. The quantified iron content was higher in metastatic SLNs than non-metastatic SLNs, but the difference was not statistically significant.

Superparamagnetic iron oxide for sentinel lymph node biopsy

**Table 3.** Patient- and SLN-based detection, using SPIO, by injection site, injection dose, and timeframe.

	Patient-based detection		p-value*	SLN-based detection		p-value*
<b>Whole population</b>			p = 0.01			p <0.001
SPIO	841/868	96.9%		1535/1653	92.9%	
Tc <sup>99m</sup>	679/720	94.3%		1307/1473	88.7%	
<b>Dose</b>	<b>n = 908</b>		p = 0.09	<b>n = 1653</b>		p <0.001
0.04–0.5 mL	103	94.5%		155	86.1%	
1.0 mL	171	98.8%		342	97.4%	
1.5 mL	157	96.3%		293	87.5%	
2.0 mL	437	94.4%		745	94.7%	
<b>Injection site</b>	<b>n = 908</b>		p <0.04	<b>n = 1653</b>		p <0.001
Intra-tumoral	44	89.8%		55	80.9%	
Peri-tumoral	96	99.0%		190	94.5%	
Subareolar	414	94.7%		716	92.5%	
Peri-areolar	314	96.6%		574	94.1%	
<b>Timeframe</b>	<b>n = 818<sup>#</sup></b>		p = 0.28	<b>n = 1463<sup>†</sup></b>		p = 0.8
0–2 h	366	95.1%		653	93.0%	
>2–36 h	113	98.3%		183	92.9%	
>36–168 h	189	96.4%		346	94.3%	
>168 h	114	93.4%		186	94.4%	

\*Chi-square test

<sup>#</sup>missing data for 90 patients

<sup>†</sup>missing data for 190 SLNs

## 5. DISCUSSION

The results of this thesis shed light on questions regarding the use of SPIO as a tracer for SLN detection, aimed at refining the technique. The safety and feasibility of using a lower dose of SPIO was demonstrated for both patients with breast cancer and cutaneous melanoma. Also, SPIO-enhanced MRI was found to potentially be able to predict SLN status, thereby advancing non-invasive diagnostic capabilities. Notably, the ultra-low dose of 0.1 mL SPIO contributed to minor negligible skin discoloration without posing any cosmetic inconvenience, and also minimized MRI artefacts in most patients, thus resulting in minimal effects on the diagnostic interpretation of the breast examination findings.

Before implementation of a new technique in clinical practice, various considerations are necessary. The safety of the intervention, i.e., the de-escalated dose examined herein, is paramount. The results presented in paper I indicated that a dose as low as 0.02 mL SPIO was sufficient for detecting SLNs in patients with cutaneous melanoma. Indeed, the lowest dose could potentially play a role in non-invasive SLN diagnostics with SPIO-enhanced MRI. Further, we achieved 100% SLN detection across all three dose cohorts, but this finding has to be demonstrated in a larger trial. Beyond demonstrating the feasibility of SLN detection with SPIO as a tracer, the study's further contributions included the establishment of a structured framework for facilitating future use of SPIO in combination with MRI. Building on these results, a larger phase II trial is planned, the MAGMEN-II trial, which may serve as a cornerstone for transforming clinical practice.

Transforming clinical practice by introducing new methods requires thorough clinical research. The limitations of the new method and areas to improve efficacy, while maintaining safety, must critically be identified. Furthermore, demonstrating that the new method is non-inferior to the conventional approach is crucial. The results in paper II illustrated that SLN detection in patients with breast cancer was feasible with 0.1 mL SPIO. The magnetic technique was easily implemented, without necessitating complex logistical coordination in scheduling the surgical procedures. SPIO was administered in the outpatient clinic, thereby eliminating the need for access to a nuclear medicine department and thereby overcoming a frequent restriction in accessibility to the radioactive method, especially in developing countries. Moreover, patients were spared exposure to radiation. Based on the overall

positive experience described above, the need to investigate the non-inferiority of 0.1 mL become apparent. The comprehensive experience gained has laid the groundwork for a larger national study which could potentially change practice.

Refinement of a technique and its adverse effects is essential before implementation, to minimize or eliminate potential adverse effects associated with the new method. Additionally, ensuring the well-being of the participants and upholding ethical standards throughout the research process are crucial. The two major side effects of SPIO, skin discoloration and MRI artefacts were addressed in this thesis. The results in paper III demonstrated that patients receiving intradermal injection of 0.1 mL SPIO showed only minimal skin discoloration through the 12 months follow-up, which did not influence the cosmetic outcomes. In this study, compared with other studies injecting patients with 1–2 mL SPIO (15, 16), the skin discoloration was limited to a smaller area (Figure 4 and 5).



**Figure 4.** Skin discoloration follow-up in a woman after intradermal injection of 0.1 mL SPIO (108).

The skin discoloration at the 10 o'clock position in the 1-month photo is due to BD.



**Figure 5.** Skin discoloration in a woman after peri-areolar injection of 2 mL SPIO diluted with 3 mL saline (16).

Photos from an earlier series at Uppsala Academic Hospital.

Even if a 0.1 mL dose of SPIO induced MRI artefacts, these MRI artefacts were primarily confined to the subcutaneous tissue and extended no more than 30 mm into the FGT, thereby enabling clear diagnostic interpretation of most of the breast tissue. Through structured monitoring and regular photographic documentation, valuable observations were made regarding both skin discoloration and MRI artefacts. Patients who received a precise intradermal SPIO injection tended to exhibit skin discoloration, whereas those who received a deeper subcutaneous injection showed no such discoloration. These differences have implications regarding MRI artefacts in patients experiencing skin discoloration exhibiting superficial artefacts compared with those without such discoloration. These findings underscore the need for further refinement of this method, by considering the injection technique. Time and diligent assessment of the learning curve are key factors for achieving flawless execution of a technique and optimal outcomes.

To optimize the assessment of a procedure's effectiveness and identify areas for improvement, data must be gathered to establish a benchmark for comparison. When collecting data associated with the procedure and its outcomes for comparison, appropriate measurement tools are essential to prevent biases that might affect the results. Furthermore, interpreting outcomes with non-validated instruments can be challenging, since their validity has not been tested to ensure their applicability across different populations, contexts, and settings. This is a limitation in paper III, but since no tools were available, these tools can now be used in future research projects asking the same questions.

The results of paper IV provided interesting and valuable data regarding the optimal dose, injection site and timeframes for the use of SPIO as a tracer for SLN detection through a comparison of individual patient data pooled from eight clinical studies. In the context of patient-based detection, the 1.0 mL dose achieved a higher rate of SLN detection (98.8%) than the other doses; the intratumoral injection site was associated with the lowest SLN detection (89.8%); and the optimal injection timeframe before surgery was more than 2 hours. A recent meta-analysis including 20 studies comparing SPIO to  $Tc^{99m} \pm BD$  has reported no discernible differences in SLN detection across injection doses or injection sites. However, the findings suggested that injecting SPIO more than 1 day before surgery might enhance SLN detection (109). Pooled data from multiple studies in meta-analyses can provide a comprehensive understanding of the research question and increase statistical power. However, the pooled studies may be subject to potential biases due to methodological differences

between studies, which must carefully be considered and addressed when interpreting results. Since the distribution of SPIO dosage, injection site, and timing remained consistent across the included studies, no significant definitive interpretation was made in terms of identifying optimal parameters. Regardless of the outcomes of paper IV, the findings contributed insights, data, and observations in this field that may serve as building blocks for future research.

## 6. ETHICAL CONSIDERATIONS

All studies were conducted in accordance with the Declaration of Helsinki. Papers I–III were approved by the regional Ethical Board of Gothenburg and were registered at [clinicaltrials.gov](https://clinicaltrials.gov) before the start of patient recruitment. Approval from the Swedish Medical Product Agency (MPA) was also obtained for papers II and III, this was not deemed to be necessary by the MPA for the MAGMEN study reported in Paper I. All patients provided written and signed informed consent. Paper IV was approved by the Netherlands Ethical Review Authority and the Swedish Ethical Review Authority.

Several considerations must be made to maintain ethical principles when conducting clinical trials. It is paramount that participants are fully informed about the study's nature, potential risks, and benefits, with thorough oral and written consent obtained. Researchers must ensure that the benefits of the study outweigh the risk for the participants. Inclusion of participants should be fair and equitable, without discrimination based on race, ethnicity, gender, or socioeconomic status. Additionally, the privacy and confidentiality of the participants' data must be protected to maintain their integrity. The participants should have the right to withdraw from the study at any time, and their decision should be respected. Furthermore, a scientifically well-conducted study design must be ensured, resulting in a valid report that includes both positive and negative outcomes comprehensively. Ethical review boards play an important role in approving the studies and making sure that they meet the ethical standards and protect the participants' rights. Continuous monitoring throughout the study is essential to ensure that the ethical aspects remain unchanged.

## 7. CONCLUSIONS

- I Detection of SLN was demonstrated to be feasible with an ultra-low dose of SPIO injected intradermally in patients with cutaneous melanoma. Notably, SPIO-enhanced MRI could potentially predict SLN status and be used as a non-invasive modality in the future.
- II Detection of SLN was demonstrated to be feasible with an ultra-low dose of 0.1 mL SPIO injected intradermally in patients with breast cancer.
- III An ultra-low dose of SPIO injected intradermally resulted in minimal MRI artefacts, thus enabling the diagnostic interpretation of most fibroglandular tissue in the breast. Additionally, the ultra-low dose of SPIO led to minor skin discoloration not impacting patients' quality of life.
- IV Detection of SLN with SPIO could potentially be more effective compared to Tc<sup>99m</sup> and BD. However, the preferable SPIO dose, injection site and timeframe for SLN detection, and the amount of iron nanoparticles in the lymph nodes, has to be further investigated, although an early and an intra-tumoral injection of SPIO seemed less effective.

## 8. FUTURE PERSPECTIVES

The surgical approach for lymph node assessment has evolved over the past century, with the aim of de-escalating surgical interventions to minimize unnecessary procedures and to avoid complications. In general, traditional open surgeries performed through large incisions are continually being replaced by minimally invasive surgery, thus leading to faster recovery time, and decreasing post-operative complications. Individual tailoring of treatments according to tumor biology characteristics, along with multidisciplinary decisions grounded in evidenced based treatments, has increased cancer-specific survival.

The development of the SLNB procedure aimed to spare the node- negative patients from lymph node dissections and its associated morbidity. However, also the necessity of lymph node dissections in patients with positive SLN was questioned in the Z-0011 trial, which suggested that the node-positive patients did not significantly benefit from additional surgical intervention. This prompts consideration of whether SLNB is truly necessary when preoperative imaging shows no evidence of metastasis in SLN staging.

Furthermore, advancements in imaging technologies, such as MRI, have enhanced tumor visualization. In paper I, we demonstrated that SPIO-enhanced MRI could potentially serve as a non-invasive modality to predict SLN status in vivo. Looking ahead, the integration of advanced techniques, such as machine learning algorithms and artificial intelligence into analysis of imaging data may play an important role in staging procedures. These innovations could offer valuable guidance for surgeons or surgical oncologists in determining patient tailored treatment strategies.

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