MAGNETIC

SENTINEL LYMPH NODE DETECTION AND METASTASES EVALUATION

CONFIDENTIAL

Master Thesis

Anke Christenhusz
University of Twente, Enschede
September 29th, 2017

UNIVERSITY OF TWENTE.

Magnetic sentinel lymph node detection and metastases evaluation.

Anke Christenhusz
Technical Medicine
Faculty of Science and Technology
University of Twente, Enschede, The Netherlands

Exam committee:

Prof. dr. ir. C.H. Slump
MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede

Dr. J.M. Klaase
Department of Surgery, Medisch Spectrum Twente, Enschede

Dr. ir. B. ten Haken
MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede

Dr. J.J. Pouw
MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede

Drs. A.G. Lovink
University of Twente

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Abstract

Background: In the Netherlands, 14.500 people receive the diagnosis of breast cancer annually and one out of five will develop metastases. For a good prognosis, early detection of metastases is extremely important. The current clinical practice for sentinel lymph node biopsy (SLNB) involves a combined detection procedure, using locally injected blue dye and a radioactive tracer. However, the use of radioactive tracers is a major disadvantage. A promising radioisotope free technique for SLNB is associated with the use of an interstitial injection of Super Paramagnetic Iron Oxide (SPIO). The use of a low dose SPIO tracer for SLNB, and the potential of using pre-operative MRI for non-invasive metastases detection will be investigated in this thesis.

Methods: Patients with histologically confirmed invasive breast cancer received an intra-tumoral injection with SPIO tracer. The SLN was intraoperatively detected according to the normal protocol and with the Sentimag® magnetic probe. Imaging was perfomed using a 3T MR system.

Results: In order to perform a complete magnetic sentinel node detection and evaluation procedure, an intra-tumoral injection of 2 mg iron should be perfomed, 1 day before surgery. A pre-operative MRI will be performed prior to surgery to evaluate the lymph nodes on the presence of metastases. If the iron distribution across the lymph node gives no suspicion for metastases, the SLNB can be avoided. In case of doubt, the SPIO in de lymph nodes can be used for intra-operative detection, after which histopathological research will follow. The SPIO tracer in the tumor can also be used to detect occult breast lesions, so a wire localization (lot of complications) or iodine seed (radiation) is no longer necessary.

Discussion: By increasing the amount of iron, the successful intra-operative detection rate of the sentinel node will probably improve, but its now known that a high dose of iron causes a disturbance on pre- and post-operative MRI diagnostics by the presence of large artefacts. If a transcutaneous measurement fails due to the low dose of iron, the pre-operative MRI can be used to determine the incision site. It seems that the own magnetism of the human body creates the largest disturbance during the current measurements with the Sentimag Magnetometer. It would be a great addition to examinate the Diffmag magnetometer (Utwente) for the SLNB, which not respond to the human body's diamagnetic signal and can also be used in combination with the surgical equipment.

1. General Introduction

In the Netherlands, 14.500 people receive the diagnosis of breast cancer annually. Breast cancer can occur in several ways. In most cases, it develops from the mammary ducts (ductal mamma carcinoma) and from the mammary glands (lobular mamma carcinoma). In some cases there is a genetic predisposition.

One out of five women with breast cancer develop metastases, but in only one in twenty of these women, these metastases are diagnosed during the initial examinations. Metastases usually appear later, often even years after the primary tumor has been treated. The prospects of patients where the metastases are diagnosed at a later moment in the course are poor. Curing the disease is then often no longer possible. Depending on the aggressiveness of the breast cancer this terminal phase may take months to years.

For a good prognosis, early detection and treatment of metastases are extremely important. When breast cancer spreads, it usually dissemminates through the lymph nodes in the armpit. Cancer cells detach from the tumor, and enter the lymphatic fluid. The lymphatic fluid is then discharged to the lymph nodes. From there, the cancer cells are absorbed into the blood and spread into the rest of the body. To determine whether cancer cells are spreading, the [first?] lymph nodes [in the armpit?], the sentinel Nodes (SNs), are surgically removed. The presence or absence of cancer cells in these SNs as detected during pathological investigation provides information about the extent of breast cancer [and therefore the prognosis for the patient]. In about 80% of the patients, no metastases are present in the SN. It then is very likely that there are no cancer cells in the rest of the lymph nodes. If their metastases are found in the SN, additional treatment of the lymph nodes is generally necessary. This may be surgery, radiation, or chemotherapy, depending on the size and type of the tumor and the nodal metastases.

1.1 Sentinel lymph node biopsy

Until the early 90's, the status of the regional lymph nodes could only be assessed by an elective lymph node dissection in combination with histopathological evaluation of all excised lymph nodes[1]. However, this method is associated with high morbidity and costs [2], while a significant number of patients is node negative, and no cancer cells are found in the sentinel node. [1]

Therefore, the Sentinel Lymph Node Biopsy (SLNB) procedure was devoloped as a minimally invasive procedure in which the sentinel lymph node is identified, removed and examined to determine whether cancer cells are present. A negative sentinel lymph node SLN result suggests that the breast cancer has not developed the ability to spread to nearby lymph nodes or other organs. A positive SLN indicates that cancer is present in the SLN and may be present in other nearby lymph nodes and possibly other organs. This information can help to determine the stage of the cancer and develop an appropriate treatment plan.

The current clinical practice for SLNB usually involves a combined detection procedure, using locally injected blue dye (Patent blue) and a radioactive tracer (Tc99-m nanocolloid) in combination with a gamma-probe, the combined technique. After injection, the tracers spread through the lymphatic system to the SLNs, like metastatic tumor cells. A lymphoscintigraphy is then made to determine the location of the SN, followed by surgical excision and evaluation of the lymph nodes at the histopathology department. [3] Only in case of a tumor-positive sentinel node, other axillary nodes must be removed to prevent the malignant cells from further spreading to other organs (see figure 1). Currently radiation or chemotherapy may also be a possibility to treat metastatic nodes

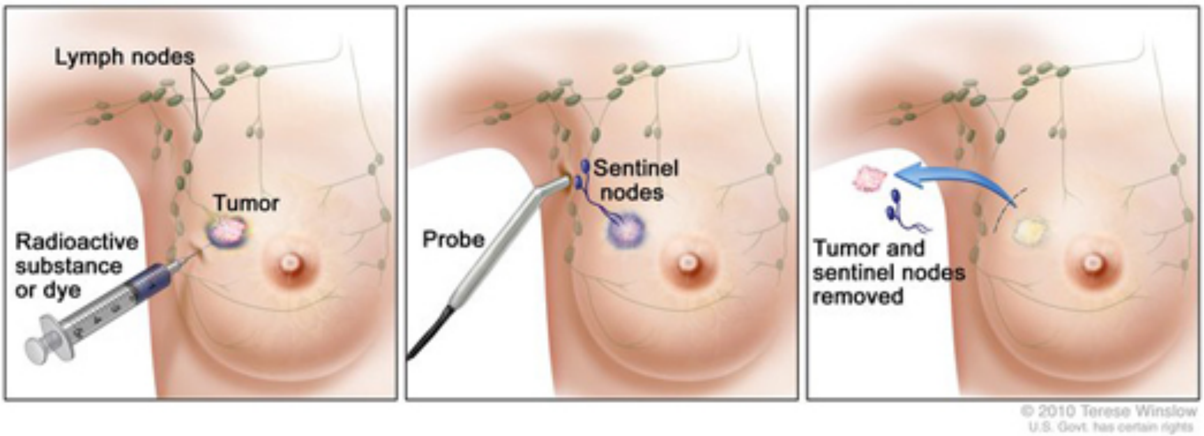


Figure 1 Overview of the current clinical practice for sentinel lymph node biopsy using locally injected Patent Blue dye and a radioactive tracer Tc99-m.

The SLNB procedure has proved to perform equally well to the complete lymph node dissection in terms of patient survival, but significantly better in terms of patient morbidity. However, the combined technique still suffers from disadvantages. The use of radioactive tracers exposes patients and medical staff to radiation, and provides poor pre-operative spatial resolution on lymphoscintigraphy. In addition, less than 60% of eligible patients in the developed world have access to the procedure. The use of intraoperative Patent Blue dye injections can obscure the surgical field and frequently leaves a skin residue, which can take months to fade out. Furthermore, the use of radioisotopes involves a major logistical challenge and is not available in many less developed countries. [4 5] The production of the radiotracers is limited to a small number of reactors over the world, who are dealing with strict rules. This may lead to a shortage of radioactive tracers. It is therefore important to invest in the development of alternative radioisotope-free SLNB methods.

A tracer for the sentinel node detection without the use of radioactivity would be a huge improvement over the currently used method. The procedure could be performed in many more (less developed) countries, and the radiation exposure for the patient and medical staff can be avoided.

The use of an iron-containing tracer can provide a solution. Recently, research has been done into the use of Superparamagnetic Iron Oxide (SPIO) for detection of the sentinel node, and two magnetometers have been developed for its detection, respectively the CE certified Sentimag (Endomagnetics, Ltd, UT) and the DIffmag (currently developed by the University of

Twente, Netherlands). [6] The first results are promising, but improvement is needed to be as successful as the current dual technique (radio-active tracer and blue dye). Previous studies with the magnetic SN tracer showed that a high dose of iron has adverse effects on pre- and post-operative Magnetic Resonance (MR) diagnostics. It is therefore important to see whether magnetic detection is also feasible with a low dose iron injection. In addition, working with a low dose of SPIO particles in combination with a pre-operative MRI scan offers opportunities to study the uptake and distribution of iron in the nodes, and possibly evaluate the presence of axillary metastases pre-operatively. A non-invasive diagnostic and therapeutic treatment of the SN in breast cancer patients may then be achievable. The use of a low dose magnetic tracer for SLNB, and the potential of using pre-operative MRI with the magnetic tracer for non-invasive metastases detection will be investigated in this thesis.

2. Magnetic technique for SLNB

In the past, a lot of research has been done into novel methods for radiation-free SLNB procedures. The use of Superparamagnetic Iron Oxide (SPIO) has been used to perform SLNB in breast cancer [6], and is a promising radioisotope-free technique. There has also been research into the uptake of SPIO in axillary nodular tissue in the histological and diagnostic field.

Chapter 2.1. and 2.2 shows what is already known about the histological distribution of SPIO in the lymph nodes, which is important for the examination of the possibility for a pre-operative evaluation of metastases in the sentinel node using SPIO contrast. Thereafter chapter 2.3 describes how the SPIO can be used as MRI contrast agent for lymph node mapping, and how signal loss on MR can help the identification of lymph node metastases. Finally in chapter 2.4 the potential of this new magnetic technique for clinical practice is described in relation to the current technology.

2.1 SPIO in Sentinel Lymph Nodes

The SPIO injection is interstitially administered into the breast and travels via the lymphatic drainage system to the axillary sentinel lymph nodes where it is distributed over the sinuses, the subcapsular space and the parenchyma of the nodes (fig 2). The iron is sequestered within the macrophages before being broken down and distributed in the body. Johnson et al. showed that the SPIO was not found in areas containing metastases. [7] This indicates that a high resolution SPIO-enhanced MRI can help identify in-vivo metastases in the SLN in breast cancer patients. However, previous studies from preclinical porcine models have shown that only 2% of injected SPIO reaches the draining sentinel nodes when the clinical volume of 2 ml (54 mg Iron oxide) of the magnetic tracer is injected interstitially. Thus, most of the injected SPIO remains in the injection area in the breast. [8] Consequently, the largest trial (The Sentimag Trial, Douek, 2014) of the magnetic technique shows that this retention of iron can produce void artefacts on MRI that may interfere with the evaluation of the glands. The high dose of iron that is left in the mammary tissue also causes MRI artefacts. This disturbed the assessment of the MR images of the mammary tissue, and follow-up by MRI is no longer reliable in these patients. [9]

2.2 Histopathological examination of SPIO in SLN

Histopathological examination of SPIO in SLN in the Johnson trial show that iron distribution was found predominantly within the sinuses (81%), fewer nodes contained iron in the subcapsular space (24%) and iron was seen within the parenchyma in 14% of the nodes. Lymph moves from the lymphatic channels into the sinus and subcapsular space of the node, and ultimately into the parenchyma. (Fig. 2) Sentinel lymph nodes that contain iron in the parenchyma had been excised following a significantly longer interval after injection (mean 22.56 min) when compared to nodes that have iron in the subcapsular space and sinus only (mean 17.25 min) ($p<0.005$). Neither iron nor macrophages with or without iron were present in the area of the node that contained a metastases. The iron could only be found in areas of the remaining non-metastatic area of the node. SPIO deposition within the SLN can easily be identified with the standard H&E staining. A Perls staining can confirm the presence of iron in the nodular tissue. Previous studies shown that routine histological examination was unaffected by the use of SPIO. [7]

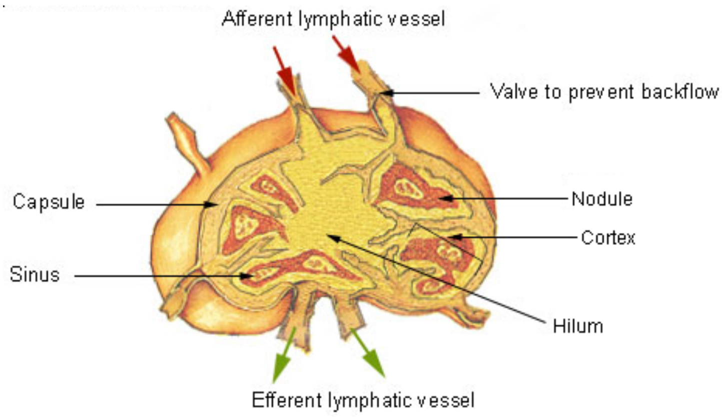


Figure 2 Diagram of a lymph node, showing the flow of lymph through the lymphatic vessels. A normal lymph node is kidney or oval shaped and range in size from a few mm to 1-2cm. (Illustration By SEER http://training.seer.cancer.gov/module_anatomy/unit8_2_lymph_compo1_nodes.html)

2.3 SPIO enhanced MRI

After intravenous administration, SPIOs are transported to the lymphatic tissue where they have a negative (dark) effect on MRI with T2 and T2* weighted imaging. A non-homogeneous distribution of iron in the SLN can help identify a metastatic lymph node. [10]

Harinsinghani et al. [11] were the first to demonstrate that intravenously administered Ultrasmall SuperParamagnetic Iron Oxide (USPIO) enhanced Magnetic Resonance Imaging (MRI) can be used to non-invasively predict the nodal status of prostate cancer patients. The uptake of the approximately 20 nm sized USPIO paticles by macrophages that accumulate in lymph nodes results in black-appearing nodes on MRI images. However, in malignancy-baring regions of the glands, macrophages are not accumulated. These regions maintain their original intensity on MRI. [10]

If imaging can be performed with sufficient resolution, the inhomogeneous signal loss in metastatic lymph nodes as compared to the homogenous signal loss in non-metastatic nodes, allows the detection of lymph node metastases. The intravenously administered USPIOs are able to reach all lymph nodes and thus are a suitable contrast agent for evaluation of the complete nodal basin.

In breast cancer, the goal is to detect the sentinel lymph node(s) and evaluate these for the presence of metastases. It's desirable to do this non-invasive. In case of uncertainty the SN can be detected and taken out for histopathological research. The some larger Super Paramagnetic Iron Oxide particles SPIOs are, if injected interstitially instead of intravenously, primarily mechanically transported to the sentinel lymph nodes only, where they are entrepped in the sentinal nodes via the uptake by macrophages. [7] The SPIO can be used for localization of the SN, for example in case of suspicion of micrometastases, which are not yet detectable with the current MR resolution.

The SentiMAG multicenter study in 350 breast cancer patients showed that a periareolar subcutaneous injection of SPIO paritcles (Sienna+® CE certified as class Iia medical device, see Appendix) in combination with a magnetometer (SentiMag ®, CE certified, fig 3) performed equally good in detection of the SLN as compared to the combined technique using blue dye and a radio-active tracer in combination with a gamma probe. [6] However, the concentration and volume of the SPIO-material injected in this study was relatively high. Such doses will cause large MR susceptibility artefacts at the injection and accumulation site, which can disturb the evaluation of potential post-surgical MRI and may troubling the evaluation of the lymph nodes for the presence of small metastases.

In 11 breast cancer patients participating to the SentiMag study, preoperative MRI was being performed before and after the SPIO injection, which made pre-operative mapping of the SLNs possible. The accumulation of iron particles in the sentinel lymph nodes led to the expected negative signal on MRI in almost every patient. In one patient the uptake of iron was probably too slow to visualize the accumulation in the SLN directly after the injection of the SPIO. One of the patients in which the SLN could succesfully be visualized by SPIO enhanced MRI, was diagnosed with macrometastases in the SLN and upon evaluation of the MR images, heterogeneous regions of higher intensity in the overall dark appearing lymph node could be observed. [12]

The first results of the SPIO enhanced SLN detection and MR evaluation are promising, but neither the MR imaging protocols, nor the injection (timing and concentration) of the contrast agent were optimized for a successful preoperative evaluation and intraoperative detection of the SLN, while both are believed to be of utmost importance for a successful performance of the magnetic technique.

Figure 3 Commercial magnetometer (Sentimag®, Endomagnetics Ltd, UT) consisting of a base unit with numerical display, and a handheld probe. The magnetic tracer (Sienna+®) consists of coated superparamagnetic iron oxide nanoparticles in sterile water for injection. (Photo: Endomagnetics)

2.4 Sentinel node localisation with Magnetic Technique

A promising radioisotope free technique for SLNB is associated with the use of an interstitial injection of Super Paramagnetic Iron Oxide (SPIO) and a handheld Magnetometer. [13] (Fig. 3) The magnetic technique has been evaluated in a number of studies and confirmed on meta-analysis in order to be non-inferior to the radioisotope dependent technique in breast cancer. The magnetic technique was found to be noninferior to the current standard with radioisotopes with a sentinel node identification rate of 97.1 and 96.8% respectively. [14] The advantages of the magnetic technique are the long shelf life and there is no decay of the magnetic tracer in contrast to the radioactive tracers. There is no need to take into account for the radiation exposure and the logistical challenges that accompany it. The iron is freely accessible, making it also applicable in less developed countries that do not have any radiation facilities.

In clinical practice, the magnetic tracer measurements are not stable. The surgical instruments largely influence the measurements and in many cases no hotspot is found transcutaneously. It should be examined how the magnetometer measurements can be perform more stable. Background effects should be eliminated and a solution must be found to replace or improve the transcutaneous localisation.

2.5 Summary

Review of many radiation free SLNB studies with the use of SPIO and a handheld Magnetometer suggested that these new methods for sentinel lymph node biopsy have clinical potential. The concentration and volume of the SPIO material injected in these studies, was relatively high. Such doses will cause large MR susceptibility artefacts at the injection and accumulation site, not only making the evaluation of potential post-surgical MRI impossible, but also troubling the evaluation of the lymph nodes for the presence of small metastases. In addition, the transcutaneous measurements are not very stable what makes the SN detection difficult, and still can not match the current standard dual technique.To be able to move forward to a radiation free and potentially non-invasive SLNB procedure, there is a need to further optimize this magnetic procedure and further assess its potential effectiveness against the current standard technique in randomized (controlled) trails.

3. Scope and outline of this thesis

As outlined in the previous chapter, the current studies concerning Magnetic sentinel lymph node detection and evaluation suffer from limitations. The aim of this thesis is to develop an effective complete magnetic procedure for a radiotion-free SLNB. The experience of different studies is used to optimize the injection, the intra-operative measurements, the MR images with SPIO contrast and the logistic process in the hospital.

3.1 Objectives of the studies performed

Primary objectives:

- To investigate the possibility of using a low dose intratumoral SPIO injection for a complete magnetic, radiation free and non-invasive procedure for sentinel lymph node detection and evaluation.

Secondary objectives:

- To test the intraoperative detectability of the SLN using a low dose intratumoral SPIO injection in combination with the SentiMag detector.
- Achieving pre-operative identification of sentinel Node metastases using MRI.
- Non-invasive metastases recognition; a 1 to 1 correlation of SPIO deposition into histopathological coupes and MR images of sentinel Nodes.

3.2 General outline of the thesis

Chapter 4 describes the impact of MRI artefacts that have occured after SPIO injections performed in earlier studies. It describes the extent of the artefact and its consequences for future diagnostic possibilities for the affected patients. There are also further recommendations to prevent these artefacts in future, and how intersitially administered iron-containing tracers can be safely used.

Based on the discovery of MRI artefacts after high dose SPIO injections, the question was raised whether it was possible to perfom a magnetic detection procedure with a lower dose of iron. This can help to improve the pre-operative evaluation of the sentinel node, which may prevent patients for unnescessary surgical removal of negative lymph nodes, en post surgery morbidity. **Chapter 5** describes the results of the Lowmag clinical trail in which a dose of 1.1 mg was used, instead of 2.7-56 mg used previous work.

Despite the fact that much research has been done already on magnetic sentinel node procedures, the perfect technique has not yet been found. **Chapter 6** summarizes the experiences of previous studies and experiences in the clinical field to get a step closer to the perfect complete magnetic SLNB and evaluation procedure. **Chapter 6** provides a general conclusion and recommendations for improvement and future perspectives of the Magnetic tracers and devices.

4. MRI void artefacts

Avoiding long-lasting void artefacts on breast magnetic resonance imaging after magnetic sentinel node biopsy

4.1 Abstract

Background: The magnetic technique for sentinel node biopsy (SNB), using an interstitially administered superparamagnetic iron oxide (SPIO) tracer and handheld magnetometer for intraoperative identification, was developed to overcome drawbacks associated with the use of radioactivity. However, residual magnetic tracer after breast conserving surgery (BCS) can alter post-operative MRI examinations. The purpose of this cohort study was therefore to determine if the magnetic technique for SNB results in long-lasting MRI void artefacts, and whether these artefacts could be avoided by using a low dose intra-tumoral injection.

Method: All patients who were treated with BCS and the magnetic technique in our centre were invited to undergo an additional post-operative T1- and T2*-weighted MRI using a 1.5T MR system to determine whether a void artefact was visible. One group of patients received a 2 ml subareolar administration (high dose group) while the other group received a 0.1 – 0.5 mL intratumoral dose (low dose group).

Results: A total of 20 patients were included (13 high dose group, 7 low dose group). In the high dose group 9/13 patients displayed an artefact on T1-weighted imaging, while no artefacts were observed on the low dose group.

Conclusion: We demonstrated that SNB using a high dose subareolar administration of magnetic tracer in patients undergoing BCS results in residual tracer in the breast in the majority of patients, which hampers follow-up MRI up to 3.6 years after the procedure. By using a low dose intra-tumoral administration, these problems can be avoided, and it therefore provides a viable alternative.

4.2 Introduction

Sentinel node biopsy (SNB) using a radioisotope tracer (^{99m}Tc-nanocolloid) is the standard of care for axillary staging of early-stage breast cancer patients. The magnetic technique, using an interstitially administered superparamagnetic iron oxide (SPIO) tracer and handheld magnetometer for intraoperative identification, was developed to overcome drawbacks associated with the use of radioactivity. Several clinical trials evaluated the new technique, and demonstrated a non-inferior identification rate to SNB with a radioisotope tracer [14-21]. The magnetic tracers used were originally developed as magnetic resonance imaging (MRI) contrast agents, and provide negative contrast on T2*-weighted images. The uptake of tracer in the lymph nodes results in a drop of signal and therefore allows for pre-operative localization of the sentinel lymph nodes (SLNs) with MRI as alternative to lymphoscintigraphy [12], in addition to intraoperative detection.

When residual magnetic tracer is retained during surgery, the negative contrast on MRI could potentially alter follow-up MRI examinations. Huizing et al. [22] retrospectively analysed all post-operative breast MRIs requested on SentiMAG Trial participants recruited at their centre, and found long-lasting void artefacts in all 6 patients due to residual magnetic tracer in the breast. These long-lasting void artefacts could compromise interpretation of follow-up MRI after breast conserving surgery (BCS). Indications for performing breast MRI after BCS consist of the exclusion of suspected local recurrence (upon clinical identification or inconsistent findings between mammography and ultrasound) and diagnosis of suspected new ipsilateral carcinoma in the treated breast [23]. The inability to perform accurate postoperative breast MRI in patients treated with magnetic SNB could impact their disease management, and should therefore be considered before using the magnetic technique. The manufacturer of the magnetic tracer warns for (long term) alteration of MRI studies in the instructions for use [24], however it is not known how long these alterations persist, and whether they can be avoided using a different injection site and dosage. In the MagSNOLL trial an intra-tumoral administered low dose of magnetic tracer (4-20 times less tracer) was used for occult lesion localization and SNB [25]. The lower dose could lead to less residual magnetic tracer after surgery, and eliminate alterations of follow-up MRI examination.

The purpose of this cohort study was therefore to determine if the magnetic technique for SNB results in long-lasting MRI void artefacts, and whether these artefacts could be avoided by using a low dose intra-tumoral injection.

To confirm that the injection of SPIO tracer leads to long-lasting MRI void artefacts, all patients treated with BCS and the magnetic technique for SNB in our centre were invited for a post-operative MRI. The influence of the injection site and dosage was evaluated by comparing the postoperative MRIs of two groups of patients: a group of patients who received a 2 mL subareolar magnetic tracer injection and a group who received a 0.1 - 0.5 mL intra-tumoral injection.

4.3 Methods

Patients

In our centre a total of 76 patients were included in the SentiMAG Multicentre [15] and MagSNOLL trials [25] between May 2012 and March 2015 (NTR3238 & NTR4691, http://www.trialregister.nl). A subareolar injection with 2 mL of magnetic tracer (Sienna+®, (27 mg iron per ml); Endomagnetics Ltd, Cambridge, UK) diluted in 3 mL saline was administered in 66 patients included in the SentiMAG Multicentre trial for SNB (*High dose group*). An intra-tumoral injection of 0.1 - 0.5 mL magnetic tracer was used in 10 patients included in the MagSNOLL trial for occult lesion localization and subsequent SNB (*Low dose group*). All patients who underwent BCS were eligible for inclusion and invited to undergo a post-operative MRI to evaluate the presence of void artefacts. Patients with contraindications against MRI were excluded. Ethics Committee approval was obtained before the start of inclusion, and all patients gave written informed consent.

MR Imaging

Imaging was performed using a 1.5T MR system (Intera, Philips Medical Systems, Best, the Netherlands). First, T1-weighted turbo spin echo (TSE) images were acquired in the transverse plane (scan parameters: TR/TE 734/16 ms; FA 90°; Slice Thickness 3.0 mm; Imaging time approx. 4 min) and coronal plane (scan parameters: TR/TE 727/16 ms; FA 90°; Slice Thickness 3.0 mm; Imaging time approx. 3 min). Since T1-weighted dynamic contrast enhanced (DCE) imaging is generally performed to diagnose breast malignancies, the T1-weighted TSE images were used to evaluate the influence of residual magnetic tracer on diagnostic breast imaging. Additionally, T2-weighted gradient echo (GRE) images (scan parameters: TR/TE 500/4.6 ms; FA 18°; Slice Thickness 3.0mm; Imaging time approx. 5 min), sensitive to susceptibility differences caused by presence of magnetic tracer, were acquired in the transverse plane to evaluate the presence of residual magnetic tracer. The transverse images were used to make a coronal reconstruction in which the artefact dimension could be determined.

Image analysis

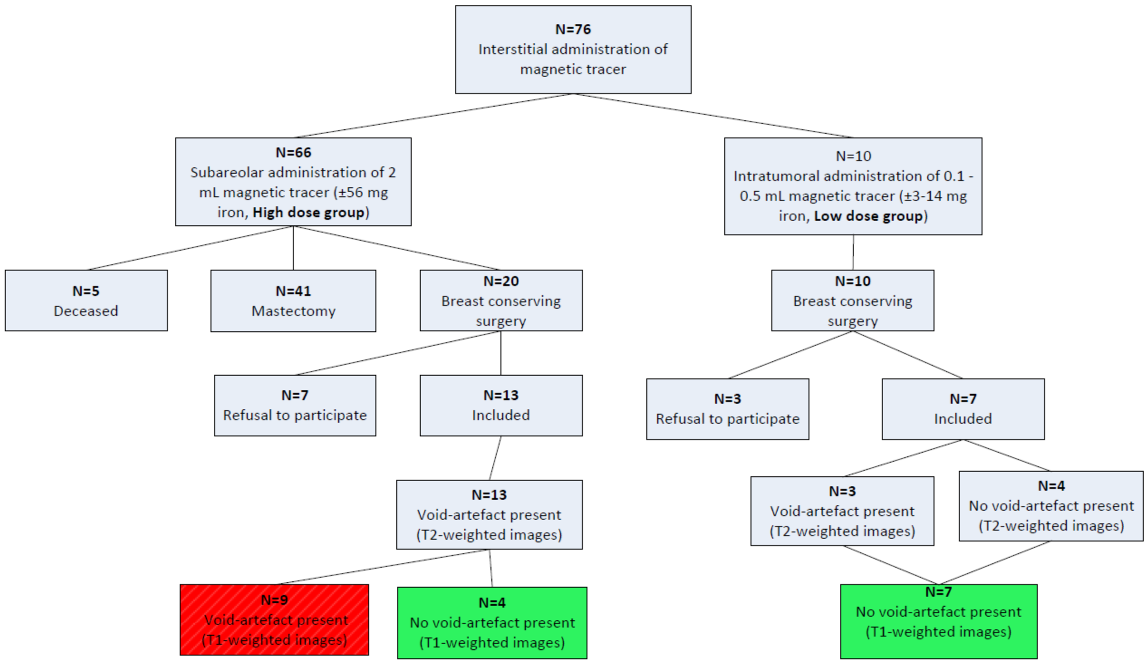
Two radiologists experienced in breast imaging (MCvdS, CAHK), analysed the magnetic resonance images, using a DynaCAD® workstation (Philips Healthcare, Best, the Netherlands). Both the T1-weighted and T2-weighted images were analysed for the presence of void artefacts. If artefacts were present, the dimensions were recorded in the slice with the largest artefact. The dimensions were recorded in three directions; firstly, the maximum diameter of the artefact in the transverse plane (d1), secondly the maximum diameter of the artefact perpendicular to this (d2), and thirdly the maximum diameter in the coronal plane (d3). Prior post-operative mammography and the surgical report were used to distinguish void artefacts originating from clip makers from artefacts due to presence of magnetic tracer. Furthermore, the location of the artefact and the time between surgery and post-operative imaging was recorded.

The possibility to perform reliable breast MRI was evaluated in all patients presenting with void artefacts. Any disagreements were solved by consensus. A breast MRI was classified as unreliable when artefacts due to the presence of magnetic tracer exceeded the dimensions of a typical breast biopsy marker artefact (>10 mm in any dimension) on T1-weighted imaging. When the breast MRI was classified as unreliable, prior post-operative mammography and ultrasound studies were additionally evaluated for presence of artefacts.

4.4 Results

Patients

In the *high dose group* (subareolar injection of 2 mL of magnetic tracer diluted in 3mL saline) 20 patients were treated with BCS surgery and were eligible for inclusion, 13 of these patients gave informed consent and were included. In the *low dose group* (intra-tumoral administration of 0.1 - 0.5 mL of magnetic tracer), all 10 patients were treated with BCS; seven of these patients gave informed consent and were included. Of the seven patients, one was administered 0.5 mL of tracer, and the other six received 0.1 mL of tracer. The details on the number of included and excluded patients, and the primary outcomes are described in Figure 1. The mean number of days between tracer injection and post-operative MRI were 1099 (SD 110) days (range 917-1308) in the *high dose group*, and 241 (SD 30) days (range 196-294) in the *low dose group*.



Figuur 1 Flow chart of the included and excluded patients, and the primary outcomes of this study.

MR imaging outcomes

In the *high dose group* all 13 patients displayed a void artefact on T2-weighted imaging. On T1-weighted imaging an artefact was present in 9 out of 13 patients. The images of these 9 patients were classified as unreliable by both independent observers. A representative example with an artefact on both T2-weighted and T1-weighted imaging in the transverse plane is displayed in *Fig.* 2. In *Fig*. 3, images of a patient displaying an artefact on T2 - weighted imaging, but not on T1 - weighted imaging is shown.

In the *low dose group,* a void artefact was observed in 3 out of 7 patients on T2-weighted imaging, however no artefact was present in any of the patients on T1-weighted imaging. All artefacts were located superficially subcutaneous. All breast MRIs in the *low dose group* were therefore classified as reliable. An example of a T2-weighted image with artefact and corresponding T1-weighted image in the same slice without artefact from a patient in the *low dose group* is shown in *Fig.* 4.

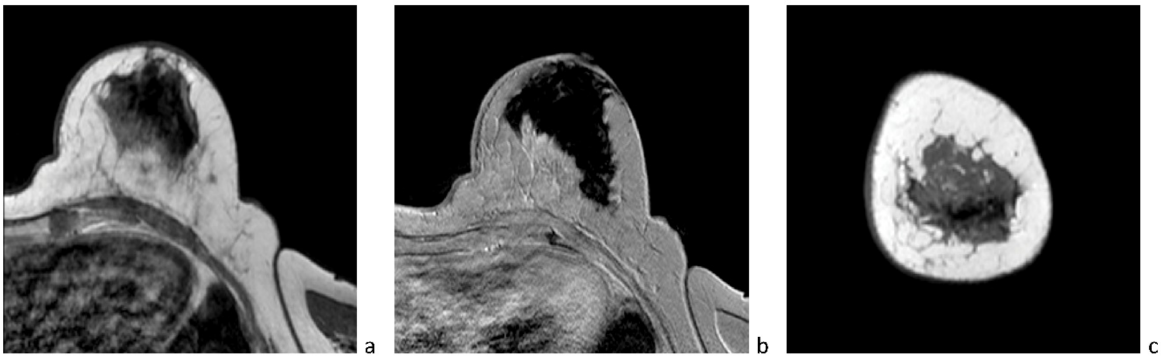
The mean dimensions of the artefacts of both groups are provided in Table 1. Except for one artefact on T2-weighted imaging in the *low dose group* (3 mm x 3 mm x 3 mm), all observed artefacts in both groups exceeded the dimensions of an artefact from a typical biopsy marker (greater than 10 mm in any dimension).

On the post-operative mammography and ultrasound studies of the 9 patients in which the MRI was classified as unreliable no artefacts due to the presence of magnetic tracer were observed.

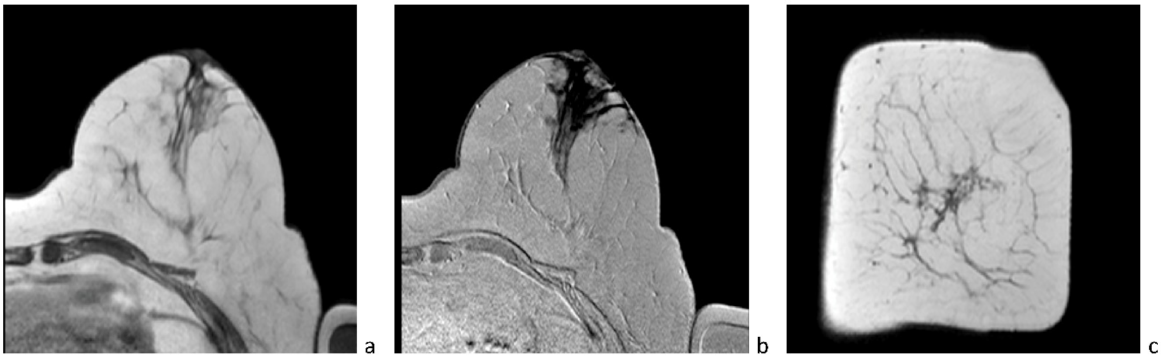
T2-weighted imaging	**d1 (cm) (mean±SD, range)**	**d2 (cm) (mean±SD, range)**	**d3 (cm) (mean±SD, range)**
High dose group (N=13)	5.6±1.9 (2.1-8.4)	3.1±1.3 (1-5.5)	3.6±1.5 (1-10)
Low dose group (N=3)	1.0±0.7 (0.3-1.2)	0.6±0.2 (0.3-0.8)	1.2±0.8(0.3-2.4)

T1-weighted imaging	**d1 (cm) (mean±SD, range)**	**d2 (cm) (mean±SD, range)**	**d3 (cm) (mean±SD, range)**
High dose group (N=9)	3.2±1.2 (1.5-5.6)	1.7±0.8 (0.7-3.7)	1.9±0.6 (0.8-2.8)
Low dose group (N=0)	No artefacts	No artefacts	No artefacts

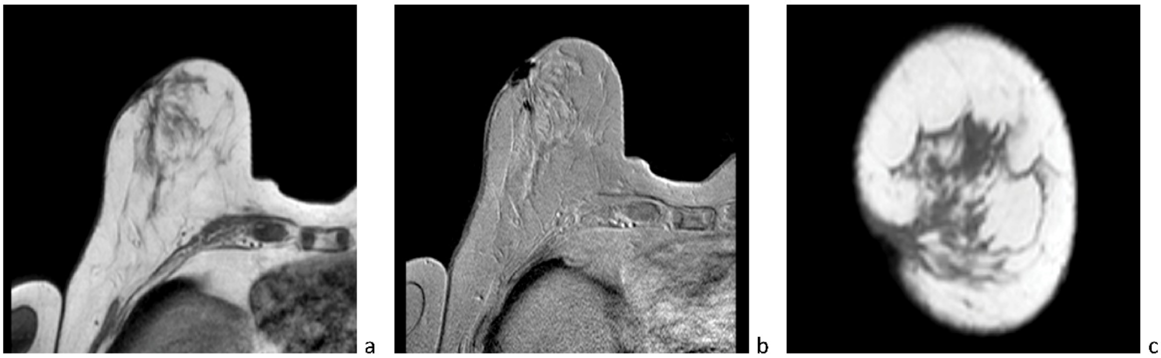
Table 1 Mean dimension of the void artefacts. Means were calculated using the measurements of both observers.



Figuur 2 Images from a patient in the High dose group in which the MR was classified as unreliable a) T1-weighted image with large void artefact in the transverse plane b) T2-weighted image in the corresponding plane with prominent artefact c) T1-weigted image in coronal plane with visible artefact.



Figuur 3 Images from a patient in the High dose group which was deemed reliable. a) T1-weighted image in the transverse plane without artefact. b) T2-weighted image in corresponding plane with artefact. c) T1-weighted image in coronal plane without artefact.



Figuur 4 Images from a patient in the Low dose group. a) T1-weighted image in the transverse plane without artefact. b) T2-weighted image in corresponding plane with artefact. c) T1-weighted image in coronal plane without artefact.

4.5Discussion

The magnetic technique for SNB, using an interstitially administered magnetic tracer, can be used to overcome problems associated with the use of radioisotope tracers. However, there were strong indications that residual interstitial magnetic tracer compromises the possibility to perform postoperative MRI in patients undergoing BCS. In this study, we confirmed that subareolar administration of 2 mL of magnetic tracer diluted in 3 mL saline (recommended dosage and injection site according to the manufacturer's instructions for use [24], *high dose group*) interferes with follow up MRI in a large proportion of patients (9 out of 13 patients) up to 3.6 years after surgery. When an intra-tumoral injection of 0.5 - 0.1 mL of undiluted tracer was used (*low dose group*), it was deemed not to alter follow-up MRI after a mean postoperative interval of 8 months in all 7 patients.

It is important to discuss the consequences of these findings on daily clinical practice. In this study, it was demonstrated that the residual magnetic tracer does not interfere with the commonly used mammography and ultrasound examinations. Currently breast MRI is not the standard of care for post-operative follow-up after BCS in the Netherlands. However, it is an important tool to evaluate suspected recurrence detected by clinical examination, or when mammography or ultrasound produce non-corroborative findings [23]. This evaluation is not possible after a high subareolar dose of magnetic tracer is administered, and could therefore impact the disease management of patients. This is particularly the case in patients with a high risk of recurrence (young patients, patients with BRCA1/2 expression) the inability to perform reliable post-operative breast MRI for many years is not acceptable. The use of an intra-tumoral low dose of magnetic tracer avoids these problems, and is therefore recommended - particularly in high-risk patients.

While the lower intra- tumoral administration avoids long-lasting MRI artefacts, the sentinel node detection rate is lower than when the high dose is used (85 per cent *versus* 94 per cent) [15,25]. However, when the low dose is used in combination with blue dye, the combined detection rate is 96 per cent - comparable to the standard SNB technique using radioisotopes [25]. Furthermore, a longer waiting time between injection and surgery is also expected to improve the tracer uptake in the sentinel nodes, which would facilitate identification [8].

The influence of the used injection site and dosage of magnetic tracer on follow-up MRI examination was investigated in this study, however the mean interval between surgery and imaging was considerably shorter in the *low dose group* compared to the *high dose group*. Since the residual magnetic tracer is expected to be cleared from the body over time [26] this could be of influence when comparing both groups. Despite the larger time interval in the *high dose group*, more and larger artefacts were observed. This indicates that the additional clearance does not compensate for the injection site and dosage difference.
Even though no artefact was visible in the T1-weighted images of all patients in the *low dose group,* residual magnetic tracer was visualized in the T2-weighted images in 3 out of 7 patients. Previously, interaction of intravenously administered SPIOs on Gadolinium contrast enhanced MRI has been described [27,28], however since our patients were not administered a gadolinium contrast agent, it is not possible to rule out interactions of the residual tracer on contrast-enhanced images.

In conclusion, we demonstrated that SNB using a high dose (2 mL tracer in 3 mL saline) subareolar administration of magnetic tracer in patients undergoing BCS results in residual tracer in the breast in the majority of patients, which hampers follow-up MRI up to 3.6 years after the procedure. This should be avoided, especially in patients with a high risk of recurrence such as BRCA patients undergoing BCS or mammographically and ultrasound occult lesions. By using a low dose (0.1 – 0.5 mL tracer) intra-tumoral administration, these problems can be avoided, and it therefore provides a viable alternative.

*This chapter is submitted as: Joost J. Pouw, **Anke Christenhusz**, Muneer Ahmed, Margreet C. Van der Schaaf, Caroline A.H. Klazen, Michael Douek, Joost M. Klaase, Bennie ten Haken. Avoiding long-lasting void artefacts on breast magnetic resonance imaging after magnetic sentinel node biopsy.*

4.6 Contribution of Anke Christenhusz in this publication:

AC acted as trial coordinator for this study; obtained ethics approval for the conduction of the trial, was responsible for making financial arrangements with all departments involved, analysed all data of patients potentially eligble for inclusion in this study, subsequentially recruited and scheduled all patients. AC performed the first data analysis together with MCvdS and CAHK, provided all the data for the publication and was involved as second author in compiling the manuscript. AC provided input on the first draft (and subsequent versions) of the manscript for improvement. Furthermore AC coordinated communication on legal issues with patients and parties involved in the hospital.

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5. Lowmag trail

Low dose SPIO injection for a complete magnetic, radiation-free, procedure for sentinel lymph node detection and metastases evaluation.

5.1 Abstract

Background: Multiple studies in breast cancer patients showed that periareolar subcutaneous injection of SPIO particles in combination with a handheld magnetometer performed equally well in detection of the SLN. [14] However, the concentration and volume of the SPIO material injected in these studies, was relatively high. This will cause large MR susceptibility artefacts at the injection and accumulation site. The purpose of this study is to make a first evaluation of the possibility of using a low dose magnetic procedure for intra operative SLN detection and pre-operative evaluation of lymph node metastases.
Method: Patients with histologically confirmed invasive breast cancer received an intra-tumoral injection of 1.1 mg Iron containing magnatic tracer. The SLN was intraoperatively detected according to the normal protocol and the Sentimag® magnetic probe. Imaging was perfomed using a 3T MR system in the orientation comparable to the conventional histopathology protocol. Additional staining was undertaken on 4 lymph nodes that containing metastases, using Perls' Prussian blue.
Results: Successful intraoperative localization of the SLN was achieved in 12 of 19 procedures. After a 1 to 1 correlation of the ex-vivo MR images and the histopathology slices it can be seen that there is no iron visible at the metastases site. This could indicate that at tumor regions, no iron deposition is possible.
Conclusion: The first results of this feasibility study show that the use of an extremely low dose of SPIO has the potential for a Magnetic SLN detection and evaluation. Inferior detection rate of the Magnetic procedure is partly due to the fact that the measurements were unstable. Time between injection and measurment/MRI and the amount of SPIO tracer should be considered to improve the measurements. First results shown that no iron is visible at the metastases site on the MR image and in the pathology slices. In order to recognize a clear pattern in the correlation between the pathohistology slices and MR images, more research needs to be done. Given this is an interim evaluation; the expectation is that more clarity will come forward.

5.2 Introduction

The SLN biopsy procedure has proven to perform equally well to the complete lymph node dissection in terms of patients' survival, but it is significantly better in terms of patient morbidity. [29] However, the combined method still suffers from disadvantages' such as the use of radioactive materials and the fact that node-positive patients require multiple surgical procedures. [29] Although the radioactive dose is low, the requirement on radioactive tracers leads to complicated logistics and regulations and makes that the sentinel node procedure is out of reach for patients in less developed countries. Besides, for axillary SLNB there is a minor risk of treatment related morbidity, as lymphedema, limitations in shoulder range of motion, loss of strength, numbness, dysesthesias and pain. [30 31]

A magnetic technique, using a Superparamagnetic Iron Oxide (SPIO) tracer and handheld magnetometer for a radiation free SLNB procedure was developed and have succesfully been investigated in earlier studies to overcome drawbacks associated with the use of radioactivity. [6 21 20]

Harinsinghani et al. [11] were the first to demonstrate that intravenously administered Ultrasmall Superparamagnetic Iron Oxide (USPIO) enhanced Magnetic Resonance Imaging (MRI) can be used to non-invasively predict the nodal status of prostate cancer patients. USPIO and Superparamagnetic Iron Oxide (SPIO) particles exert negative contrast on MRI. The uptake of the (U)SPIO particles by macrophages that accumulate in lymph nodes, results in black-appearing nodes on MR images. In malignancy-baring regions of the lymph node, no macrophages are accumulated and the specific regions keep their original intensity. [32] If imaging can be performed with sufficient resolution, the inhomogeneous reduction of signal in metastatic lymph nodes as compared to the homogenous reduction of signal in non-metastatic nodes, theoretically allows for the detection of lymph node metastases.

Multiple studies in breast cancer patients showed that periareolar subcutaneous injection of SPIO particles (Sienna+®) in combination with a magnetometer (Sentimag®) performed equally well in detection of the SLN. [14] However, the concentration and volume of the SPIO material injected in these studies, was relatively high. Such doses will cause large MR susceptibility artefacts at the injection and accumulation site, not only making the evaluation of potential post-surgical MRI impossible, but also troubling the evaluation of the lymph nodes for the presence of small metastases. [9] In addition, the unstability in localization measurements results in difficulties to detect the SN, which is inferior to the current standard dual technique.

The purpose of this study was to make a first evaluation of the possibility of using a low dose magnetic procedure for intra operative SLN detection and non-invasive pre-operative evaluation of lymph node metastases. The goal is to optimize the individual steps of the magnetic SLN procedure to develop one complete magnetic procedure.

5.3 Methods

Patients

Patients, with histologically confirmed invasive breast cancer visible on ultrasound imaging and suitable for SLNB, were included in the Lowmag trial between November 2016 and June 2017 (NTR 4858, http://www.trialregister.nl). All patients had to be available for a minimum of 12 months follow-up. Patients who received neo-adjuvant treatment or who are allergic or hypersensitive to iron or dextran compounds were excluded. Ethics Committee approval was obtained before the start of inclusion, and all patients provided written informed consent.

Magnetic sentinel node localization

Within one week before surgery an intra-tumoral injection of 0.04 ml magnetic tracer (Sienna+®, Endomagnetics, Cambridge, UK) diluted in 0.46 ml normal Saline (1.1 mg Iron) was performed under ultrasound guidance by an experienced mamma radiologist. The injection is combined with the standard Tc99-m nanocolloid and Patent Blue injection for the conventional SLNB procedure. During surgery, the SLN was intraoperatively detected according to the normal protocol (gamma probe and blue dye) and by use of the CE-certified Sentimag® (Endomagnetics) probe. The γ probe was used only when the magnetic procedure was not successful, or as a control measurement. Magnetometer counts were repeated on the excised sentinel node(s) and in the axilla incision after removal of the SN. The sentinel node was fixated in formaldehyde for transport to the clinical MRI system.

a b

Figure 4.2 (a) Overview of an Ultrasound guided intra-tumoral Sienna injection by a Radiologist. [33] *(b) ex-vivo MR phantoom made of Agar, a seaweed based binder. Small jars filled with formaldehyde and the sentinel nodes are placed into the holes, to create more volume for scanning.*

MR Imaging

Imaging was perfomed using a 3T MR system (Ingenia, Philips Medical Systems, Best, The Netherlands) by use of a phantom (fig 4.2b Agar, seaweed based binder) and a dedicated head coil in the orientation comparable to the conventional histopathology protocol. The Agar phantom was used to fix the jars filled with formaldehyde and the SNs, to create more scan volume and create a water peak required to create the MR signal.

A T1-weighted turbo spin echo (TSE) was acquired in the transverse plane (scan parameters: TR/TE 734/16 ms; FA 90°; Slice Thickness 1.0 mm; Imaging time approx. 4 min) and coronal plane (scan parameters: TR/TE 727/16 ms; FA 90°; Slice Thickness 1.0 mm).

Then a T1-weighted dynamic contrast enhanced (DCE) sequence was perfomed and additionally, T2-weighted gradient echo (GRE) images (scan parameters: TR/TE 500/4.6 ms; FA 18°; Slice Thickness 1.0 mm), sensitive to susceptibility differences caused by presence of

magnetic tracer, were acquired in the transverse plane to evaluate the presence of the magnetic tracer. Imaging time of this MR protocol was approx. 10 minutes. The transverse images were used to make a coronal reconstruction in which the SPIO disposition and dimensions could be determined. On top of the nodes in the formaldehyde, a small bag with formaldehyde is placed in order to prevent the sentinel node from drifting into the artefact of the transition from air to liquid.
The MR images were independently evaluated, before the histopathological diagnosis, by two radiologists. Per node, the suspisciousness for metastases (yes or no), and several descriptive parameters are documented. The amount of fat and nodular tissue was appointed, and the amount and dispensation of the iron artefacts.

Histopathology

The lymph nodes were investigated for the presence of metastases according to the standard protocol, with standard H&E staining. Nodes were reported as normal, containing macrometastases (larger than 2mm), micrometastases (0.2mm – 2mm) and isolated tumor cells (0.2mm or smaller). To confirm that any brown discoloration was due to iron deposition, additional staining was undertaken on 4 lymph nodes that containing metastases, using Perls' Prussian blue (Sigma-Aldrich, Poole, UK).

5.4 Results

A total of 19 patients were included in the study. The patients, all women, had a mean age of 59 years and 5 months (range 49-73). Neoadjuvant chemotherapy was one of the exclusion criteria for participation in the study, so all observed nodes were not systemically treated. All patients underwent SLN biopsy at the time of the mastectomy or breast conserving surgery. In total, 17 patients had invasive breast cancer. Two patients had surgery for large areas of Ductal Carcinoma in Situ (both grade 2). Other tumor/patient characteristics are shown in table 1.

Sentinel Node localisation

Successful intraoperative localization of the SLN corresponding to the presence of peak magnetometer counts was achieved in 12 of 19 patients (table 2), in 16 of 19 procedures by the combined magnetic and blue dye technique, and in 17 of 19 procedures with the standard dual technique. Discordance between the standard and magnetic technique was observed in 7 of 19 procedure, and in 3 of 19 for magnetic techinique + blue dye versus standard dual technique. Of the 19 procedures performed, there were four instances of nodal involvement; one micrometastases and three macrometastases. The micrometastases and one macrometastases were identified independently by both techniques. One macrometastases was identified only using the standard dual technique, and one macrometastases was only identified by blue discoloration. A transcutaneous measurement was only achievable in 8 of 19 procedures.

	No. Of tumors*
Age (years)**	
29-50	4
51-69	12
≥ 70	3
Type of surgery	
Mastectomy	2
Breast-conserving surgery	17
Node status by largest metastases	
No metastases	15
ITC	0
Micrometastases	1
Macrometastases	3
Lymphovascular invasion	
Yes	2
No	17
Tumor grade	
1	9
2	6
3	4
Tumor size	
T1	12
T2	5
T3	0
Oestrogen receptor status	
Positive	13
Negative	4
HER2 status	
Positive	2
Negative	15
Tumor type	
Invasive, nst	14
Invasive, pst	3
DCIS	2

Table 1 Patient and tumor characteristics.

**Unless indicated otherwise; **number of patients.*

ITC; isolated tumor cells. HER2; Human epidermal growth factor receptor 2.

	Radioisotope + blue dye		
	Failed detection	Successful detection	Total
Magnetic Technique			
Failed detection	1	6	7
Successful detection	1	11	12
Total	2	17	19
Magnetic Technique + blue dye			
Failed detection	1	2	3
Successful detection	1	15	16
Total	2	17	19

Table 2 SN identification (detection) rates and discordant procedures by method of detection. Discordance in 7 of 19 procedures for magnetic technique alone versus standard dual technique, and 3 of 19 for magnetic technique + blue dye versus standard dual technique.

	Radioisotope + blue dye		
	Failed detection	Successful detection	Total
Magnetic Technique			
Failed detection	1	6	7
Successful detection	1	17	18
Total	2	23	25
Magnetic Technique + blue dye			
Failed detection	1	2	3
Successful detection	1	21	22
Total	2	23	25

Table 3 Number of nodes removed in the study cohort (19 tumors).

Histopathology

The 19 patients who received an intra-tumoral SPIO injection pre-operatively, had a total of 34 excised sentinel lymph nodes, which were subjected to histological assessment (mean 1,8 range 1-4). Seven of these nodes were magnetic, radioactive and blue, 4 were magnetic and radioactive, and 1 was solely magnetic. A correlation between the number of SLNs identified by ex-vivo MR imaging, histopathological examination and the number of SLNs detected by magnetic and radioactive detection during surgery is shown in table 4. In one patient (Patient 13, table 4) no SLNs were detected by the magnetic or standard dual (radioactive and Patent blue) technique; this node was removed during clinical examination of the surgeon.

All SLNs were assessed by a histopathologist after Haematoxylin and Eosin (H&E) staining. Additional Perls' Prussian Blue staining was undertaken on the four affected sentinel nodes to confirm the iron deposition in the nodes. Iron distribution was found to a certain extent in all H&E coloured sections. Iron was depicted as a brown discolouration (fig1). In the Perls' Prussian Blue colored sections the iron is shown blue. In this colouration much more iron is visible and the distributrion is easier to observe.

Iron distribution was predominantly found within the sinuses of the lymph nodes, but iron was also seen in other parts of the lymph nodes, the subcapsular space. There was no iron visible in the central part of the nodes. There is a large difference visible in iron uptake between the various nodes. The pathologist noted that there was significantly less iron present in areas where tumor tissue is present. This is illustrated in Fig. 4.4. However, insufficient material has yet been collected (4 nodes containing metastases) to extract true conclusions about it.

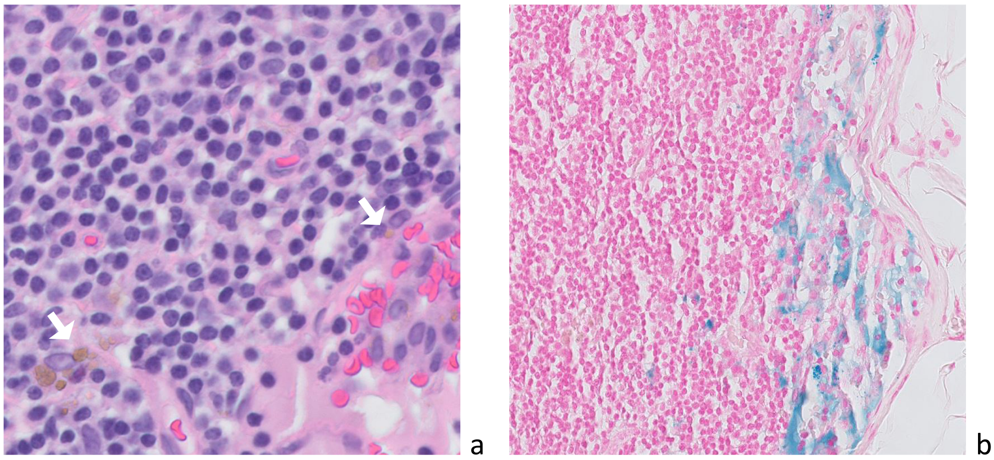

Figure 4.3 The appearance of iron deposition in H&E staining vs Perls' Prussian Blue staining in the sentinel lymph node. (a) H&E: Standard Haematoxylin and Eosin staining (iron=brown, white arrows) (b) Perls'Prussian Blue staining (iron=blue)

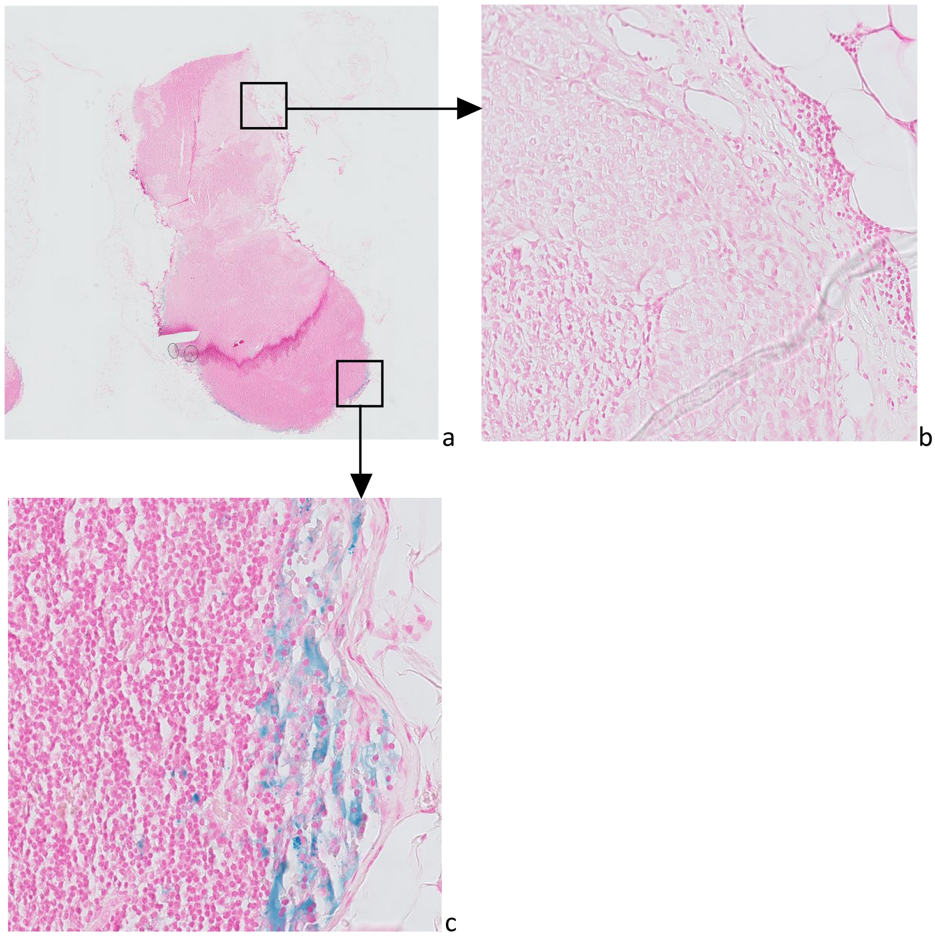

Figure 4.4 Image of a sentinel node coupe with Perls Prussian Blue staining that contains metastases. (a) Overview of the entire node. The ligther coloured part of the node indicates the presence of tumor tissue. At the bottom of the node also a darker part of the nodular tissue is visible without tumor cells. (b) An enlargement of the tumor tissue. There is no iron visible in this part of the sentinel node. (c) An enlargement of the normal nodular tissue. Here, iron is visible in the skin of the sentinel Node and the sinus.

Superparamagnetic iron oxide Ex-vivo MRI

The day after surgery, ex-vivo MRI was performed with a 3T system (Ingenia, Philips Medical Systems, Best, The Netherlands). The head Coil (Philips) was used to fix the Agar phantom. (See figure 4.2b)
A total of 33 SLNs could be identified on post-contrast T2* weighted images. (Fig. 4.5) In 11 of 19 procedures (58%) the number of excised nodes correspond on pathological and radiological (ex-vivo MRI) examination. Of the remaining 8 procedures, four patients showed one or two additional SLNs on MRI (21%), and four patients showed one or two additional SLNs during the histopathology examination (21%). (Table 4)

Patient	SLNs on ex-vivo MRI (n)	SLNs on histopathology (n)	Magnetic SLNs detected (n)	Radioactive SLNs detected (n)
1	3	1	1	1
2	1	1	1	1
3	1	1	1	1
4	2	3	1	1
5	2	1	1	1
6	1	1	0	1
7	1	1	1	1
8	2	4	0	1
9	2	2	1	1
10	2	2	2	2
11	1	3	1	1
12	3	3	1	2
13	1	1	0	0
14	2	3	2	2
15	2	1	1	1
16	1	1	1	1
17	1	1	1	1
18	3	2	2	2
19	2	2	2	2

Table 4 Correlation between the numbers of sentinel lymph nodes (SLNs) identified by ex-vivo MRI, histopathology examination and magnetic and radioactive detection during surgery.

In 18 of 19 procedures, areas of low signal, possibly iron, are visible in the lymph nodes on MR imaging. In the first MRI performed no area of low signal could be identified. The node was fixed in formaldehyde, but drifted to the surface. This resulted in the node being exactly in the artefact of the transition from water to air, and no distinction could be made between the two types of artefacts, there was no certainty that any iron was present in the lymph node based on the evaluation of solely this ex-vivo MRI. In all following MR procedures, a small plastic bag filled with formaldehyde was placed on top of the sentinel node, so it was completely surrounded with liquid. In the remaining MRIs, the surface artefact was avoided this way.

In all other fragments, a linear configuration with low signal is surrounded by tissue with fat intensity. The low signal indicates the presence of iron. In view of the configuration, the iron appears to be in the cortex of the lymph node. The cortex is not thickened and the artefacts reveal no recognizable recesses of the cortex. In some nodes a larger area with lower signal is visible than in the other node, which may be related to the presence of metastases. (Fig 4.6)

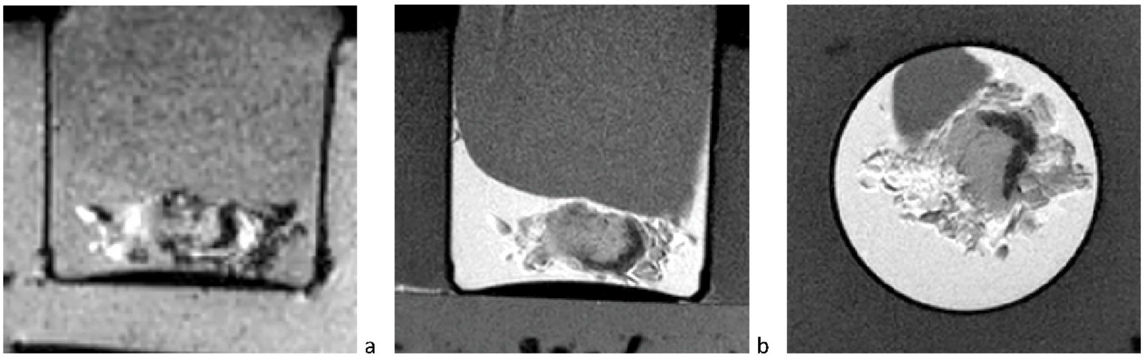

Figure 4.5 Ex-vivo MR imaging according to the lowmag protocol, by use of a phantom (made of Agar, a seaweed based binder) (a) A T1 weighted image showing the anatomy and morphology of the surgical excised tissue, including fat tissue, nodular tissue and possibly tumor tissue, in the transverse plane. (b) The same axillary lymph node on a T2 weighted MR image. (c) T2 weigthed image of the node in coronal plane.

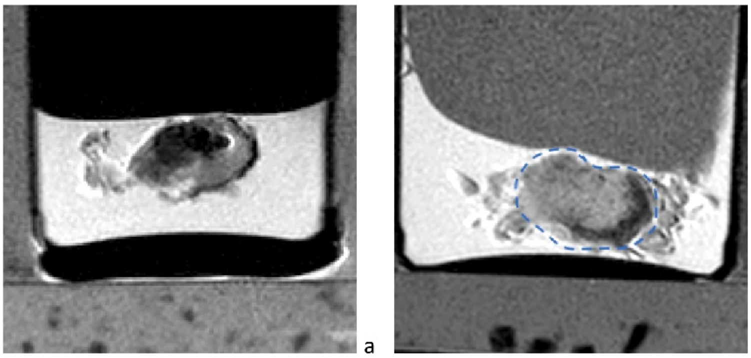

*Figure 4.6 Ex-vivo T2 MR imaging of sentinel nodes. (a) MR imaging of a healthy sentinel node **without** any **metastases**. In a large part of the node iron deposition is visible. (the black discolouration within the node) (b) MR imaging of a sentinel node **with metastases**. The entire node is plotted with a blue dotted line. There is a small iron deposit visible (black discolouration) in the cortex of the gland.*

The ex-vivo MR images show that all depicted nodes contain an amount of iron, both the nodes with metasates and the nodes without metastases. On order to make a better distinction a 1 to 1 correlation with the histopathology slides is necessary. Figure 4.7 shows a comparison between an H&E staining, Perls staining and T2* MR image of a sentinel node coupe with the presence of a macrometastase. De metastases in the depicted gland is 1.8cm in size. No clear metastase is visible on the corresponding ex-vivo MR image. However, it can be seen that there is no iron visible at the metastases site on the MR image and in the pathology slides. This could indicate that at tumor regions, no iron deposition is possible.

However, we have collected too little data (3 nodes with macrometastases and 1 micrometastase) to make hard statements about this phenomenon.

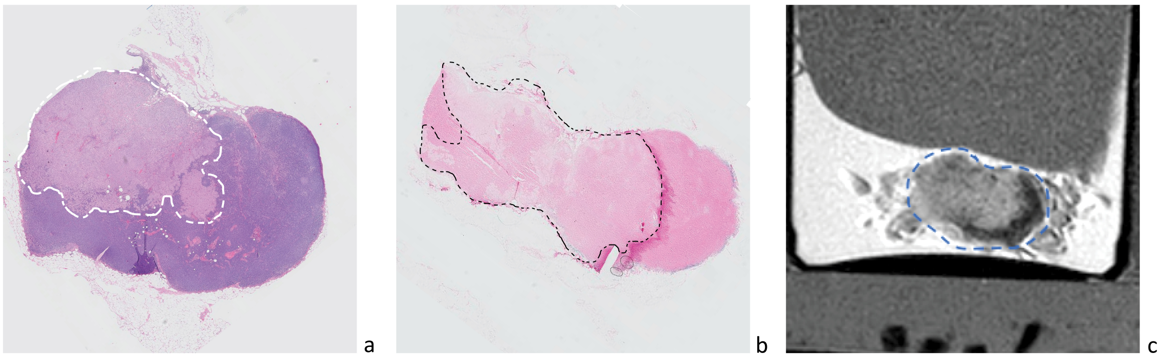

Figure 4.7 (a) Histopathology (H&E) section of a sentinel node with metastases. The macrometastase is visible whithin the white dotted line. (b) Histopathology slice (Perls staining) of the same sentinel node. As seen in fig. 4.4 in the metastatic area (within black dotted line) no iron is visible in this Perls staining section. In the normal/healthy part of the node iron is visible in the skin of the node. (c) T2 MR imaging of the corresponding slice; the node is visible whithin the blue dotted line. the regions were the metastase is present is much lighter than the region were the iron depostion is visible (black discolouration).

5.5 Discussion

The first results of this feasibility study, including 19 eligible patients with primary breast cancer show that the use of an extremely low dose of SPIO has the potential for a Magnetic SLN detection and evaluation. We evaluated whether it is possible to intra operatively measure any iron in case of a small dose of SPIO, in combination with pre- and post operative MR imaging of the iron distribution in the axillary nodes. In this study, 1.1 mg Iron was used instead of 3-56 mg Iron in previous research.

Table 4 showed that there was some discrepancy between the number of nodes measured during the operation, the number of nodes visible on MR and the number of nodes visible with pathology examination. It is very likely that during the intraoperative magnetometer measurements closely located sentinel nodes may appear as one hotspot because no distinction can be made at such a small distance, and besides the nodes, also some surrounding (fat) tissue is removed.

Intratumoral injection of the magnetic tracer allowed a successful localization and concurrent SLNB in 12 of 19 procedures. When the magnetic technique was combined with a sub-areolar Patent blue injection a successful identification was achieved in 16 of 19 procedures. The successful SLN identification rate was higher when the standard dual technique was used (successful in 17 of 19 procedures).
The underestimation of the Magnetic procedure has partly due to the fact that the measurements were unstable, and often high countings were noted at irrelevant places.

Considering the Sentimag is also sensitive to surrounding ferrous metal, we have tried to exclude these factors as much as possible. We therefore switched to the use of disposable plastic operating tools for performing the SLNB after 12 procedures. However, the measurements were still not stable. The assumption was that the iron is not only limited to 1 or 2 sentinel nodes, but extends in many other nodes and a pre- and post-operative and post- Sienna+ contrast MRI was performed in 1 patient to confirm this suspicion. The performed pre-operative MRI confirmed this suspicion. This partly could explain the great fluctuation during the SLN localization procedure. The high number of residual counts that was measured in the axilla after the elimination of the SN would substantiate this finding.

Another explanation for the unstable Magnetic measurement and high peak counts may be due to an undesired diamagnetic signal. The SPIO particles produce a positive signal in the surrounding of the probe, while the diamagnetic tissue produces a negative signal. [34] The resulting displayed signal is the sum of both the positive and negative counts. A positive measurement of the SPIO can be obscured by a negative signal of the surrounding tissue. This can result in failed sentinel lymph node detection. By balancing the probe on the skin on beforehand, it is attempted to prevent this phenomenon. However, it appears that during intraoperative measurements, more pressure and movements are used, which is eliminating the effect of balancing. It can even increase the signal, resulting in peak counts at places where no SPIO is present.

Further optimization of the technology is necessary to get the measurements stable. The time between injection and measurment/MRI and the amount of SPIO tracer should be considered. It is desirable that sufficient iron is injected so the nodes are detectable during intraoperative measurements with the Sentimag magnetometer, but not that much so artefacts occur on the pre-operative and ex-vivo MRI for the SN localization and metastases evaluation.

In addition, it is important to improve the limitations of the probe regarding the depth performance. Nowadays, there are many patients with a high fat percentage, which greatly increases the distance between the skin and the nodes. A transcutaneous measurement becomes almost impossible. Especially when using a low dose of iron. The range is now up to 3.5 cm, with a minimum of 8.5 cm desired.

First results shown that no iron is visible at the metastases site on the MR image and in the pathology slices. In order to recognize a clear pattern in the correlation between the pathohistology slices and MR images, more research needs to be done. In this study only 3 patients with macrometastases have been analyzed so no statistical analysis is possible. It is possible that micrometastases with a size of less than 2 mm cannot be distinguished on a SPIO contrast MRI. MR imaging has a limited resolution in the present settings and it is the question whether metastases smaller than 1 mm are visible with a MR slice thickness of 1mm. Because we measure a relatively high amount of iron in the nodes (with the Sentimag Magnetometer) it is possible that small metastases are underestimated in this way of

analyzing. The detection of isolated tumor cells is not feasible in this way. Therefore, it will be necessary to have a good look at the amount of iron that reaches the sentinel lymph nodes and the metastases will have to be recognized on a large scale according to a correlation pattern between MR images and histopathology slices.
It should be noted that fat containing nodes and scar tissue could distort the distribution of SPIO to the sentinel nodes. This can result in a lower iron intake in the node, while no metastases is present, leading to a false positive result.

Currently, there is no evidence that micrometastases have a prognostic value for survival. In two multicenter studies, the impact is investigated of the omission to remove all axillary lymph nodes in case of micrometastases, on survival. Based on the low incidence of micrometastases, the routine histological examination of the sentinel node biopsy should be aimed at detecting macrometastases. However, when a micrometastase is found, additional axillary lymph node treatment is indicated.[oncoline]

Further pathological research is necessary to gain information about the normal distribution of iron over the nodes. The iron that is visible at the pathology slices can also be the result of degradation after bleeding. For example, after a puncture for diagnostic purposes. It is advisable to carry out some iron staining on sentinel nodes without metastases, as well as nodes of patients who did not receive an SPIO (iron) injection. In this way a better distinquish can be made between iron distribution on metastatic and non-metastatic nodes.

The assessment of the MR images may require some explanation considering the fact these are not everyday findings. The T1 sequence depicted both iron and air black. For differentiation between these two, it is assumed that air gives an artefact with a small white peel on the T1 because it is a Gradient echo image (water-fat shift). This occurs because the 180-degree pulse is missing and there will not be any compensation for field inhomogeneity. This will show an artefact (white) next to the air on T1 sequences.
The water-fat shift is related to the entering width and field of view (FOV) and represents the total frequency range from one side of the FOV to the other. The used bandwidth of the scan protocol sequences could be examined to determine if this artefact can be avoided in the future.

Taking the occurence of the arise of the post-operative MR artefacts into account, as described in chapter 3, it is advisable to perform also some post-operative MRI's in the Lowmag trial, to ensure that no large amounts of iron remain in the axillary space or breast that could complicate follow-up with MRI in the future.

Since this study was a feasibility study, the number of patients is small (19 patients) en insufficient to perform any additional meaningful statistical analysis. The number of patients with metastases is too small (4 patients) to draw valid conclusions about the efficiacy of SPIO MRI to characterize and evaluate the SLNs. The first findings indicate that this is certainly feasible in the future, and further research will have to show this.

At this moment, the measurement with the Sentimag magnetometer in combination with the low dose SPIO injection does not work satisfactorily yet. The measurements fluctuate and a transcutaneous measurement is often unapplicable due to insufficient signal. Multiple sites in the axilla give high residual counts, after removing the sentinel node. This delayed the current method with the combined technique.
The SPIO injection has been moved after 19 patients from 6 to 4 days prior to surgery, with the aim of reducing the spread of the SPIO tracer over multiple lymph nodes. The expectation is that the SPIO tracer have less time to migrate, and will fill less lymph nodes with SPIO. This will result in a less extensive diffusion of SPIO in the axillary lymph nodes, and make the intra-operative measurements probably more stable. It is advised to try this in 5 patients, after which an interim analysis will follow about the intra operative measurements and MR images for metastases evaluation. In case of insufficient improvement of the intra operative measurements, an interval of 2 days between injection and surgery can be considered.

5.6 References

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6. The road to a complete radiation-free SLN evaluation and detection.

Different groups around the world are experimenting with radioisotope-free techniques, and the use of Magnetic detection seems the most promising. [13] This magnetic technique comprises an interstitial injection of Superparamagnetic Iron Oxide (SPIO) nanoparticles, which are identified intraoperatively with a handheld magnetometer, and has been evaluated in a number of studies. [6]

JJ Pouw evaluated in a previous study the depth performance of a handheld magnetometer. In ideal conditions the maximum 'probe-lymph node' distance at which the SLNs can be detected was about 3.75cm with a high iron uptake (500µg iron) measured in a phantom. [34] Because SLNs can reach 8.5 cm deep [35], a transcutaneous measurement in this way is not successful. An option would be to perform a preoperative MRI after administering the SPIO. This way, the optimal incision site can be determined and the transcutaneous measurement is obsolete.

In chapter 4 was demonstrated that the SPIO concentration/injected iron dose has impact on the usability of the magnetic procedure in clinical practice. The relatively high doses used in the phantom study[34] and previous clinical magnetic detection studies[6] resulted in large MR susceptibility artefacts at the injection and accumulation site.[9, chapter 4] This does not only make the evalutation of the post surgical MRI impossible, but it is also troubling the evaluation of the SLN localization in a pre-operative MRI.

The first results of the Lowmag trail[chapter 5], in which a lower iron dose was used in combination with pre-operative SPIO contrast MRI[36], demonstrated that this problem was avoided. In addition, a lot of information about the possible presence or absence of tumor cells in the SLN can be provided by following this procedure. It may be feasible to prevent patients for unnecessary surgival removal of a negative node, and post surgery morbidity, like edema formation and movement limitations of the arm [37], can be avoided. However, the Lowmag trail also showed that the intraoperative Sentimag measurements are not yet stable enough to perform a complete Sentinel node procedure. The time between the SPIO injection and measurement/MRI must be further optimized, as well as the exact amount of SPIO injectected.

Thus, a complete Magnetic sentinel node detection and evaluation procedure has the potential to actually be used in clinical setting but first; there are several factors to be improved. First, the iron dose of the intra-tumoral Sienna+ injection has to be optimized so enough iron is present in the nodes to detect them during surgery, but not too much so artefacts occur during pre- and post-operative MR imaging. The following chapter describes whether a very low dose of iron can be measured in the nodes during surgery. Second, it is examined whether it is possible to detect/evaluate node metastases during pre-operative MR imaging non-invasively. This may result in the ability to only remove the SNs that are suspicious for metastases. If a SLNB is not necessary, morbidity associated with the biopsy can be avoided.

6.1 Sentinel lymph node localisation

In previous studies patients were included in the MST, Enschede in various Magnetic SLN Detection trials between May 2012 and June 2017 (NTR3238, NTR4691 and NTR 4858, http://www.trialregister.nl). In the SentiMAG Multicentre study patients received a subareolar injection with 2mL (56mg iron) Sienna+® magnetic tracer (Endomagnetics Ltd, Cambridge, UK) diluted in 3mL normal Saline. In the MagSNOLL trial an intra-tumoral injection of 0.1-0.5 mL Sienna+® (2.7mg-13.5 mg iron) was administered. In the LowMAG trial patients received an intratumoral injection of 0.04 mL Sienna+® (1.1 mg iron) diluted in 0.46 mL Saline. (Table 5.1) All participating patients had undergone a sentinel lymph node biopsy at the time of a breast conserving surgery or mastectomy. In the three studies to be analyzed the Magnetic tracer was injected in different ways and in various periods as seen in table 5.1.

	Sienna+® (mL)	Iron (mg)	Total volume injected (mL)*	Injection site	Time between injection and SLNB (h)
SentiMAG trial	2	56	5	Sub-areolar	Intra-operative
MagSNOLL trial	0.1-0.5	2.7-13.5	0.1-0.5	Intra-tumoral	22-26
LowMAG trial	0.04	1.1	0.5	Intra-tumoral	92-145

*Table 5.1 Study protocol details.*Sienna+® diluted in normal Saline.*

In the Sentimag Multicentre Trial the sentinel node identification rate was 95% with the standard dual technique and 94 % with the magnetic technique, with a discordance rate (disagreement) between the standard and magnetic technique of 6.9%.
In the MagSNOLL Multicentre Trial a successful identification rate of 78% was achieved with the magnetic detection technique. However, this was greatly improved to 90% when the magnetic technique was combined with the Patent blue injection. This was also noted at the LowMAG trial. The successful SLN detection rate improved from 63% with the magnetic technique to 84% with the combined magnetic and patent blue technique. Table 5.2

	Successful detection Standard dual technique	Successful detection Magnetic technique	Successful detection Magnetic + Patent Blue technique	Discordance
SentiMAG	95%	94%	94%	6.9%
MagSNOLL	93%	78%	90%	18%
LowMAG	89%	63%	84%	5.3%

Table 5.2 Successful detection rate of SLNB in 3 Magnetic localization studies.

The magnetic technique has the ability to carry out the SLNB procedure, but the successful identification rates are not yet superior to the standard dual technique. It greatly improves when the magnetic technique is combined with the patent blue injections, but further optimization has to been done. The discordance between the standard and magnetic technique occurs because the Sentimag measurement are much less stable than the Gamma probe measurements. Besides the second Sentimag probe is a bit more unstable than the first Sentimag probe [34], there are also some differences in the magnetic study protocol that can exacerbate the fluctuations.

During the Lowmag trial, more residual counts were found in the axillairy space, after removing the sentinel node, than at the Magsnoll trial (fig 5.3). While even less iron was injected. 1.1 mg iron in the Lowmag trial vs 2.7-13.5 mg iron in the Magsnoll trial. Another difference in both study protocols was the time between injection of the iron particels and the intra-operative measurments, 1 day at the Magsnoll patients vs 6 days in the Lowmag patients. This may indicate that the longer time between injection and measurements cause iron to migrate to the underlying nodes, and not only in 1 or 2 sentinel nodes.
To substantiate this phenomenon, pre-operative MR imaging was performed to show the presence of the iron distribution over the axillary nodes.
The pre-operative MRI perfomed one hour after the intra tumoral 0.1ml Sienna+® injection showed a large susceptibility artefact at the injection site (black arrow). In addition, three normal axillary lymph nodes are visible without any iron uptake (white arrows) (fig 5.1a). The pre-operative MRI performed 20 hours after the intratumoral 0.1 ml SPIO injection shows beside the artefact at the injection site (black arrow) and normal axillary glands (white arrow), also 2 glands with iron uptake. The 119 hours (5 days) post 0.04 ml Sienna+® MRI shows a large spread of iron particles in 8 glands over multiple levels. Axillary, parasternal and subpectoral nodes and in several regions dorsal of the pect. Major against the thoracic wall are multiple susceptibility artefacts visible due to the SPIO injection. This large amount of iron is remarkable because significantly less iron is used at the intratumoral injection (0.04 ml Sienna+®). (Fig 5.2) The long time between injection and MRI appears to increase the SPIO spread over glands in multiple levels.

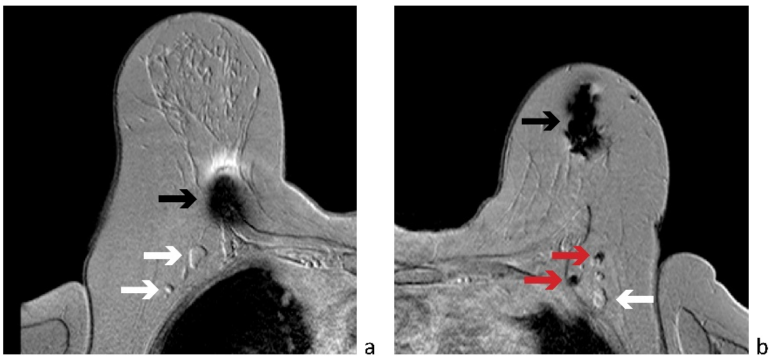


*Figure 5.1 Pre-operative SPIO MRI of 2 patients. Both patients received a 0.1 ml intratumoral Sienna+ injection.(a)T2 weighted MR image. Time between injection and MRI is **1 hour**. A large susceptibility artefact at the point of injection is visible (black arrow). Multiple normal axillary nodes are visible without any SPIO uptake (white arrows). (b) T2 weighted MR image. Time between injection and MRI is **20 hours**. A large susceptibility artefact at the point of interest is visible (black arrow). 2 axillary nodes are visible with SPIO uptake (red arrows), also some normal axillary nodes are visible without any SPIO uptake.*

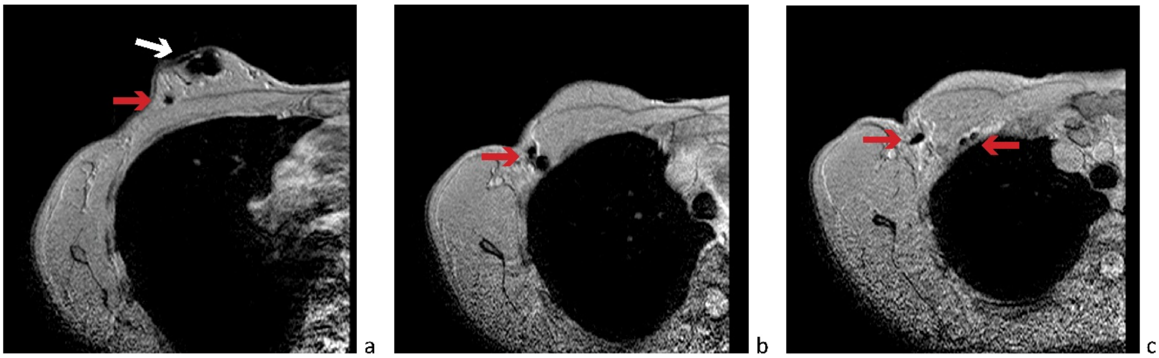


*Figure 5.2 Pre-operative SPIO MRI of 1 patient. This patient received a 0.04ml intratumoral Sienna+ injection. Time between injection and MRI is **119 hour**. A large susceptibility artefact at the point of injection is visible (white arrow). Multiple axillary nodes with SPIO uptake are visible at multiple levels (red arrows).*

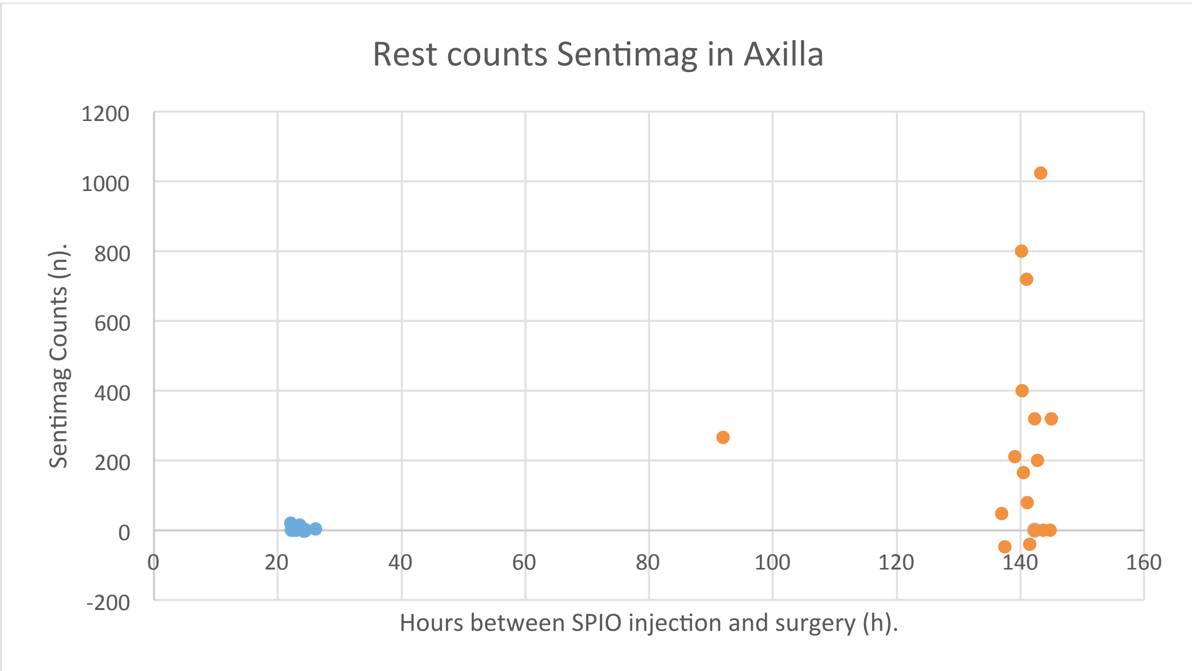


Figure 6.3 Rest Counts Sentimag in Axilla after sentinel node removal.
Blue dots: 0.1 ml Sienna+ (2,7mg iron) Magsnoll trial
orange dots: 0.4 ml Sienna+ (1,1mg iron) Lowmag trial the lowmag and magsnoll trial were performed with different probes. Therefore, the measurements of the lowmag trial are scaled to make a better comparison.

Table 5.2 shows that during the Sentimag trial the highest successrate for the magnetic detection was achieved. This would assume that a high dose of iron injected sub-areolar would be the most successful. The large amounts of iron have a big downside, which appears in the Artefact study (chapter 3) [9]. This study shows that a large amount of iron injected sub-areolar creates large artefacts, which could be a barrier for the diagnostic evaluation in the follow-up of breast cancer patients.
The MRI is not a standard follow-up method for breast cancer patients, it's actually used in patients with lobular carcinoma, patients with dense breast tissue and patients with a high risk by the breast cancer gene mutation. The loss of MRI as a diagnostic tool is therefore not

desirable.
A low amount of iron in combination with an intra-tumoral injection leaves no residual iron at the injection site and no artefacts show up at a post-operative MR image. This injection technique is therefore recommended for any subsequent studies.

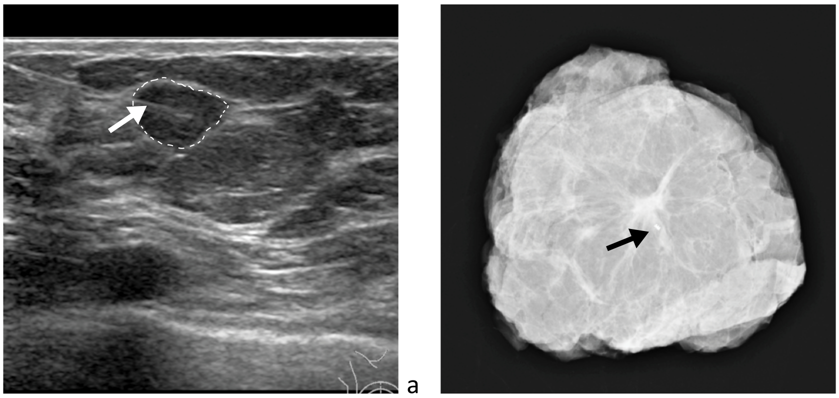
6.2 Occult lesion localisation

The current method for the localisation of occult lesions is done using a wire location or iodine seed. At the wire localisation a metal wire is placed in the tumor under ultrasound guidence. This wire is very sensitive for migration and involves an increased chance of complications. The iodine seed is a good alternative, but has the disadvantage of being radio active. This entails enormous logistical disadvantages, so only 18% of the Dutch hospitals work with this technique currently.

In the MagSNOLL trial [33], the intra-tumoral Sienna+® SPIO tracer was used for occult lesion localisation. The magnetometer was used to localize the centre of the tumor, and an excision of the breast lesion was undertaken with an oncological margin. After removal of the lump, a control mammography was made of the lump at the radiology department. It was verified that the marker, which was previously left in the tumor, was in the center of the lump (Fig 5.4b).

In the MST hospital in Enschede, 10 procedures were performed within the Magsnoll trial in which the non-palpable tumor was located by the intra-tumoral Sienna+ injection and intra-operative magnetometer measurements. The wire localization (currently golden standard for these procedures) was not perfomed, so the surgeon could not be affected by the (visible) presence of the wire. Instead, a hydrophilic marker was placed in (or next to) the tumor so the precize location could be radiographically checked after surgery.
The ultrasound guided intra-tumoral Sienna+ injection ran without any problems (Fig 5.4a). The patients experienced little inconvenience from the injection. In some cases, patients experienced mild pain when injecting the SPIO suspencion. This pain was disappeared right after the procedure.

In all 10 patients, the tumor was located precisely. All tumors have been removed radically, and no re-excision was necessary.
In the first 2 procedures, iron was also measured in the course of the tumor to the skin. This caused some fluctuations during the measurements, making it less accurate or stable. During the following injection procedures, a small bubble of air was injected after the iron suspencion. This ensured there was a clean needle without iron drawn towards the skin. The contamination of the measurement was over then.



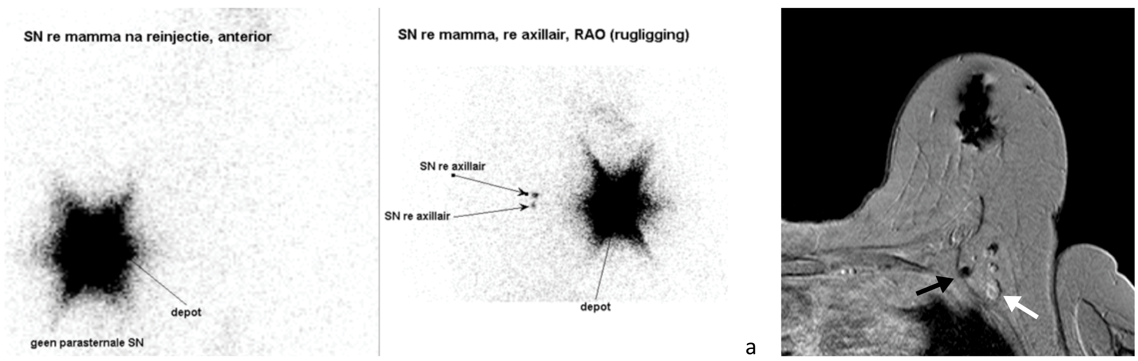
a b

Figure 4.4 (a) An Ultrasound image of the intra-tumoral Sienna+ injection. The tumor is visible within the white dotted line. The injection neelde is visible going in the centre of the tumor (white arrow). (b) An intra-operation Mammography of the lump. In the centre a star-shaped lesion is visible, this corresponds with the aberration on the mammogram. Right next to the lesion a coil-shaped hydrophilic marker is visible.

6.3 Pre-operative MRI

In the current combined technique for SLNB using radioactivity, a lymphoscintigraphy is performed prior to surgery to determine the presence, location and amount of SNs. This lymphoscintigraph is quite inaccurate given the large amount of noise, and the lack of anatomical structures (fig. 5.5a).

In case of a complete magnetic procedure, a pre-operative MRI would be of great value. In addition to locate the SN, pre-operative MRI could also be considered for the evaluation of the presence of possible metastases (fig. 5.5b). The first results of the Lowmag trial show no clear metastase is visible on the corresponding ex-vivo MR image. But there can be seen that there is no iron visible at the metastases site on the MR image and in the pathology slides. This could indicate that at tumor regions, no iron deposition is possible. [36] A pre-operative MR image of a metastatic node could show an inhomogeneous distribution of the iron, as opposed to an homogeneous iron distribution in nodes without metastases.
In order to substantiate this assumption, a large observational study of SPIO-enhanced 3T MRI versus sentinel node biopsy is needed to confirm the accuracy of this technique, and may avoid sentinel node biopsy in patients with node-negative breast cancer.



a b

Figure 5.5 Radioisotope guided lymphoscintigraphy (a) vs SPIO guided MRI (b).

6.4 SPIO metastases evaluation

During the analysis of the intra operative measurement data, it has been observed that the Sentimag counts in metastatic nodes are much lower than de Sentimag counts in non-metastatic nodes. This would suggest that tumor tissue couldn't absorb the iron. Table 5.6 shows that the median of the sentimag counts in metastatic nodes are lower in both studies. However, in the Lowmag trial, the measurements are still unstable, hence the spread is bigger and there are also some large outliers (peak at 7000 counts). The outliers cannot be explained, and further research will need to be done.

In case of a few tumor cells (micrometastases or isolated tumor cells), the flow/iron uptake in the nodular tissue is not be disturbed. Only in case of large metastases (macrometastases >2mm) the flow will be disturbed, and the nodular tissue may absorb less iron. However, for clinical practice it is not so relevant. The detection of isolated tumor cells does not affect the prognosis or further treatment. The presence of micrometastases will indicate regional treatment, but its incidence is very low regarding the current histopathological detection, which currently affecting its clinical relevance also.

It is questionable whether sentinel nodes will be missed (false-negative sentinel node procedure) if the entire node is metastatic, and no iron can be absorbed in the nodular tissue. To provide information about the extent of the cancer, research on the status of the first lymph node is necessary and reliable examination of the sentinel lymph node (SLN) is crucial, as a false-negative finding may result in undertreatment of the cancer.

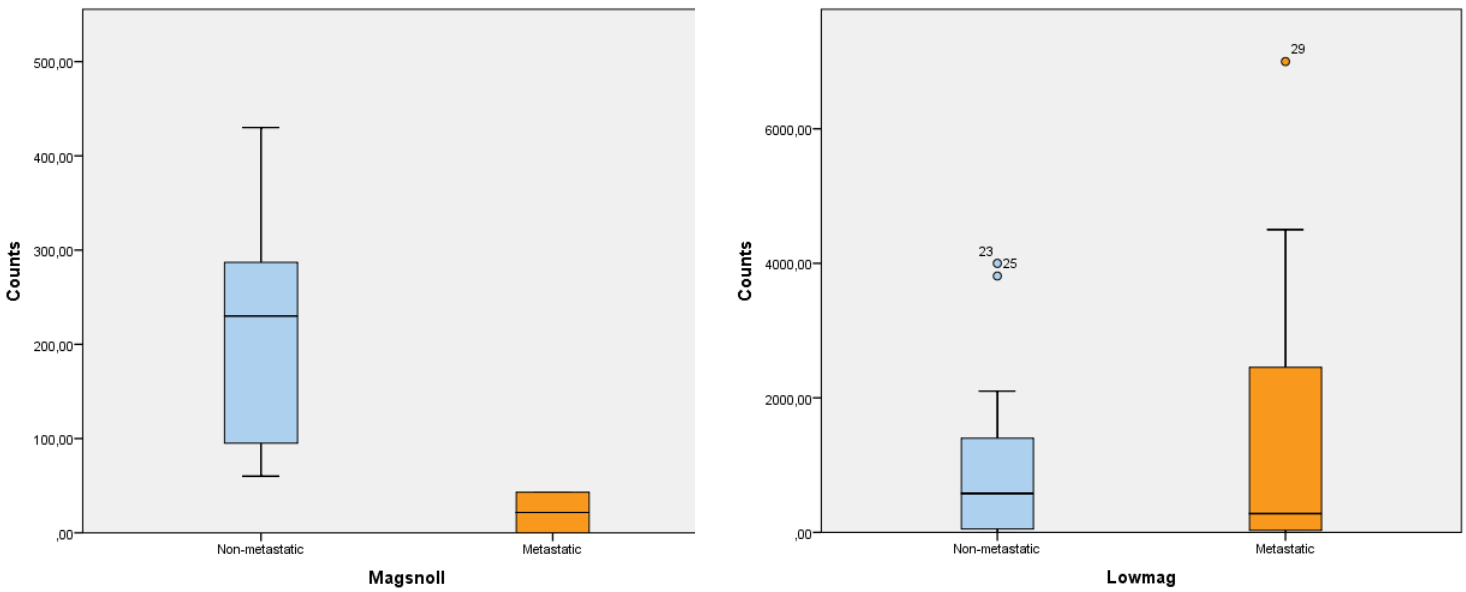


Figure 5.5 Sentimag Magnetometer counts in metastatic and non-metastatic nodes, in the Magsnoll and Lowmag trial. It should mentioned that different Sentimag probes are used in the Magsnoll trial and Lowmag trial.

It is remarkable that the range of graph 5.6 at the Magsnoll trial reaches 500 where it is up to 7000 in the Lowmag trial. This indicates that in the Magsnoll trial less iron has ended up in the nodes, while significantly more iron is injected. Since the time between injection and measurement is the only deviating factor, it appears that the long time interval in the Lowmag trial ensures a higher absorption of iron in the nodes.

5.5 Complete magnetic sentinel node detection and evaluation procedure

If we combine the results of the above studies, it is possible to optimize the magnetic SN procedure.

The sentinel lymph node localization was best performed within the Sentimag trial. Here a high SN identification rate was performed close to the successful gammaprobe procedure. This is due tot the high concentration of iron present in the SNs. In the Magsnoll and Lowmag trial the intra-operative SN detection was a lot more difficult, and transcuateous localization was often not feasible. A logical step would be to continue with the high iron doses. However, it turns out that this entails many disadvantages, mainly major artefacts in the axillary region and in the breast tissue on pre- and post-operative MR imaging even 5 years after surgery.[chapter 4] In order to prevent post-operative artefacts in the breast area, it is advisable to continue working with a **low dose of iron** between 1,1 and 2,7 mg. In this way, post-operative diagnostics can be ensured, and optimal use can be made of MR diagnostics during follow up. No postoperative artefacts were found in the Magsnoll Trial (2,7 mg iron injected), so we don’t expect these artefacts at a lower iron dose in the Lowmag trial (1.1 mg iron injected). For the same reason, it is advisable to inject iron particles **intra-tumorally** (Magsnoll and Lowmag trial) instead of sub-areolar (sentimag trial). This will ensure the removal of the most iron within the extracted breast tissue, and less iron is left in the healthy breast tissue. In addition, the intra tumoral SPIO injection can also be used for the **localisation of occult lesions**. Wire localisation or iodine seeds will no longer be needed if the iron particles in the tumor are used to locate these non-palpable tumors.

The pre-operative MR images have shown that a prolonged interval between injection and measurement/MRI increases the distribution over the lymph nodes. If there is a long interval after the injection, the iron will not only limit to 1 or 2 SNs, but will further migrate through the lymphatic system to other lymph nodes across different layers in the axillary and thoraric regions. In this way, it is impossible to recognize the SN, and the intraoperative measurements will be disturbed because iron is present in all layers of the surrounded tissue. By carrying out the **iron injection 1 or 2 days before surgery**, the spread of the iron will be limited to the SN. It is also expected that the intraoperative measurements will be less distorted in this way.

Chapter 4 showed that is is possible to **pre-operative evaluate the SNs** whether there are metastases present, using the iron distribution pattern over the nodular tissue. If a small amount of iron (1.1 – 2.7 mg) is used, pre-operative MRI can be performed without iron-related artefacts disturbing the image. In this study, the iron distribution has been analyzed by detailed ex-vivo MR imaging. Further research should indicate whether this metastases evaluation is also possible during a normal pre-operative MRI. The MR protocol may need to be optimized so the nodes in the axillary region can be visualized more in detail. You should be able to determine where these nodes are located prior to this scan, using the Sentimag Magnetometer.

In order to perform a complete magnetic sentinel node detection and evaluation procedure an ultrasound-guided intra-tumoral injection of 2 mg iron should be perfomed, 1 day before surgery. 2 mg iron containing SPIO tracer is desirable because the intraoperative measurements in the Lowmag trial (1.1 mg) are not good enough. By increasing the amount of iron, the successful intra operative detection rate of the sentinel node will probably improve. The iron dose should not be too high so adverse effects of the high dose as mentioned in chapter 4 can be avoided. The spread of the SPIO tracer will be limited to 1 or 2 sentinel nodes and the risk of post-operative MRI artefacts is negligible, so follow-up by MRI will not give any problems. A pre-operative MRI will be performed just before surgery (1 day after SPIO injection) to determine the exact location of the sentinel node pre-operatively. If the iron distribution across the lymph node gives no suspicion for metastases in the lymph nodes, the sentinel node biopsy can be avoided. A non-invasive evaluation then suffices. In case of doubt about the metastases status in the lymph nodes, the SPIO in de lymph nodes can be used for intra-operative detection, after which histopathological research will follow. If a transcutaneous measurement fails due to the low dose of iron, the pre-operative MRI can be used to determine the location of the incision. The SPIO tracer in the tumor can also be used to detect occult breast lesions, so a wire localization (high chance of migration and complications) or iodine seed (radiation) is no longer necessary.

6.6 General Discussion

A low dose Sienna of 1.1 mg is not accurate for intraoperative detectability of the SLN using the SentiMag magnetometer system. The perfect Sienna+ dose will have to be further investigated. It is now known that a high dose of iron causes a disturbance on MRI evaluation and detection of the nodes by the presence of artefacts. As described in Chapter 5 a low iron dose makes the detection measurements during operation very difficult. For this time, a correct dose seems between 1.1mg and 2.7 mg of iron. However, the effect of the iron dose will coincide with the time interval between injection of the tracer and measurement/imaging as described in chapters 6.1 and 6.4.

The magnetic technique has the ability to carry out the SLNB procedure, but the successful identification rates are not yet equal to the standard dual technique. It greatly improves when the magnetic technique is combined with the patent blue injections, but further optimization has to been done.

To overcome the limitations of the probe regarding the depth performance, the pre-operative MRI can be the solution. Nowadays, there are many patients with a high BMI and fat percentage, which greatly increase the distance between the skin and the nodes. A transcutaneous measurement becomes almost impossible. Especially when using a low dose of iron. The range is now up to 3.5 cm, with a minimum of 8.5 cm desired. The pre-operative MRI can be used to determine the optimal incision site prior to surgery, and so replace the inaccurate transcutaneous measurement. The MRI will take place approximately 20 hours (1 day) after Sienna+ injection, since 1 hour is too short, en 119 hours too long as been described in chapter 6.1. The pre-operative SPIO MRI provides the surgeon also detailed anatomical information.

The secondary goal of this study was to evaluate the potential of SPIO MRI evaluation to identify Lymph node metastases pre-operatively and prevent patients for unnescessary surgical removal of negative lymph nodes, en post surgery morbidity. Chapter 5.4 describes that a difference in iron uptake between normal and tumor tissue is visible on SPIO MR imaging. This phenomenon will need to be further investigated so a pattern can be found in recognizing iron uptake in healthy vs tumor nodular tissue. It should be noted that the differences are only seen in macrometastatic nodes. In case of micrometastases and isolated tumor cells, detection of metastases by SPIO MRI will be difficult because such small metastases will not disturb the flow/uptake in the nodes to that extent that it is visible on MR imaging. The time interval between injection and measurement/imaging should be carefully considered. In a short perio of time, no iron is absorbed in the nodes, which can cause a false positive result, if the node is still half filled with iron.

The manufacturer of the Sentimag Magnetometer (Endomagnetics Ltd, Cambridge, UK), is working on an improvement of the technology. The probe has undergone further development in the past years, meaning that the Sentimag and Magsnoll trials were both performed with probe 1, were the Lowmag trial was performed with probe 2. J. Pouw [34] has shown in his article that there is a difference in sensitivity between the two probes. Probe 2 shows a higher mass sensitivity (Sentimag Signal/mg iron) than probe 1. In order to make a better comparison, the results of probe 2 are scaled to match the probe 1 data. Besides the higher mass sensitivity of probe 2, the measurement of this probe is less stable, reflected in higher standard deviations. The signal fluctuates very fast, and any signal smaller than 50 were difficult to record.

The costs of this new technology must also be considered. SPIO-enhanced MRI, after further improvement, may be sufficient for evaluation of the sentinel node. Besides, this Magnetic evaluation technique can lead to avoidance from axillary surgery, in patients with negative nodes on imaging, with less post-surgery morbidity.
Working with SPIO is at first sight not cheaper than the current radioactive tracers. However, The SPIO tracer is not dealing with a half-life time, which makes it easier and cheaper to transport, store and work with it. Because we are no longer exposed to radioactivity, the logistic workflow will be easier and no facilities need to be purchased/maintained.

First results shown that no iron is visible at the metastases site in histopathology slices. In order to recognize a clear pattern in the correlation between the pathohistology slices and

MR images, more research has to be done. With the Perls Fe staining in histopathology, free FE^{3+} is stained. In the presence of the iron-cyanide ions in the worksolution, the free iron precipitates prussian (berlin) blue (= ferric ferrocyanide). As anti-dye, kernel red is used, which causes the cores to stain red and the cytoplasm becomes pink. The sienna + contains a hydrophilic colloidal suspension of superparamagnetic iron oxide particles. However, these iron particles have a biocompatible organic coating. It is the question of whether this coating affects Perl's staining, and more iron is present in the pathological slices than is now visible.

From previous research it has been found that only 2% of the injected iron reaches the nodes. In case of an injection of 1.1 mg whitin the Lowmag trial, this will be +/- 0.022 mg of iron. Further research into the sensitivity of the Perl's iron staining has to show if this amount of iron is sufficient to visualize with this coloring technique. Besides, iron stacking in the axillary nodes may also arise due to abnormalities in iron metabolism or local accumulation of broken erythrocytes, after bleeding.

It is possible that nodular involvement can only be detected by SPIO MRI in case of macrometastases. Micrometastases and isolated tumor cells may not be noted because this causes too little disturbance in the nodular iron uptake. However, it is the question if these ‘missing’ micrometastases and isolated tumor cells are such a major disadvantage. Recent research indicates that the detection and evaluation of these small metastases only give a minimal clinical benefit for patients’ treatment and prognosis. [38] It is the question how valuable the detection of very small metastases is, in case of the increasing use of axillary radiotherapy.

The main drawbacks of the Magnetic technique using the Sentimag Magnetometer include the need to remove all metal retractors and other iron containing items before the magnetometer readings during surgery. Prior to each intra operative measurement the probe must be balanced on the skin of the patient, to compensate for undisired diamagnetic influences. Balancing interrupts the surgeon at his work, which negatively affects the patience. Because we are unable to filter out all undesired diamagnetic signals, it would be desirable to develop a technique without these characteristics. The new develeped Diffmag technology (University of Twente, Netherlands) is not sensitve to any diamagnetic signal and can be used in conjunction with the normal surgical equipment. It is very likely that the intraoperative measurement should be more stable and the technique more user friendly with a quicker workflow. Not only in logistics but also in the technical field there is still a profit to be achieved.

The main conclusions are allready discussed. In summary, with the work in the previous years with the magnetic detection procedures many things have already been improved by making adjustments in the logistic process and the work experience that is gained during the measuments. Unfortunately, it has not been possible to overcome all the drawbacks in this period of time. But I am convinced that is the technology is still improving, and the measurements can become more stable, the magnetic detection and evaluation technique certainly has potential for the future.

It seems that the own magnetism of the human body creates the largest disturbance during the current measurements with the Sentimag Magnetometer. Balancing on a neutral spot (the sternum) must compensate for this diamagnetic signal, but in practice it doesn't work properly. In addition, it is very disturbing that the Sentimag magnetometer responds to the surgical equipment. It would be a great addition to examinate the Diffmag magnetometer (University of Twente, Netherlands) in the Lowmag trial. The Diffmag does not respond to the human body's diamagnetic signal and can also be used in combination with the surgical equipment. However, the Diffmag must first have a CE certification before it can be used in clinical setting.

7. Bibliography

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