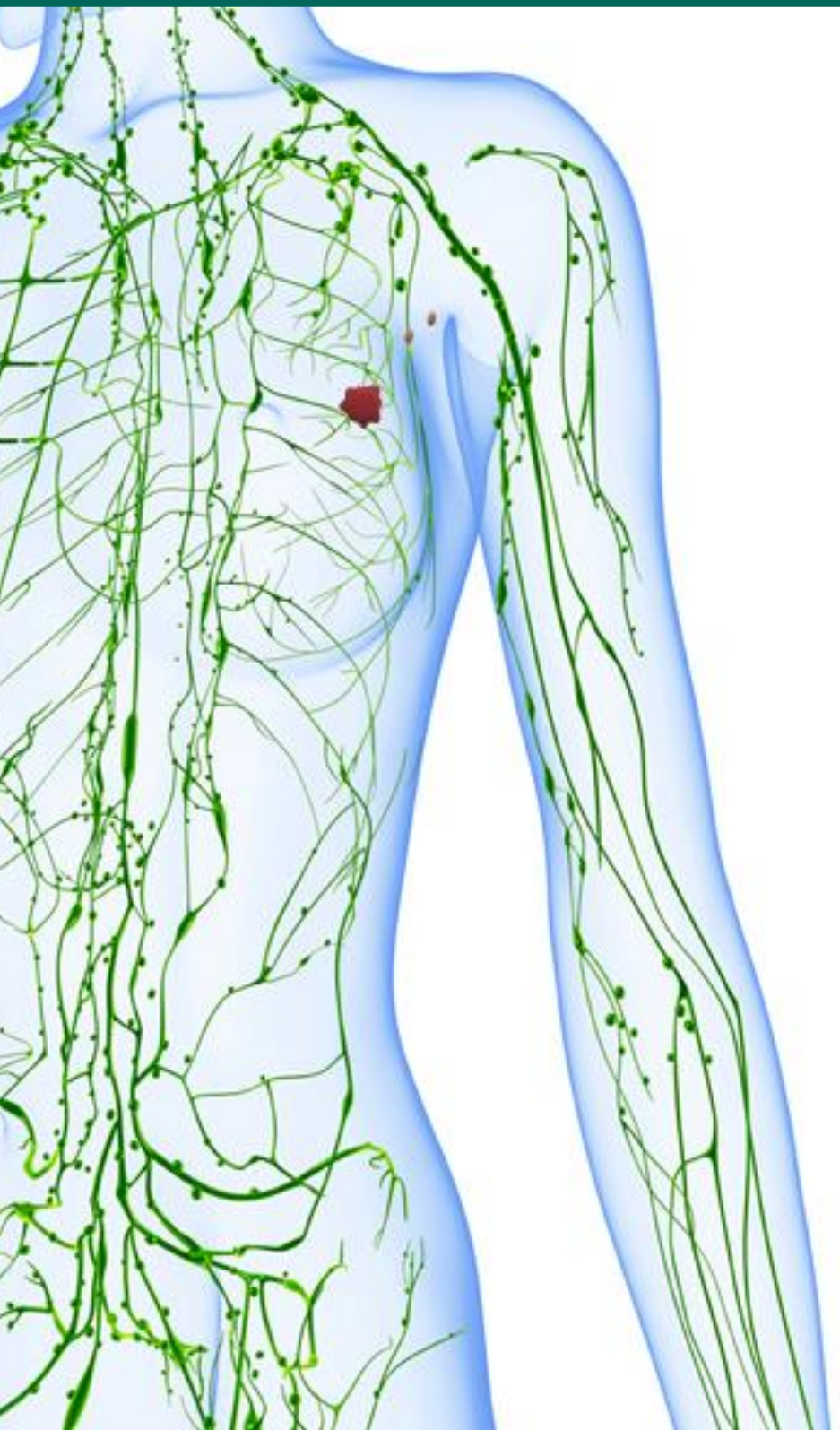
Optimisation of the Magnetic Procedure to Detect the Sentinel Lymph Node in Breast Cancer Patients



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Technical Medicine

23 September 2020

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Optimisation of the magnetic procedure to detect the sentinel lymph node in breast cancer patients

Master Thesis

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Technical Medicine

Medical Imaging and Interventions

23 September 2020

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Surely God is my help;
the Lord is the one who sustains me.

~ Psalm 54:4 NIV

Zie, God is mijn helper,
de Heer is het die mijn leven draagt.

~ Psalm 54:6 NBV

Summary

Investigation whether tumour cells are metastasized to lymph nodes is necessary for appropriate treatment of breast cancer patients. If no suspected lymph nodes can be found during clinical examination, sentinel lymph node biopsies (SLNBs) are performed using radioactive tracers and blue dye. However, this procedure has major disadvantages in use because of short half-lives of radioactive tracers and strict guidelines for working with radioactivity. To overcome these limitations, magnetic SLNB procedures using superparamagnetic iron oxide nanoparticles (SPIOs) have been developed. Initial studies showed detection rates of the sentinel node (SN) non-inferior to conventional SLNBs. However, high SPIOs doses induced artefacts on postoperative magnetic resonance imaging's (MRIs). Therefore, a pilot study using low SPIOs doses, named the LowMag trial, has been performed. Surgeons expressed that it was difficult and sometimes impossible to magnetically detect the SN. Therefore, the aim of this thesis was to investigate how to improve magnetic detection of SNs using the Sentimag magnetometer and low dose SPIOs.

First, we performed phantom studies to determine the correlation between the number of counts detected with the magnetometer and the iron dose. Subsequently, we performed retrospective analyses using data from the LowMag trial and two prior breast cancer studies, the SentiMag trial and MagSNOLL trial. These studies used respectively an intratumoural SPIOs injection containing 1.1 mg iron, a periareolair SPIOs injection containing 56 mg iron, and an intratumoural SPIOs injection containing 2.8-14 mg iron. We compared the iron content in SNs. In addition, we investigated whether the iron content in SNs correlated with the time interval between injection and surgery, and following personal factors: age, body mass index (BMI), breast size, tumour size, and tumour location. Subsequently, for ten patients, the iron content in the SN after a peritumoural injection has been examined. For eight of them, the injected iron dose was increased to 4.4 mg iron. The results of these ten patients were compared to the iron content in the SN after an intratumoural injection from prior LowMag trial results. In addition, we compared this magnetic SLNB with conventional SLNB regarding detectability of the SN. At last, for five patients, we evaluated the presence of SPIOs induced artefacts on postoperative MRIs of the breast made six weeks after surgery.

We found a correlation between the detected number of counts and iron dose for a fixed position of the SPIOs to the magnetometer. In addition, we showed that the distance between SPIOs and the magnetometer strongly affects the detected number of counts. We found a significant difference in iron content in the SN between the LowMag trial and SentiMag trial (p=.000), and no significant difference between the LowMag trial and MagSNOLL trial (p=.705). Furthermore, no significant correlation was seen between iron content in the SN and age (p=.241), BMI (p=.314), breast size (p=.770), tumour size (p=.609), tumour location (p=.065), and time interval (p=.433). In addition, we found no significant difference between the iron content in the SN using intratumoural or peritumoural injections (p=.723). SNs were

magnetically detectable in 50% of the procedures, whereas all SNs were detectable using the conventional procedure (n=10). The surgeons agreed that it was easy to magnetically locate the SN in 80% of the procedures in which it was possible to magnetically detect the SN. It was easy to locate the SN using the conventional SLNB procedure in all procedures. At last, no void artefacts induced by remaining SPIOs in the breast were visible in all postoperative MRIs.

We confirmed that it is difficult to magnetically detect the SN during the LowMag trial, since the SN was magnetically detected in 50% of the procedures (n=10). A promising finding is that surgeons agreed that it was easy to locate SNs using the magnetic SLNB procedure in 80% of the procedures in which it was possible to magnetically detect the SN. Furthermore, we found that postoperative MRIs showed good assessable images of breast tissue, without SPIOs induced artefacts after a peritumoural SPIOs injection. In addition, we found no significant correlation between personal factors and iron content in SNs, which suggests that SPIOs injection procedures do not have to be person specific. For implementation in conventional care, detection rates of magnetic SLNBs using a low dose SPIOs have to be further increased. The possibility to magnetically detect the SN is mainly affected by the following three factors: the size and dose of the magnetic tracer, the technique of the magnetometer, and the experience and understanding of the surgeon.

Samenvatting

Voor de juiste behandeling van borstkanker is het belangrijk om te weten of tumorcellen gemetastaseerd zijn naar de lymfeklieren. Als er geen verdachte lymfeklieren gevonden worden tijdens klinische onderzoeken dan krijgt de patiënt een schildwachtklierprocedure (SWK-procedure). Tijdens de SWK-procedure wordt gebruik gemaakt van een radioactieve tracer en een blauwe kleurstof. Er gelden strikte richtlijnen voor het werken met radioactiviteit en de radioactieve tracers hebben korte halfwaardetijden, waardoor de uitvoering en planning van de SWK-procedures beperkt worden. Om deze beperkingen te overkomen is een magnetische SWK-procedure ontwikkeld. Deze procedure maakt gebruik van een magnetische tracer, de super-paramagnetische ijzeroxide nanodeeltjes (SPIOs). Eerste studies laten zien dat de detectiepercentages van de schildwachtklier (SWK) vergelijkbaar zijn voor de magnetische en conventionele SWK-procedures. De hoge SPIOs dosissen induceerden echter artefacten op postoperatieve magnetic resonance imaging-scans (MRI-scans). Daarom wordt er een pilotstudie, de LowMag-studie, uitgevoerd waarbij een lage dosis SPIOs geïnjecteerd wordt. De chirurgen lieten echter weten dat ze het lastig en soms onmogelijk vonden om de SWK magnetisch te detecteren. Daarom was het doel van deze thesis om te onderzoeken hoe de magnetische detectie van de SWK verbeterd kon worden gebruik makend van de Sentimag magnetometer en een lage dosis SPIOs.

Allereerst hebben we fantoomstudies uitgevoerd om de correlatie te bepalen tussen de ijzerdosis en het aantal tellingen gedetecteerd door de magnetometer. Vervolgens hebben we retrospectieve analyses uitgevoerd op de data van de LowMag-studie en twee eerdere borstkankerstudies, de SentiMag- en MagSNOLL-studies. Deze studies gebruikten respectievelijk een intratumorale SPIOs injectie met 1.1 mg ijzer, een periareolaire SPIOs injectie met 56 mg ijzer en een intratumorale SPIOs injectie met 2.8 – 14 mg ijzer. We vergeleken het ijzergehalte in de SWKs. Daarnaast hebben we onderzocht of het ijzergehalte in de SWKs een correlatie heeft met het tijdsinterval tussen de injectie en de operatie. Ook hebben we onderzocht of het ijzergehalte in de SWKs een correlatie heeft met de persoonlijke factoren: leeftijd, body-mass index (BMI), borstgrootte, tumorgrootte en tumorlocatie. Vervolgens is voor tien patiënten onderzocht wat het ijzergehalte in de SWKs is na een peritumorale injectie. Voor acht van de tien patiënten was de geïnjecteerde ijzerdosis opgehoogd naar 4.4 mg. Deze resultaten zijn vergeleken met het ijzergehalte in de SWK na een intratumorale injectie uit eerdere resultaten van de LowMag studie. Daarnaast hebben we de magnetische en conventionele SWK-procedure met elkaar vergeleken betreffend de detecteerbaarheid van de SWKs. Ten slotte hebben we onderzocht of er door SPIOs geïnduceerde artefacten aanwezig zijn op postoperatieve MRI-scans van de borst. Hiervoor hebben zes weken na de operatie MRI-scans gemaakt bij vijf patiënten.

We hebben de correlatie tussen de ijzerdosis en het aantal tellingen bepaald voor een vaste positie van de magnetometer en SPIOs. Aanvullend hebben we gevonden dat het aantal

tellingen sterk werd beïnvloed door de afstand tussen de SPIOs en de magnetometer. Daarnaast hebben we een significant verschil in ijzergehalte in de SWKs gevonden tussen de LowMag- en SentiMag-studie (p=.000) en geen significant verschil tussen de LowMag- en MagSNOLL-studie (p=.705). Verder hebben we geen significante correlatie gevonden tussen het ijzergehalte in de SWK en leeftijd (p=.241), BMI (p=.314), borstgrootte (p=.770), tumorgrootte (p=.609), tumorlocatie (p=.065) en tijdsinterval (p=.433). Daarnaast hebben we geen significant verschil gevonden in het ijzergehalte in de SWK na een intratumorale en peritumorale injectie (p=.723). In 50% van de procedures konden de SWKs magnetisch gedetecteerd worden, terwijl in alle procedures de SWKs gevonden konden worden met behulp van de conventionele SWK-procedure (n=10). De chirurgen waren het er mee eens dat de SWK makkelijk magnetisch te lokaliseren was in 80% van de procedures waarbij het mogelijk was de SWK magnetisch te detecteren. In alle procedures waren de SWKs makkelijk te lokaliseren met behulp van de conventionele SWK-procedure. Ten slotte hebben we gevonden dat in alle postoperatieve MRI-scans geen door SPIOs geïnduceerde artefacten zichtbaar waren.

We hebben bevestigd dat het lastig is om de SWK magnetisch te detecteren tijdens de LowMag-studie, aangezien in 50% van de procedures de SWK magnetisch werd gedetecteerd (n=10). Een veelbelovende bevinding is dat in 80% van de procedures waarbij het mogelijk was de SWK magnetisch te detecteren, de chirurgen het er mee eens waren dat de SWK makkelijk te lokaliseren was met behulp van de magnetische SWK-procedure. Bovendien zagen we geen door SPIOs geïnduceerde artefacten na het geven van een peritumorale SPIOs injectie op de postoperatieve MRI-scans, waardoor het borstweefsel goed te beoordelen was. Daarnaast hebben we geen significante correlatie gevonden tussen persoonlijke factoren en het ijzergehalte in de SWKs. Dit suggereert dat de SPIOs injectie procedure niet persoonsspecifiek hoeft te zijn. Voordat de magnetische SWK-procedure met een lage SPIOs dosis geïmplementeerd kan worden in de standaard zorg moet het detectiepercentage worden verhoogd. De mogelijkheid om de SWKs magnetisch te detecteren wordt voornamelijk beïnvloed door de volgende drie factoren: de grootte en dosis van de magnetische tracer, de techniek van de magnetometer en de ervaring en kennis van de chirurg.

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I. Introduction

Approximately 15,000 women and 120 men were diagnosed with invasive breast carcinoma in the Netherlands in 2018 [2, 3]. In addition, in situ carcinoma were found in circa 2,300 women [2, 3]. To determine the appropriate treatment for the patient, it is necessary to investigate whether tumour cells are metastasized to lymph nodes. If no palpable lymph nodes are found during physical examination and no abnormal lymph nodes are found during ultrasound examination a sentinel lymph node biopsy (SLNB) procedure will generally be performed [4].

The sentinel lymph node (SN) receives the first lymphatic drainage from the tumour tissue, and subsequently, the lymph flows to the other lymph nodes, as can be seen in Figure 1. If no metastases are found in the SN, it is suspected that other lymph nodes do not have metastases either. The status of axillary lymph nodes is determined during a SLNB procedure [4].

Conventional SLNB procedures consist of the following five steps [6]. Firstly, a radioactive tracer, technetium-99m (^{99m}Tc), is preoperatively injected in the affected breast quadrant. Secondly, a lymphoscintigraphy is made to determine the number and location of the SNs, which are marked by ^{99m}Tc. Thirdly, a blue dye is injected periareolar or peritumoural a few minutes before surgery. Subsequently, the SN is searched using the blue colour and the radioactive signal, which is detected with a handheld gamma probe. Finally, the SN is removed and sent to the pathology department. The pathologist checks the SN for metastases.

Conventional SLNB procedure, using a radioactive tracer and blue dye, has a high identification rate of 96% and is considered as the gold standard nowadays [6, 7]. However, this method has some limitations. Firstly, ^{99m}Tc and its parent molybdenum-99 both have relatively short half-lives of 6 h and 66 h respectively [8]. Therefore, there is a limited timeframe between production and surgery. This results in less flexibility in surgical planning of SLNB procedures. Moreover, SLNB procedures cannot be performed at a great distance of a nuclear reactor, due to the short half-lives. For that reason, no SLNB procedures can be performed in many less developed countries. Secondly, the use of ^{99m}Tc results in exposure to radiation for both the patient and the therapist. Although small exposure to radiation is

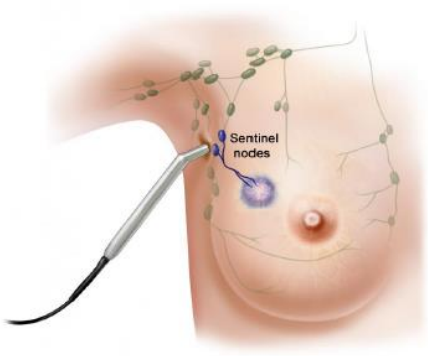

Figure 1 Identification of the sentinel lymph nodes using visual guidance due to the blue dye and a handheld gamma probe for the radioactive tracer. [5]

considered safe, it is known that a small amount of radiation can still negatively influence the body [9]. Thirdly, strict guidelines exist for transport, storage, and waste processing of radioactive material, resulting in logistical drawbacks [10].

To overcome these limitations new techniques for SLNB procedures are developed [11]. A first technique is a magnetic SLNB procedure using superparamagnetic iron oxide nanoparticles (SPIOs) as magnetic tracer to detect the SN [12]. A second technique is fluorescence imaging, which uses indocyanine green as tracer to locate the SN [13]. A third technique uses contrast enhanced ultrasound with microbubbles to locate the SN [14]. This thesis contributes to the development of the magnetic SLNB procedure.

Many studies conclude that the magnetic SLNB procedure is non-inferior to the gold standard [12, 15–18]. However, Christenhusz et al. showed that large artefacts caused by SPIOs in the breast can be found on magnetic resonance imaging (MRI) made five years after surgery [19]. This means that the human body was not able to clear the SPIOs within five years. This is undesirable, since the still present SPIOs make it impossible to evaluate the tissue using MRI. MRI is an important diagnostic imaging tool to diagnose a suspected cancer recurrence in cases that mammography and ultrasound cannot confirm a recurrence. The risk of local recurrence is especially high for patients who were younger than 40 at the time of the first diagnosis [20]. Young patients have dense breast tissue, which makes it difficult to diagnose a breast tumour using mammography [20]. MRI screening significantly increases the detection of breast cancer for women with dense breast tissue [21]. Nonetheless, the magnetic SLNB procedure also has major advantages compared to the conventional SLNB procedure. The magnetic tracer has a shelf-life of years, which makes it easy to store until use [22]. In addition, it can be injected up to seven days preoperative, which creates more flexibility in planning. Besides, exposure to radioactivity and strict actions associated with the use of a radioactive tracer can be prevented. Therefore, there is an ongoing study using a low dose of SPIOs at the department of surgery in the Medisch Spectrum Twente (MST) in Enschede, the LowMag trial.

The LowMag trial is a pilot study started in November 2016. The aim of this study is to investigate the feasibility of using a low dose of SPIOs for magnetic, intraoperative, SN detection in breast cancer patients. In addition, SPIOs are used to determine the presence of lymph node metastases using preoperative MRIs. The information acquired during the LowMag trial will be used to create a complete magnetic SLNB procedure, which can be used in future conventional care. This magnetic SLNB procedure is visualised in Figure 2. The current LowMag trial procedure is as follows. Patients who had breast cancer with a clinical negative lymph node and were scheduled for a SLNB procedure, were asked to cooperate in the LowMag trial. These patients received both the magnetic SLNB procedure with SPIOs and the conventional SLNB procedure with ^{99m}Tc and blue dye during the same surgery. First, the magnetic SLNB procedure was performed. This result was confirmed with the conventional SLNB procedure. A flowchart of the procedure for the patients who participated in the LowMag trial is given in appendix A.

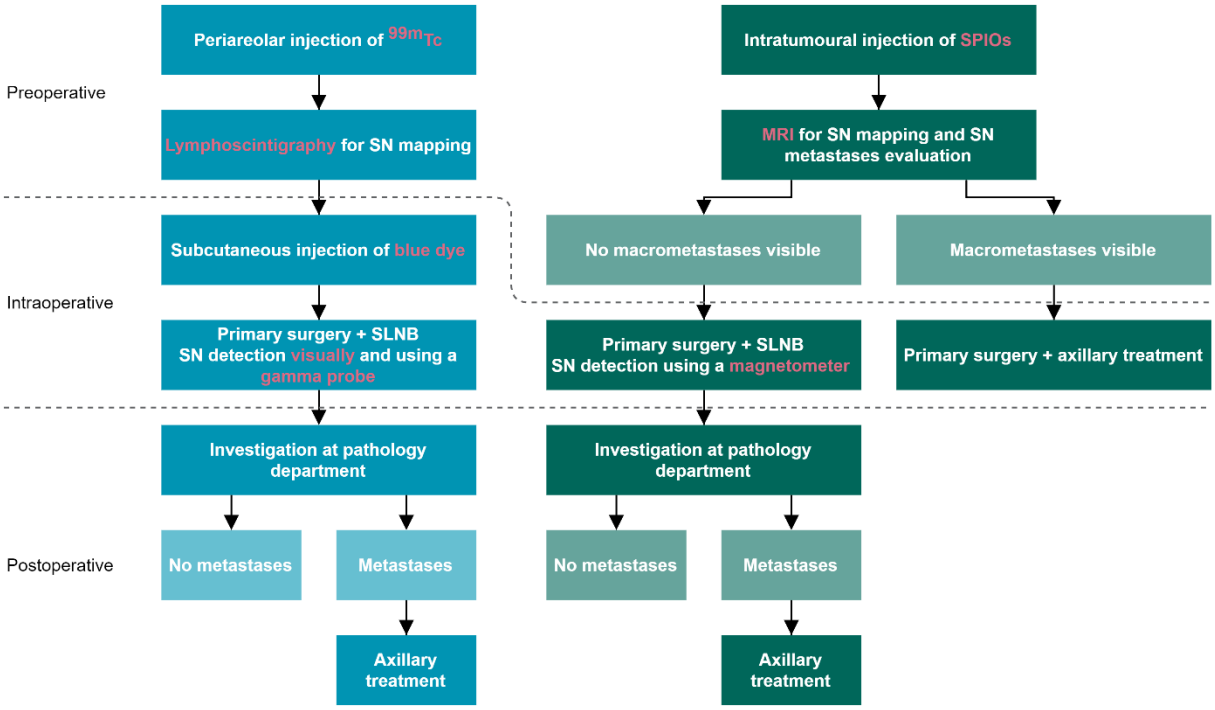

Figure 2 In blue the steps of conventional sentinel lymph node biopsy (SLNB) procedures using technetium-99m (^{99m}Tc) and blue dye. In green the steps of the ideal magnetic SLNB procedures using superparamagnetic iron oxide nanoparticles (SPIOs). Magnetic resonance imaging (MRI) is used to map and evaluate the sentinel lymph nodes (SN) preoperatively.

The surgeons expressed that it was difficult and sometimes impossible to magnetically detect the SN during the first 39 procedures of the LowMag trial. The output, a number of counts, of the magnetometer, Sentimag (Endomagnetics Limited, Cambridge, United Kingdom), strongly fluctuated during the search for the SN without a clear peak at a specific location. Therefore, the surgeons experienced that it was difficult to locate the SN.

The aim of this thesis is to investigate how to improve the magnetic detection of the SN using the Sentimag magnetometer and a low dose magnetic tracer.

This thesis is organised in the following way. Chapter II – IV examine the reasons why it is difficult to magnetically detect the SN using the Sentimag magnetometer. Chapter II describes the clinical background of the breast and lymphatic system and the technical background of the magnetometer and SPIOs. Chapter III describes a phantom study, which was performed to determine the correlation between the number of counts detected by the Sentimag magnetometer and the iron content in the SN. Chapter IV describes an interim evaluation of the LowMag trial. The LowMag data was compared to prior available MST breast cancer studies, using the Sentimag magnetometer and its magnetic tracer. In addition, the LowMag data was used to analyse whether the iron content in the SN is affected by factors, such as time, BMI, and injection site.

The findings from Chapter II – IV were used to make an adjustment to the LowMag trial protocol. Chapter V analyses whether this adjustment resulted in a higher iron content in the SN and furthermore evaluates the detection rate of the magnetic SLNB procedure. Chapter VI describes a discussion referring to the aim of this thesis.

II. Clinical and technical background

This chapter contains information about the anatomy and physiology of the breast and the lymphatic system. Furthermore, information about the SPIOs and an explanation of the operation of the magnetometer is given.

The breast

The female breast extends vertically from the second through the sixth ribs and horizontally from the midaxillary line to the border of the sternum [23, 24]. The breast lies on the facias of the musculus pectoralis major and musculus serratus anterior. A retromammary space is between the breast and the facias. This is a loose connective tissue plane, which allows the breast some amount of movement [23, 24].

The female breast mostly consists of mammary glands embedded within a fatty matrix, see Figure 3 [23, 24]. The suspensory ligaments attach the mammary glands to the dermis of the overlying skin. Each lactiferous duct connects 15 – 20 lobules of the mammary gland with the lactiferous sinus in the areola. The volume and contour of the breast are mainly formed by subcutaneous fat. However, the volume of the breast can increase during pregnancy when the mammary glands enlarge and new glands form [23, 24].

Breast cancer usually arises in the lobules or ducts of the breast [25, 26]. The most common form of breast cancer is invasive carcinoma of no special type, which has an incidence of 47 – 70% of all invasive carcinomas. Other types of invasive carcinoma are the following: tubular carcinoma (incidence 1 – 4%), medullary carcinoma (incidence 3 – 5%), mucinous carcinoma (incidence 1 – 6%), micropapillary carcinoma (incidence 1 – 2%), and cribriform carcinoma (incidence 5 – 6%). The second common form of breast cancer is invasive lobular carcinoma, which has an incidence of 5 – 15% of all invasive carcinomas. In addition to invasive breast cancers, non-invasive breast cancers can be diagnosed. Ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS) are non-invasive breast cancers. When the carcinoma is confined by the gland's basement membrane it is carcinoma in situ. When the malignant cells have infiltrated through the basement membrane into adjacent breast stroma it is invasive carcinoma [25, 26].

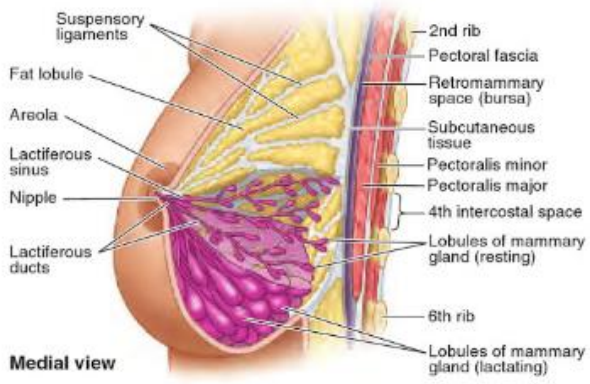

Figure 3 A sagittal section of the female breast. [24]

Anatomy and physiology of the lymphatic system

The lymphatic system is an open system. It takes up waste products from the body and has an important function in the immune system [27, 28]. Lymph, existing of extracellular fluid and their carried cells, flows from interstitial spaces, via lymphatic vessels, to lymph nodes. The lymph is propelled by internal wall motion generated by smooth muscle cells, by compression of neighbouring arterial pulsation, and by muscle contractions [29]. A midsagittal view of a lymph node is shown in Figure 4. A constant stream of lymph enters the subcapsular sinuses of the lymph node via afferent lymphatic vessels. Each afferent vessel receives lymph from a different area and delivers it into its corresponding lobule. Subsequently, the lymph is spread over subcapsular sinuses and flows through transverse sinuses into the medullary sinus. All purified lymph leaves the lymph node in one efferent lymphatic vessel and is returned to the blood circulation via the thoracic duct [27, 28].

The reticular meshwork is the framework of lobules and crosses the lumen of the sinuses [27, 28]. Macrophages stick to this framework and filter particles, such as bacteria and cell debris, from lymph. Antigen-presenting cells (APCs) in lymph look for their matching lymphocytes to present their antigen. Lymphocytes enter the lymph node by the venule in the hilus. This venule turns into high endothelial venules (HEVs) in the paracortical area of the lymph node. Lymphocytes attach to vascular addressins of HEV walls and leave HEVs due to amoeboid movements. B lymphocytes migrate to the superficial cortex, to primary follicles. The T lymphocytes migrate around HEVs in the paracortex and interfollicular cortex. The reticular meshwork creates many channels and interstices, to facilitate interaction between lymphocytes and APCs. Lymphocytes meander through the reticular meshwork and spend hours to days to search their matching antigen. If they meet their antigen a clonal expansion occurs in specific areas of the lobule. In addition, the antigen stimulation increases the import of lymphocytes via HEVs, which results in an increased lymph node. Created plasma cells and

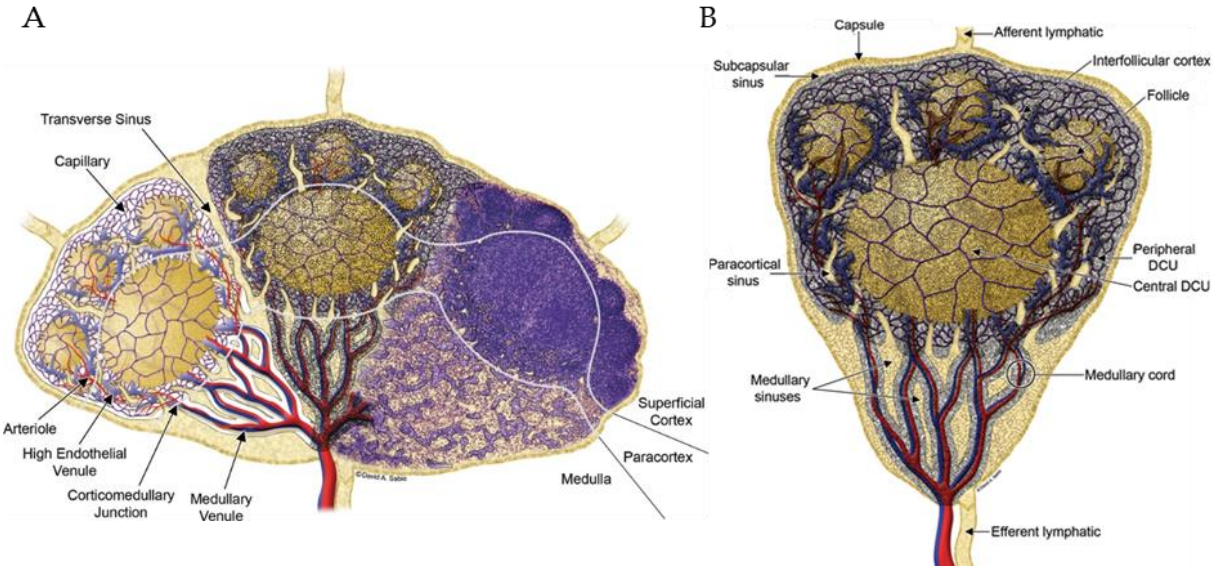

Figure 4 A) A midsagittal section of a lymph node, which consists of three lobules. B) A midsagittal section of one lymphoid lobule. [27]

lymphocytes, which are not involved by an immune reaction, emigrate from the lobule in paracortical sinuses to medullary sinuses and leave the lymph node via the efferent lymphatic vessel [27, 28].

Lymph drainage of the breast

Lymph fluid from the breast flows mostly to axillary lymph nodes [23]. A smaller part, mainly from the medial breast quadrant, flows to parasternal lymph nodes, which are located along the internal thoracic vessels [23]. In literature, however, two different conclusions are mentioned about lymph drainage in the breast. The first conclusion is that the lymphatic system of the breast drains to the same lymph node [30]. The second conclusion is that a different injection site could lead to a different SN because the lymphatic system of the breast does not drain to one lymph node [31]. Several studies showed a better and quicker marking of the SN for superficial injection (peri or subareolar) compared to a deep injection (peri or intratumoural) [32, 33]. Other studies showed a similar detection rate of the SN for the superficial injection and the deep injection [34–36]. In addition, a difference in lymphatic vessel density is found between the two deep injection sites. Several studies found a significant higher lymphatic vessel density peritumoural compared to intratumoural [37–40]. Furthermore, in some patients, no intratumoural lymphatic vessel was found.

In most breast cancer patients the SN is found in the axilla [41]. The axillary lymph nodes can be subdivided into the following five groups: apical, central, humeral, pectoral and subscapular axillary lymph nodes, visualized in Figure 5. In clinical use, the axillary lymph nodes are subdivided into three levels. The humeral, pectoral and subscapular axillary lymph nodes are defined as level I nodes. The central lymph nodes are defined as level II nodes, and the apical lymph nodes are defined as level III nodes. Most lymph of the breast drains through the pectoral, central and apical axillary lymph nodes to the subclavian lymph trunk [23]. The majority of the SNs identified during surgery are found in the subscapular, pectoral and humeral lymph nodes groups [42]. The axillary lymph nodes are located at an average depth of four centimetres and can be located up to a depth of eight centimetres from the skin [43, 44].

Superparamagnetic iron oxide nanoparticles

There are different types of SPIOs. All SPIOs consist of an iron oxide core. The iron oxide core of clinical SPIOs are covered with a biocompatible coating [45]. These SPIOs have a size of 50 to 500 nm. Particles with a size smaller than 50 nm are called ultrasmall SPIOs (USPIOs). SPIOs can be used as a magnetic tracer during the SLNB procedure.

The SPIOs are subcutaneous injected during the SLNB procedure. The SPIOs follow one of the two possible pathways: a passive flow through the lymph vessel to the SN, or an active transport by macrophages. The SPIOs are stuck to the filter in the SN. Prior studies using an intravenous SPIOs injection shows that the SPIOs are cleared in the liver and spleen [46, 47]. The assumption is that the subcutaneous injected SPIOs are similarly eliminated from the body. However, not all SPIOs transport to the SN, some of them are enclosed by macrophages

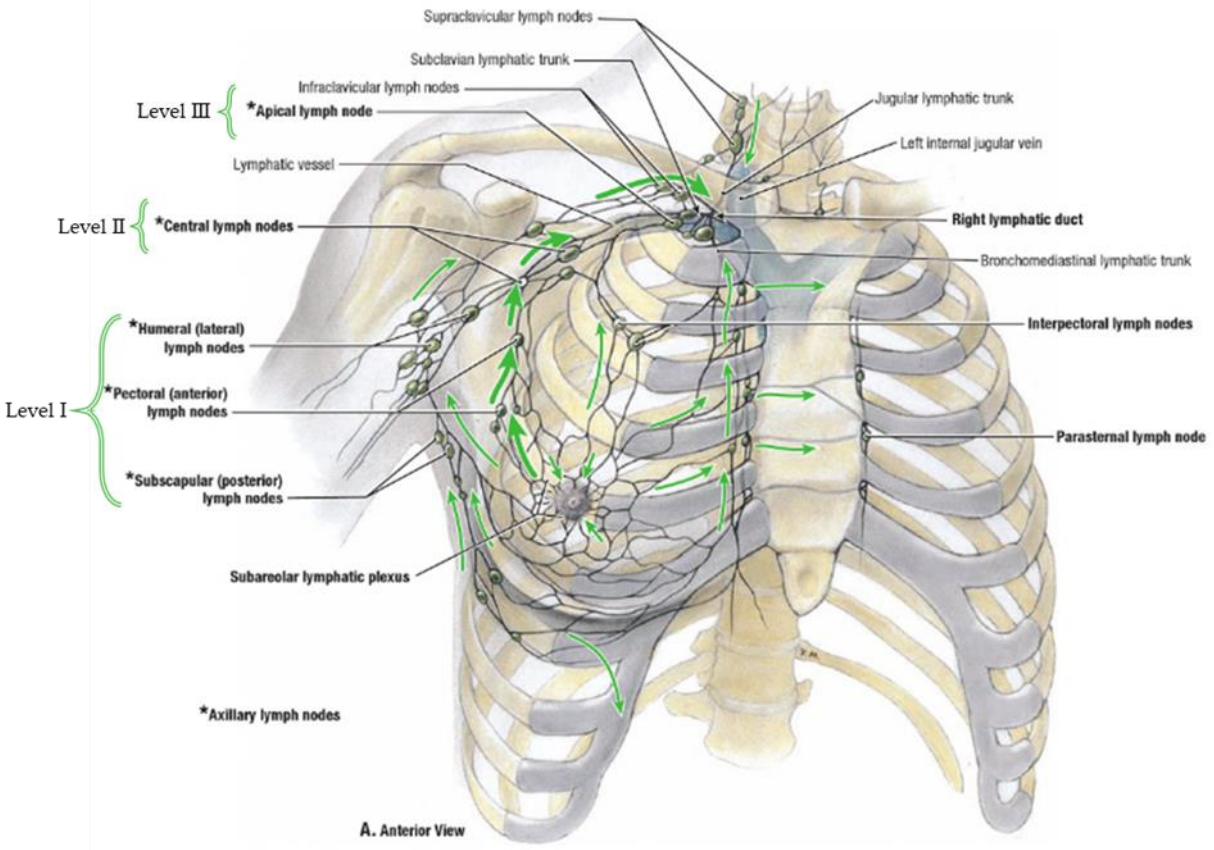

Figure 5 Lymphatic drainage of the breast. The five groups of axillary lymph nodes are divided into three clinical used levels. [23]

and nested in the subcutaneous tissue [19, 48]. These SPIOs can stay here for years, in a similar way as a tattoo.

Merely a small volume of the injection dose drains to the SN. Waddington et al. show that up to 5.1% of the injection doses of a radioactive tracer enters the SN of breast cancer patients [49]. Animal studies using SPIOs show that 0.004 – 4.7% of the injection doses enters the lymph node [50–52]. The SPIOs are mostly found in the sinuses and subcapsular space of the lymph node, as can be seen in Figure 6 [19, 53]. No SPIOs are found in areas that contain metastases [19, 53]. It seems that the SPIOs only drain to the first order lymph nodes, the assumed SNs, which is comparable with the drainage of the radioactive tracer [15, 53, 54].

The SPIOs uptake in an in-vivo lymph node can be visualised by comparing the signal intensity in the lymph node on a pre-injection MRI and post-injection MRI [55]. The SPIOs cause a decrease in signal intensity. Therefore, no dropped signal intensity or partially dropped signal intensity indicates a lymph node with a metastasis, as shown in Figure 7.

The magnetic tracer used in this thesis is Sienna+ (Endomagnetics Limited, Cambridge, United Kingdom) [56]. Sienna+ contains SPIOs coated with carboxydextran and is recommended by the manufacturer as a magnetic tracer when using the Sentimag magnetometer [22, 56]. These SPIOs have a size of 60 nm. Sienna+ has a brownish colour and is therefore, potentially, both magnetic and visual traceable. The manufacturer recommends an injection of 2 mL Sienna+,

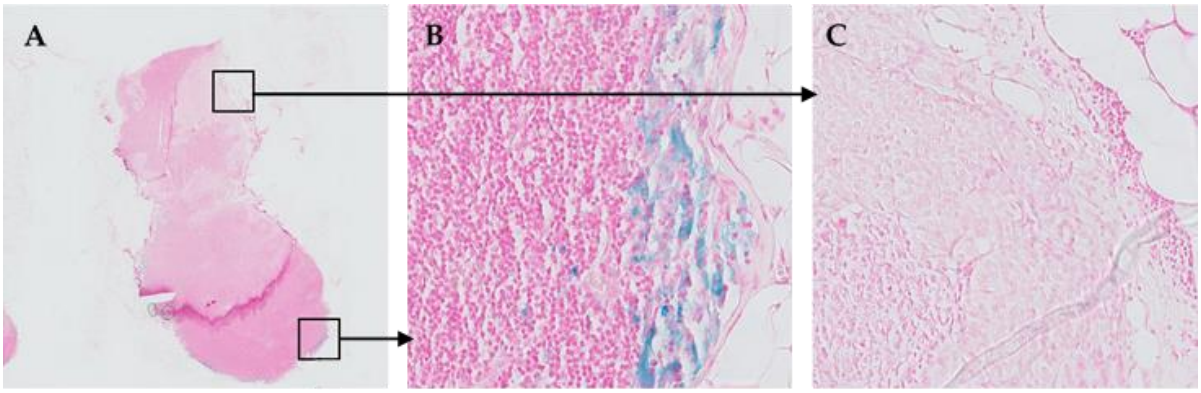

Figure 6 A histological image of a sentinel lymph node (SN) coupe stained with Perls Prussian Blue. A) An overview of the SN. B) An enlarged image of the healthy tissue. The iron particles are coloured blue and are located in the subcapsular space. C) An enlarged image of the tumour tissue. No iron particles are visible in this part of the SN. [19]

which contains 56 mg iron and 64 mg carboxydextran. According to the manual, the tracer should be injected subcutaneously between 7 days and 20 minutes prior to the SLNB surgery and followed by a 5 minutes massage.

Magnetometers

Magnetometers are instruments for measuring the intensity of a magnetic field or for detecting ferrous or magnetic materials. Magnetometers make use of the magnetic properties of materials. The extent to which a material becomes magnetized when it is placed in an external magnetic field is described as the magnetic susceptibility [57]. The following three categories of magnetic susceptibility exist: diamagnetic, paramagnetic, and ferromagnetic. Diamagnetic material slightly reacts in opposite polarization to the applied field, for example, water and fat. Paramagnetic material slightly polarizes in the same direction as the external field, for example, aluminium and oxygen. Ferromagnetic material strongly polarizes in the same direction as the external field, for example, iron and cobalt.

Three different magnetometers were used during this study. Two handheld magnetometers, the Sentimag [58], and the DiffMag [59], and one table-top magnetometer, the SuperParamagnetic Quantifier (SPaQ) [60]. The Sentimag magnetometer is approved for clinical use and is developed by Endomagnetics Limited in Cambridge. The DiffMag

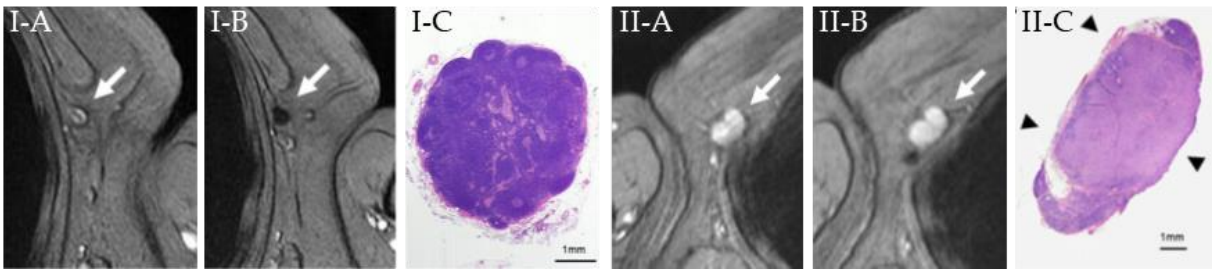

Figure 7 MRI images and histological images to visualise the sentinel lymph nodes (SN) of two patients, patient I and patient II. Image A is a pre SPIOs injection MRI image, and image B is a post SPIOs injection MRI image. Image I-B shows a dropped signal intensity of the SN (white arrow) compared to image I-A and was diagnosed as a benign SN. This diagnosis was confirmed with the histological image I-C. Image II-B shows no dropped signal intensity of the SN (white arrow) compared to image II-A and was diagnosed as malignant. This diagnosis was confirmed with the histological image II-C, which shows metastatic tissue in almost the entire SN (black arrowheads). [55]

magnetometer and SPaQ magnetometer are not yet approved for clinical use and are developed by the Magnetic Detection & Imaging group (MD&I) of the University of Twente (UT) in Enschede. These three magnetometers induce a magnetic field by passing a current through a coil, according to Ampère's law [57]. The strength and therefore the penetration depth of this magnetic field is determined by the following equation [61]:

$$B = \frac{\mu_0 \cdot N \cdot I}{L}$$

$$\begin{aligned} B &= Magnetic\ field\ strenght\ (T) \\ \mu_0 &= Magnetic\ permeability\ (H/m) \\ N &= Number\ of\ windings \\ I &= Current\ (A) \\ L &= Length\ of\ coil\ (m) \end{aligned}$$

Following this equation, the number of windings and the current determine the magnetic field strength. The number of windings is limited, because the diameter of the probe must be small enough to be able to use during surgery. In addition, the International Commission on Non-Ionizing Radiation Protection (ICNIRP) has strict guidelines for the use of magnetic fields in patients [62].

Sentimag magnetometer – The Sentimag magnetometer consists of a base unit and a probe, shown in Figure 8 [58]. The probe contains an excitation coil and detection coils. An alternating current passing through the excitation coil induces an alternating magnetic field. SPIOs subjected to this alternating magnetic field polarize in the direction of this magnetic field. The orientation of SPIOs in a magnetic field is visualized in Figure 9. The magnetisation of SPIOs induces a changing magnetic flux. According to Faraday's law [61], a changing magnetic flux induces a voltage, which creates an electric current in the detection coil. An algorithm convert this electric current to a number of counts, which is the output of the Sentimag magnetometer [58]. The Sentimag magnetometer has three sensitivity settings. The number of counts displayed on setting 2 is twice as high as on setting 1, and half that displayed on setting 3.

A limitation of the Sentimag magnetometer is that the counts are based on the net magnetic susceptibility of the total tissue volume and not only based on the net magnetic susceptibility of SPIOs. The diamagnetic human tissue has a magnetic susceptibility which is roughly seven orders of magnitude smaller than that of SPIOs [59]. However, a small SPIOs concentration induces a resulting signal which is in the same order as signal from diamagnetic tissue. This makes it more challenging to localize SPIOs. A second limitation is that conventionally used

Figure 8 The Sentimag magnetometer. [58]

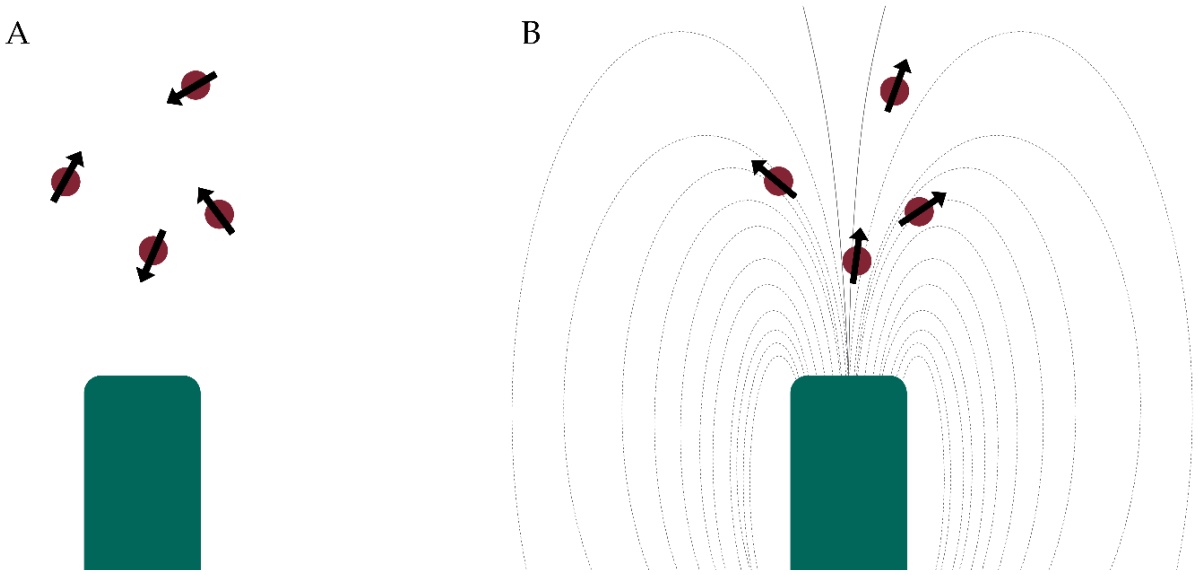

Figure 9 Orientation of the superparamagnetic iron oxide nanoparticles (SPIOs) in a magnetic field excited by a handheld magnetometer. A) The magnetometer is turned off and therefore no magnetic field is excited. The SPIOs are oriented in all different directions. B) The magnetometer induces a magnetic field. The SPIOs are oriented in the direction of this field.

surgical instruments also induce signal picked up by the Sentimag magnetometer, which make it almost impossible to localize SPIOs using current standard of care procedures. Surgical instruments made of plastic do not induce signal picked up by the Sentimag magnetometer. However, surgeons prefer the use of conventionally used surgical instruments.

SPaQ magnetometer – The SPaQ magnetometer is a table-top magnetometer in which a homogenous magnetic field can be generated [60]. A sample can be placed in the centre of the excitation coil and detection coils, see Figure 10. The technique of the SPaQ magnetometer omits mentioned limitations of the Sentimag magnetometer. SPIOs have a non-linear magnetization dependent on the applied magnetic field. Human tissue and metallic surgical instruments have a linear magnetization dependent on the applied magnetic field. The

Figure 10 A) The SuperParamagnetic Quantifier (SPaQ) magnetometer. B) Schematic visualisation of the inside of the SPaQ magnetometer. A vial with a sample (blue) can be placed into the shaft (green). The excitation coil (yellow) applies a magnetic field and the detection coils (red) measure the magnetization of the sample. The shielding coil (purple) improves the homogeneity of the excitation field. [63]

magnetization of superparamagnetic materials, such as SPIOs, and the magnetization of diamagnetic materials, such as human tissue, are visualised in Figure 11A. It can be seen that the magnetization of superparamagnetic material levels off by applying a negative or positive offset to the standard alternating magnetic field. Diamagnetic material has a linear magnetization. The applied magnetic field is strong enough so that almost all superparamagnetic particles are oriented in the direction of this field. As a result, there is almost no changing magnetic flux induced by superparamagnetic particles. This phenomenon is called saturation. This difference in properties of diamagnetic material and superparamagnetic material is used in the technique of the SPaQ magnetometer. The SPaQ magnetometer induces four magnetic fields one after the other, as can be seen in Figure 12B. The first field is a standard alternating excitation field. Subsequently, a standard alternating excitation field plus an additional positive offset field is applied, the second field. In the third interval again the standard alternating excitation field is applied. Then, the fourth field is applied, which consists of a standard alternating excitation field plus an additional negative offset field. The detected magnetization of diamagnetic material hardly changes over these four applied fields. However, a difference in detected magnetization over these four applied fields could be measured for superparamagnetic materials. During the first and third fields, a high magnetic susceptibility could be measured, while during the second and fourth field a reduced magnetic susceptibility could be measured, as shown in Figure 12C. Subsequently, the signal from SPIOs in tissue can be determined by calculation of the difference in signal detected by the detection coil, the difference in voltage, shown in Figure 12D. The output is a number of counts.

The SPaQ magnetometer has a second function to measure the SPIOs in a sample. Therefore, the SPaQ magnetometer applies constantly an alternating current field and applies additionally a direct current field which is increased over time [60]. A measurement consists of two parts, during the first part a sample with SPIOs is measured. Subsequently, the sample is removed and the second part of the measurement is performed. This results in a magnetic susceptibility (dM/dH) versus magnetic field (H) curve of the SPIOs, see Figure 11. The

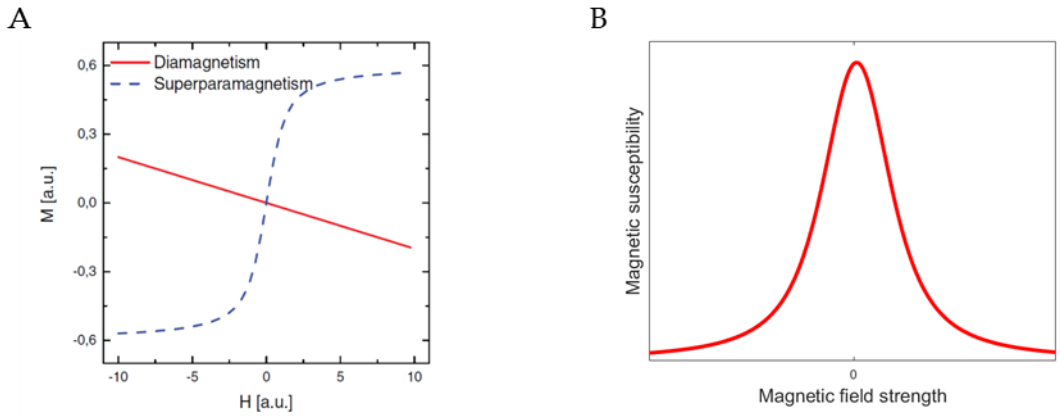

Figure 11 A) The magnetization (M) versus the applied field (H) of diamagnetic material (red) and superparamagnetic material (blue) [59]. B) A magnetic susceptibility (dM/dH) versus magnetic field (H) curve of the SPIOs. Integration of this curve creates the superparamagnetic curve as shown in A.

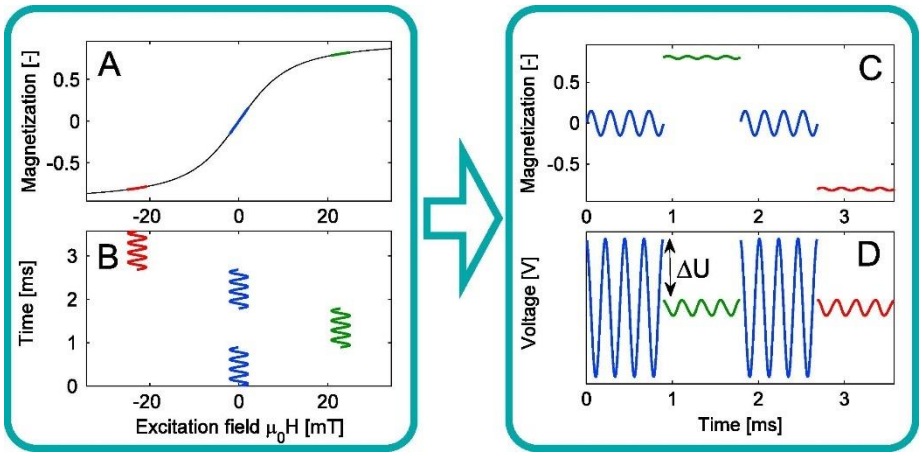

Figure 12 The concept of magnetometry used in the SPaQ and DiffMag magnetometers. A) The magnetization curve of SPIOs. B) The applied excitation fields. First, a standard alternating excitation field is applied. Second, a positive offset is applied to this alternating excitation field. Third, the standard alternating excitation field is applied. Fourth, a negative offset is applied to the standard alternating excitation field. C) The magnetization response of the measured tissue with SPIOs. A reduced response is measured when an offset is applied to the standard alternating excitation field. D) The measured magnetization is proportional to the amplitude of the induced voltage. The contribution of SPIOs can be calculated by the difference in voltage. [60]

magnetic susceptibility is proportional to the voltage. Integration of this curve results in the magnetization curve.

DiffMag magnetometer – The DiffMag magnetometer [59] is a handheld magnetometer using the same principle as the SPaQ magnetometer. The DiffMag magnetometer consists of a base unit and a probe, shown in Figure 13. The probe contains an excitation coil and two detection coils. The DiffMag magnetometer induces four inhomogeneous magnetic fields around the probe, one after the other, as can be seen in Figure 12. The contribution of the SPIOs is calculated by the difference in signal detected by the detection coil. The output of the DiffMag magnetometer is a number of counts.

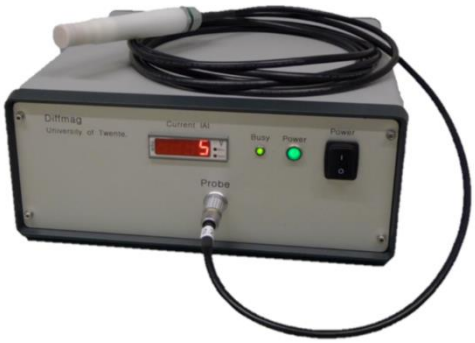

Figure 13 The DiffMag magnetometer. [59]

III. Phantom study: Correlation between counts and iron dose

Introduction

The aim of this chapter is to investigate the correlation between the detected number of counts and iron dose. The correlation between detected number of counts and iron dose was determined for all three magnetometers during a first phantom study. In this first phantom study, the assumption was made that all SPIOs are close together and positioned almost directly to the centre of the probe. However, SPIOs are scattered through the SN. Therefore, the distance to the probe differs and is in many cases larger than zero millimetres. The influence of distance between SPIOs and probe to the detected number of counts was investigated for the clinically used magnetometer during a second phantom study.

Method

Glass tubes with a diameter of 1 cm were used to make phantom nodes. Ten tubes were filled with a different amount of Sienna+, supplemented with 0.9% saline to a volume of 35 µL, the volume of a small lymph node [64]. The iron doses in the phantom nodes were 1 µg, 5 µg, 10 µg, 28 µg, 50 µg, 101 µg, 140 µg, 280 µg, 420 µg, and 504 µg. The iron content in these phantom nodes corresponded to the expected iron content in the SN based on the injected doses used in the clinical trials in the MST.

Study I: The correlation between iron dose and counts for three magnetometers

Setup

The setup for the measurements with the Sentimag magnetometer and DiffMag magnetometer were similar and can be seen in Figure 14. The probe was fixated to a standard such that the measurement side of the probe was turned upwards and surrounded by air. The phantom nodes were separately placed on the centre of the probe. A Styrofoam cubic was used to

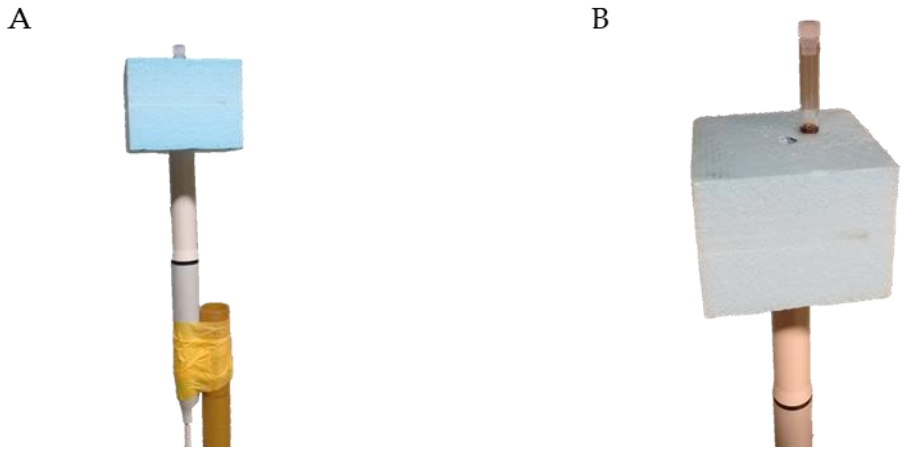

Figure 14 A) Setup of the Sentimag probe fixed to a standard, the Styrofoam cubic, and a phantom node with superparamagnetic iron oxide nanoparticles (SPIOs). B) The Styrofoam cubic had two holes. The probe was placed in one hole. The phantom nodes with SPIOs were separately placed in the other hole.

guarantee that the phantom node was placed on the centre of the probe for each measurement. This Styrofoam cubic had one hole with two different diameters, one equal to the diameter of the probe and one to the diameter of the phantom node.

The SPaQ magnetometer can be seen in Figure 10. For measurements the phantom nodes were separately placed inside the SPaQ magnetometer.

The following materials were used:

- Sentimag magnetometer
- DiffMag magnetometer
- SPaQ magnetometer
- Ten phantom nodes containing Sienna+
- Standard
- Styrofoam cubic with a hole in the centre with two diameters

Data acquisition

Data acquisition using Sentimag magnetometer – A 10 kHz alternating current, the default setting, was used to generate the magnetic field [58]. The Sentimag magnetometer was balanced with the probe surrounded by the Styrofoam cubic in the air before each measurement. Subsequently, a phantom node was placed in the Styrofoam on the probe. A measurement was performed for ten seconds and the number of counts was registered. Each phantom node was measured six times. The mean of the measurements for each phantom node was calculated and used to create a trendline. This method was performed for all three settings of the Sentimag magnetometer.

Data acquisition using DiffMag magnetometer – A 2.5 kHz alternating current, the default setting, was used to generate the magnetic field. The DiffMag magnetometer was balanced with the probe surrounded by the Styrofoam cubic in the air before each measurement. Subsequently, a phantom node was placed in the Styrofoam cubic. Each phantom node was measured six times for ten seconds, while the computer recorded the number of counts. The mean of the measurements for each phantom node was calculated and used to create a trendline.

Data acquisition using SPaQ magnetometer – A 2.5 kHz alternating current, the default setting, was used to generate the magnetic field. The SPaQ magnetometer was balanced before each measurement. Subsequently, a phantom node was placed inside the SPaQ magnetometer. Each phantom node was measured six times for ten seconds, while the computer recorded the number of counts. The mean of the measurements for each phantom node was calculated and used to create a trendline.

Study II: The influence of distance between the Sentimag probe and node

Setup

The Sentimag probe was placed in the robotic arm with its measurement side oriented upwards, as can be seen in Figure 15. A standard was enlarged with a Styrofoam bar in which

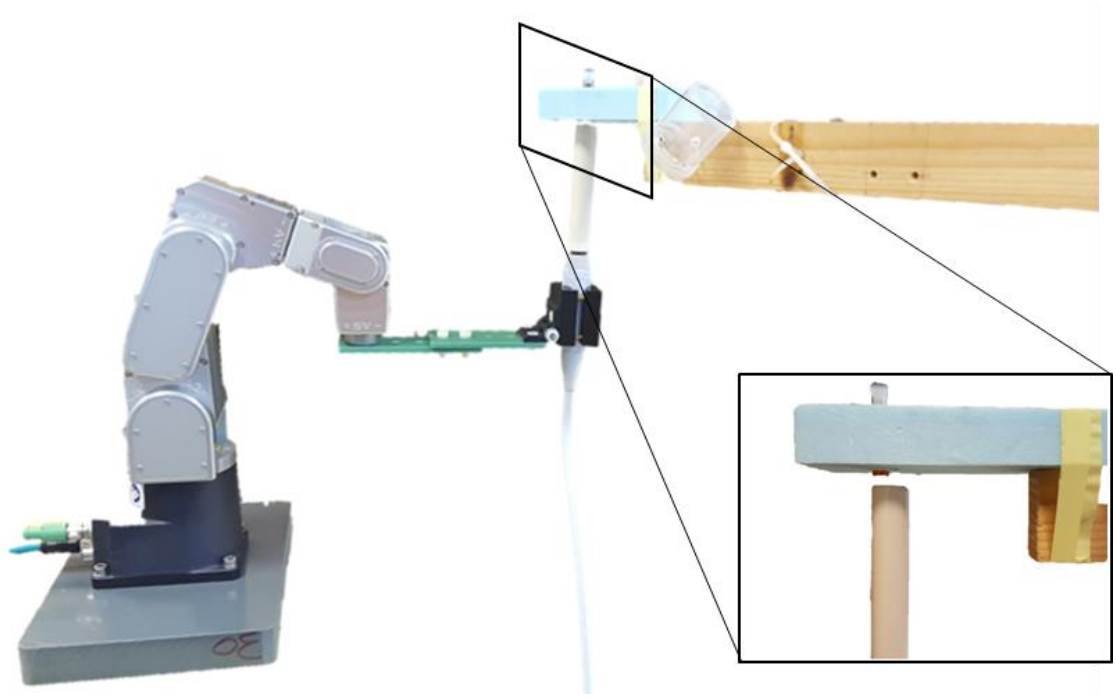

Figure 15 Setup of the robotic arm, Sentimag probe, and the phantom node with superparamagnetic iron oxide nanoparticles (SPIOs). The robotic arm was used to move the Sentimag probe to predefined positions, which were at a certain horizontal and vertical distance to the phantom node.

the phantom node was clamped. The phantom node was positioned on the centre of the probe. Subsequently, small vertically and horizontally steps were made by programming the robotic arm. All measurements were made using the Sentimag magnetometer in setting 3, which was mostly used during clinical practice.

The following materials were used:

- Sentimag magnetometer
- Three phantom nodes with 101 µg, 50 µg, and 10 µg iron
- Robotic arm MECA500 (Mecademic, Montreal, Canada)
- Standard
- Styrofoam bar with a hole in the centre with two diameters

Data acquisition

The Sentimag magnetometer was balanced with the probe in the air before each measurement. A phantom node was placed in the Styrofoam bar. The distance between phantom node and probe was changed with steps of five millimetres vertically, and three millimetres horizontally, until no counts could be measured. A schematic visualisation is shown in Figure 16. The start position of the phantom node was at the centre of the probe, so the vertical distance was 0 mm and the horizontal distance was 0 mm as well. A measurement was performed for ten seconds and the number of counts was registered. Each phantom node was measured three times.

Additional measurements were performed for the phantom node containing 50 µg iron. The distance between the phantom node and probe was changed with steps of one millimetre vertically.

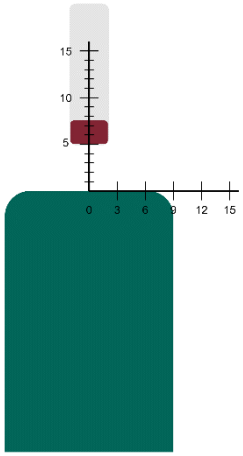

Figure 16 A schematic visualisation of a magnetometer probe, a phantom node with a solution of superparamagnetic iron oxide nanoparticles (SPIOs), and a ruler in vertical and horizontal direction to visualise the distance between probe and phantom node. In this situation, the vertical distance is 5 mm and the horizontal distance is 0 mm.

Results

Study I: The correlation between iron dose and counts for three magnetometers

The phantom nodes which had the highest iron content could not be measured accurately with the Sentimag magnetometer. The number of counts was higher than the maximum number of counts that the Sentimag magnetometer could display. All phantom nodes could be measured with both the SPaQ magnetometer and DiffMag magnetometer. The detected counts for a phantom node were different for each magnetometer. The DiffMag magnetometer showed the smallest number of counts, followed by the SPaQ magnetometer, and the Sentimag magnetometer showed the highest number of counts, as can be seen in Table 1. A higher number of counts was measured with all three magnetometers for phantom nodes with a high iron content compared to measurements of a phantom node with small iron content. The standard deviation also increased when the number of counts increased. However, the ratio

Table 1 The mean counts with standard deviation (SD) measured for ten phantom nodes with three magnetometers. The phantom nodes were measured for each setting of the Sentimag magnetometer. The magnetic fields of the SPaQ magnetometer and DiffMag magnetometer were generated using a 2.5 kHz alternating current, the default setting. The magnetic field of the Sentimag magnetometer was generated using a 10 kHz alternating current, the default setting.

	SPaQ	DiffMag	Sentimag		
			Setting 1	Setting 2	Setting 3
Iron (µg)	**mean (± SD)**	**mean (± SD)**	**mean (± SD)**	**mean (± SD)**	**mean (± SD)**
1	8 (± 3)	1 (± 0)	7 (± 5)	21 (± 3)	39 (± 5)
5	35 (± 3)	22 (± 1)	95 (± 2)	186 (± 2)	372 (± 12)
10	69 (± 3)	43 (± 1)	172 (± 1)	344 (± 1)	685 (± 2)
28	208 (± 3)	135 (± 1)	518 (± 1)	1025 (± 9)	2057 (± 5)
50	561 (± 3)	326 (± 2)	1108 (± 5)	2210 (± 3)	4416 (± 10)
101	1071 (± 5)	669 (± 3)	2149 (± 8)	4304 (± 10)	8593 (± 13)
140	1595 (± 6)	1057 (± 5)	3315 (± 11)	6620 (± 25)	Signal too high
280	3812 (± 14)	2618 (± 24)	7666 (± 13)	Signal too high	Signal too high
420	5478 (± 21)	3540 (± 17)	Signal too high	Signal too high	Signal too high
504	7868 (± 22)	4992 (± 62)	Signal too high	Signal too high	Signal too high

between the standard deviation and the mean was smaller for phantom nodes with a high iron content compared to phantom nodes with smaller iron content.

The results and the corresponding trendlines were plotted for each magnetometer, and for the Sentimag magnetometer for each setting, as can be seen in Figure 17. The linear trendlines show a very strong correlation with the measured values for all magnetometers ($0.98 < R^2 < 1.00$). The Sentimag magnetometer measured a higher number of counts for a small iron dose, resulting in a higher slope compared to the SPaQ magnetometer and DiffMag magnetometer. A conversion table to convert a number of counts to an iron dose for each magnetometer can be found in appendix B.

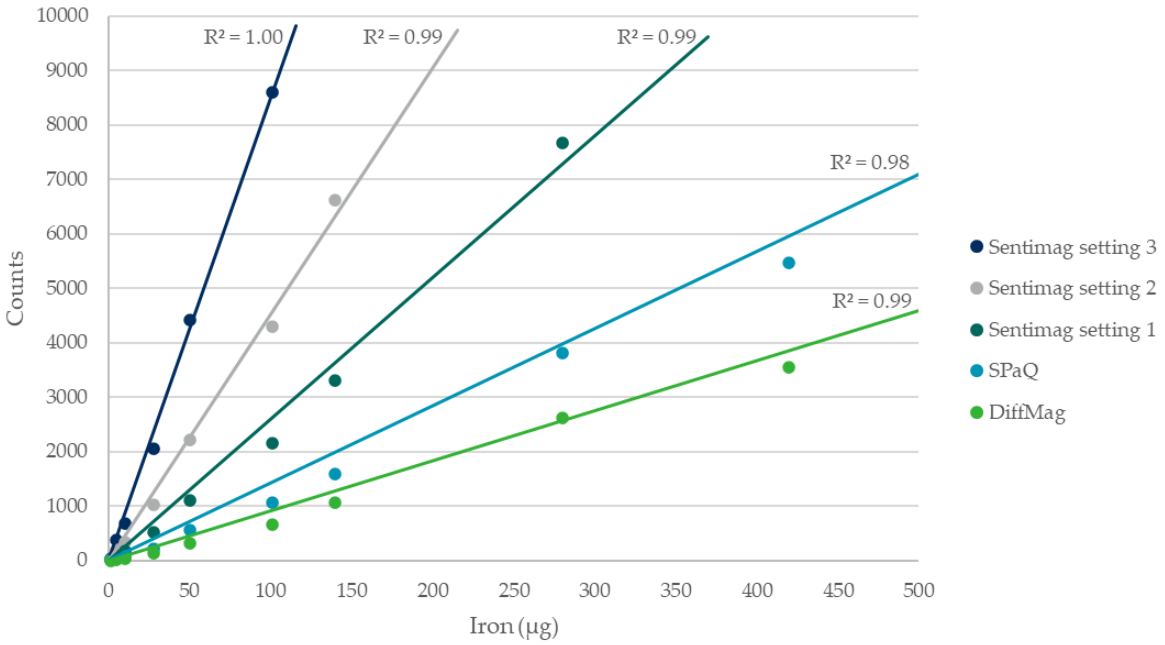

Figure 17 The correlation between iron dose and the average number of counts detected with the three magnetometers. For the Sentimag magnetometer, the results are plotted for each of the three settings.

Study II: The influence of distance between the Sentimag probe and node

Figure 18 shows the measured counts by variating the horizontal and vertical distance between the Sentimag probe and the three phantom nodes with respectively 10 µg, 50 µg, and 101 µg iron. Increasing the vertical distance between the phantom node and probe reduced the detected number of counts. An increased horizontal distance between the centre of the probe and the centre of the phantom node also resulted in a reduced number of counts, although weaker than in case of the vertical distance. In addition, it could be seen that a phantom node with a higher number of counts positioned at a larger distance from the probe resulted in a smaller number of counts than a phantom node with a smaller iron content closer to the probe. The maximum vertical distance at which it was possible to measure the phantom nodes with 10 µg, 50 µg, and 101 µg iron was respectively 5 mm, 10 mm, and 15 mm.

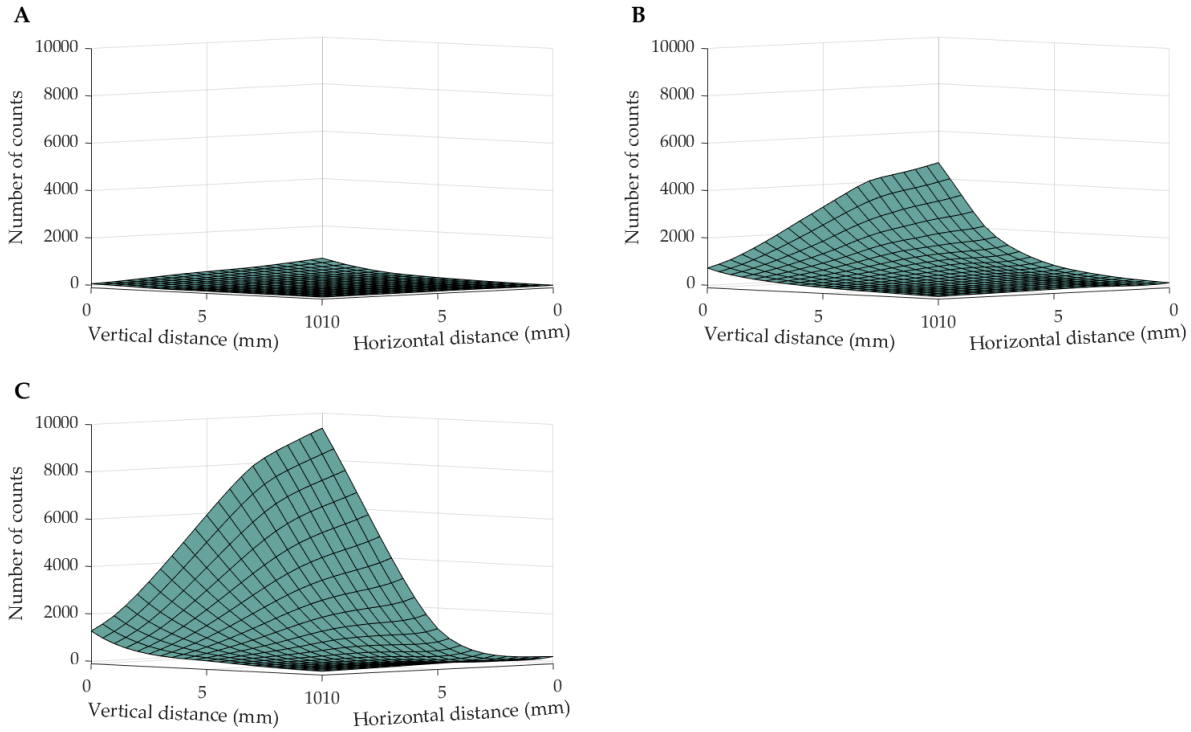

Figure 18 The correlation between the detected number of counts and the distance (both vertically and horizontally) between the phantom node and the Sentimag probe. All measurements were performed using setting 3 of the Sentimag magnetometer. A) The results of a phantom node with 10 µg iron. B) The results of a phantom node with 50 µg iron. C) The results of a phantom node with 101 µg iron.

Discussion

The detected number of counts highly depends on the distance between SPIOs and Sentimag probe. Increasing the distance between SPIOs and the centre of the probe results in a reduced detected number of counts. Therefore, a low dose of iron close to the probe could result in a higher number of counts than a higher dose of iron further from the probe. As a consequence, a superficial lymph node containing a low iron dose may therefore appear to be the SN more likely than a deeper located lymph node containing a higher iron dose. Besides, a higher number of counts could be detected for a small lymph node compared to a larger lymph node with similar iron content, which suggests a difference in iron content.

The maximum vertical distance between phantom nodes and Sentimag probe at which it was possible to detect a number of counts was between 5 and 15 mm. This distance is much lower than the average depth of 4 cm at which the axillary lymph nodes are located [43, 44]. Pouw et al. showed in a phantom study that the penetration depth for the Sentimag magnetometer to detect SPIOs in lymph nodes is up to 3.75 cm, when using a phantom node containing 500 µg iron [65]. This depth is also lower than the average depth of the lymph nodes, making it difficult, if not impossible, to detect the SN transcutaneous.

For all phantom nodes except the one with 1 µg iron, the standard deviations of the measurements are small enough to conclude that these measurements are repeatable for all three magnetometers. It was difficult to measure a number of counts for the phantom node

with 1 μg iron. During the measurements using the Sentimag magnetometer, a number of counts was detected in the first seconds. In some of these measurements, the number of counts rapidly reduced to zero counts after the first seconds, for all three settings of the Sentimag magnetometer. In those situations, the number of counts detected during the first seconds was recorded.

When using the trendlines visualised in Figure 17 to determine the iron content in clinical SNs one must be aware of the limitations. First of all, the phantom nodes are glass tubes that consist of a fluid of SPIOs diluted with saline. The SPIOs are equally distributed over the fluid. In the case of SNs, some parts contain a lot of SPIOs, while other parts do not contain any SPIOs. In addition, as mentioned before, the distance between the probe and the SPIOs greatly affects the number of counts. The excited magnetic field around the probe is strong close to the probe and weaker further away from the probe. The SPIOs will easily flip to this field when the magnetic field is strong. When the magnetic field becomes weaker, the SPIOs are less inclined to flip. In that case, the magnetic field induced by SPIOs is smaller, resulting in less detection by the detection coil, leading to a smaller number of counts. The SPaQ magnetometer excites a homogeneous magnetic field. In this situation, there is no influence of distance and also the distribution of SPIOs in the lymph node does not affect the number of counts. However, lymph nodes can only be measured ex-vivo in the SPaQ magnetometer. A second limitation is that SPIOs can move freely in the phantom node solution. However, after the injection of SPIOs in the human body most SPIOs are stuck to macrophages [53]. It is hypothesized that these macrophages limit the freedom of movements of the SPIOs and therefore it is harder for SPIOs to flip to the magnetic field.

In conclusion, a correlation between the number of counts and iron dose was found for the following three magnetometers: Sentimag, DiffMag, and SPaQ. These correlations can be used to convert the clinically detected number of counts of the SN to an iron content in the SN. In addition, it was shown that the distance between SPIOs and probe greatly affects the measured number of counts. The SPIOs were evenly distributed over the phantom nodes, which is probably not the case in clinical SNs. Therefore, it is probable that the determined correlations does not perfectly fit the clinical situation. However, these correlations can be used to determine an estimation of the iron content in SNs.

IV. Interim evaluation of the LowMag trial

Introduction

The aim of this chapter is to investigate how the LowMag procedure can be adjusted for better magnetic SN detection during surgery. The performance of the SLNB procedure using the Sentimag magnetometer was simulated on healthy volunteers using ex-vivo SPIOs in an operation room. A detailed report can be found in appendix C. It was found that balancing the magnetometer at the sternum did not result in a measurement baseline which perfectly fits the axilla. In addition, it is shown that metallic objects should be at a minimal distance of fifteen centimetres from the probe head to avoid affecting the measurement.

In this chapter a retrospective analysis is performed using the available data of the LowMag trial, and two prior MST breast cancer studies using the Sentimag magnetometer and its magnetic tracer, the SentiMag multicentre trial [66] and the MagSNOLL multicentre trial [67]. Table 2 shows an overview of the injection procedure of the three trials. The different injection sites are visualised in Figure 19. It is studied how much iron content reached the SN and in which cases it was possible to magnetically detect the SN.

The manufacturer suggests that the age and body-mass index (BMI) of the patient affect the drainage of SPIOs to the SN [22]. In addition, SPIOs have to travel a certain distance to the SN, which takes some time. It is investigated whether personal factors and time affected the iron content which reached the SN.

Method

A retrospective analysis was performed with the in the MST available data of the LowMag trial, the SentiMag trial, and the MagSNOLL trial.

Table 2 An overview of the injection procedure variables of the three trials.

	LowMag trial (n=39)	**SentiMag trial (n=31)**	**MagSNOLL trial (n=10)**
Injected iron dose (mg)	1.1	56	2.8 – 14
Injected Sienna+ volume (mL)	0.04	2.0	0.1 – 0.5
Injected saline volume (mL)	0.46	3.0	0.0
Total injection volume (mL)	0.5	5.0	0.1 – 0.5
Injection site	Intratumoural	Periareolar	Intratumoural
Time between injection and surgery	1 day (n=12) 3 days (n=2) 4 days (n=6) 5 days (n=1) 6 days (n=18)	< 1 day (n=22) 1 day (n=9)	1 day

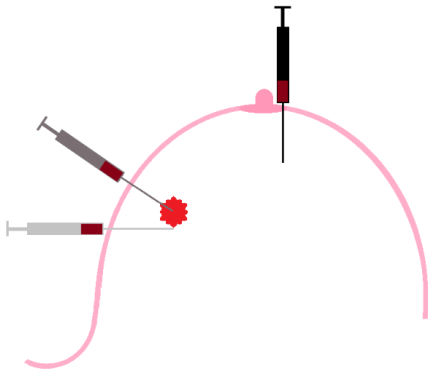

Figure 19 A schematic visualization of a breast with a syringe (dark grey) with the needle placed intratumoural, a syringe (light grey) with the needle positioned peritumoural, and a syringe (black) with the needle placed periareolar.

Comparison of the study populations – It was investigated whether the study populations of the three trials were comparable for the following variables: age, BMI, breast volume, tumour size, and tumour location. The variable age was given in the unit years, and the BMI of the patient was calculated by dividing the body mass (in kilograms) by the square of the body height (in meters). The variable breast volume was divided into groups ranging from group 1 to 16. The breast volume was based on the bra size of the patient. A conversion table, which can be found in appendix D, was used to divide the patients into the groups. The variable tumour size was defined following the T in the TNM staging system [68]. The variable location of the tumour was divided into five groups: medial upper quadrant (MUQ), lateral upper quadrant (LUQ), lateral lower quadrant (LLQ), medial lower quadrant (MLQ), and central. The subdivision of these groups is visualised in Figure 20.

Magnetic detection rate of the SN – It was investigated in how many cases it was possible to detect the SN using the magnetic SLNB procedure for each of the three trials.

Iron content in the SN – The iron content in SNs was compared between the three trials. The iron content in SNs was calculated by converting the ex-vivo counts of the SN measured with the Sentimag magnetometer to an iron dose using the correlation factors calculated by the phantom studies, as described in chapter III. One SN per patient, the SN with the highest iron

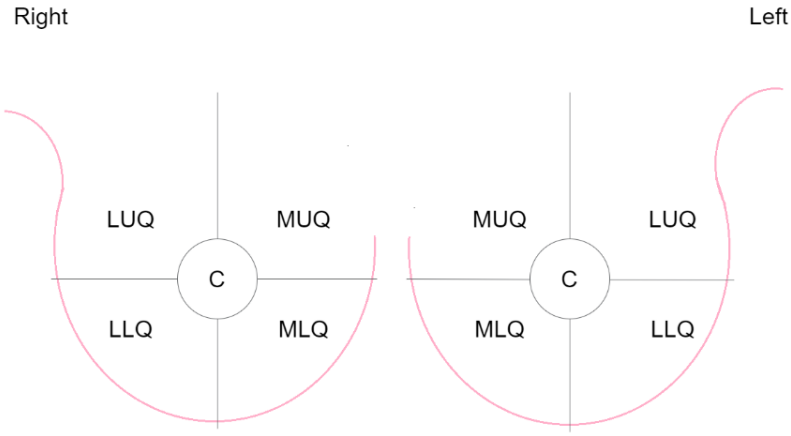

Figure 20 Schematic visualisation of the subdivision of the breast in breast quadrants. LUQ = lateral upper quadrant, LLQ = lateral lower quadrant, MLQ = medial lower quadrant, MUQ = medial upper quadrant, C = central.

content, was used for the comparison. During the SentiMag trial and MagSNOLL trial, the first edition of the Sentimag magnetometer probe was used. During the LowMag trial, the second edition was used. These probes measured a different number of counts for the same iron doses [65]. Therefore the measured counts during the SentiMag trial and MagSNOLL trial were corrected using the inverse of the scaling factor, defined by Pouw et al. [65].

Influence of personal variables and time on the iron content in the SN – It was investigated whether personal variables influence the iron content in the SN of the patients of LowMag trial. Therefore, the correlation was calculated between the iron content in the SN and the following five variables: age, BMI, breast volume, tumour size, and tumour location. In addition, the correlation was calculated between the iron content in the SN and the time interval between Sienna+ injection and surgery, which is given in days. The correlation was also determined between the number of removed SNs and the time interval between Sienna+ injection and surgery.

Statistical analyses

All statistical analyses were performed using SPSS Statistics, version 25 (IBM corporation, Armonk, United States). All numerical variables were visually checked for normal distributions. A one-way ANOVA corrected with posthoc Tukey HSD test was performed to compare the normally distributed variables of the three trials. A Kruskal-Wallis test was performed to compare the not normally distributed variables. If a significant difference was found, a Mann-Whitney U test was performed to analyse the interstudy significance for each combination of two trials. Fisher's Exact Test was performed to compare the categorical variables of the three trials. Pearson's correlation coefficients were calculated to determine the correlation for the normally distributed variables. Spearman's rank-order correlation coefficients were calculated to determine the correlation for not normally distributed variables.

Results

Comparison of the study populations – The patients' characteristics are shown in Table 3. No significant difference was found for the variables age, BMI, tumour size, and tumour location for the patients between the three trials. The bra size of the SentiMag trial patients and MagSNOLL trial patients were unknown. Therefore, no comparison could be made between the three studies for the variable breast volume.

Magnetic detection rate of the SN – It was recorded whether the SN was magnetically detectable using the Sentimag magnetometer for 20 of 31 patients in the SentiMag trial. It was possible to detect the SN with the Sentimag probe in 17 of these 20 patients (85%). It was possible to detect the SN with the Sentimag magnetometer in 1 of 10 patients (10%) in the MagSNOLL trial. However, in 3 of 10 patients, it was also not possible to detect the SN with the gamma probe. The magnetic detection rate of the SN was unknown for the LowMag trial.

Table 3 Patients characteristics for the three trials. The last column shows the significance of the four characteristics of the three trials.

	LowMag trial (n=39)		SentiMag trial (n=31)		MagSNOLL trial (n=10)		p-value
Mean age (years)	62.4 (SD=9.5)		58.8 (SD=10.2)		66.9 (SD=6.0)		.053
Mean BMI (kg/m²)	27.5 (SD=5.0)[α]		26.9 (SD=6.9)		28.3 (SD=3.7)		.777
Median tumour size (T-classification)	T1c (Q_1=1b, Q_3=1c)		T1c (Q_1=1b, Q_3=2)		T1c (Q_1=1b, Q_3=1c)		.619
Tumour location	LUQ	48.7%	LUQ	48.4%	LUQ	50.0%	
	LLQ	15.4%	LLQ	19.4%	LLQ	30.0%	
	MLQ	12.8%	MLQ	6.5%	MLQ	0.0%	.771
	MUQ	15.4%	MUQ	9.7%	MUQ	20.0%	
	Central	7.7%	Central	16.1%	Central	0.0%	

[α]n=38, one patient's BMI is unknown. SD = standard deviation, Q_1= 25th percentile, Q_3= 75th percentile, LUQ = lateral upper quadrant, LLQ = lateral lower quadrant, MLQ = medial lower quadrant, MUQ = medial upper quadrant.

Iron content in the SN – A significant difference in iron content in the SN was found between the three trials (p=.000). An overview of the iron content in the SN for the three trials is shown in Table 4. A significant difference in iron content in the SN was found between the LowMag trial (Mdn=4.1 µg, IQR=17.1 µg, n=39) and the SentiMag trial (Mdn=39.8 µg, IQR=66.3 µg, n=31, p=.000) and between the MagSNOLL trial (Mdn=3.0 µg, IQR=5.9 µg, n=10) and SentiMag trial (p=.000). No significant difference was found between the iron content in the SN in the LowMag trial and the MagSNOLL trial (p=.705). The iron content in the SN was also similar for patients who had a Sienna+ injection one day preoperative of the LowMag trial (Mdn=2.3 µg, IQR=14.3 µg) and the patients of the MagSNOLL trial (Mdn=3.0 µg, IQR=5.9 µg, p=.923).

Influence of personal variables and time on the iron content in the SN – No significant correlations were found between the four personal variables and the iron content in the SN of the patients of the LowMag trial, as can be seen in Table 5. One patient's BMI was unknown, and the bra size of eight patients was unknown. No significant difference was found between the iron content in the SN for the different tumour locations (p=.065). No significant correlation was found between the iron content in the SN and the time interval between Sienna+ injection and

Table 4 The iron content in the removed sentinel nodes for the three trials.

	LowMag trial (n=39)	SentiMag trial (n=31)	MagSNOLL trial (n=10)
Median (µg)	4.1	39.8	3.0
IQR (µg)	17.1	66.3	5.9
Median (µg) one day between injection and surgery	2.3 (n=12)	73.7 (n=9)	3.0 (n=10)
IQR (µg) one day between injection and surgery	14.3 (n=12)	142.7 (n=9)	5.9 (n=10)

IQR = interquartile range

Table 5 The correlation between four personal variables and the iron content in the sentinel node (SN), the significance value between the iron content in the SN and the fifth personal variable tumour location, and the correlation between iron content in the SN and the time interval between injection and surgery. These values were calculated using the data of the LowMag trial.

	Age	BMI	Breast volume	Tumour size	Tumour location	Time interval
Correlation Coefficient	-.192 (p=.241)	-.168 (p=.314)	-.055 (p=.770)	-.085 (p=.609)	x	.129 (p=.433)
Kruskal-Wallis test	x	x	x	x	p=.065	x
Number	39	38[α]	31[β]	39	39	39

[α] n=38, one patient's BMI is unknown. [β] n=31, the bra size of eight patients is unknown.

surgery (r=.129, p=.433, n=39). No significant correlation was found between the number of removed SNs and the time between Sienna+ injection and surgery (r=-.005, p=.976, n=39). A significant correlation was found between the number of removed SNs and the BMI of the patients (r=-.421, p=.008, n=38).

Discussion

The iron content in the SN is similar for the LowMag trial (Mdn=4.1 μg, IQR=17.1 μg, n=39) and the MagSNOLL trial (Mdn=3.0 μg, IQR=5.9 μg, n=10), even though three to fourteen times more iron was injected during the MagSNOLL trial (p=.705). Moreover, the iron content in the ex-vivo SN is almost the same, when comparing the patients who received the Sienna+ injection one day preoperative for the LowMag trial (Mdn=2.3 μg, IQR=14.3 μg, n=12) and the MagSNOLL trial (Mdn=3.0 μg, IQR=5.9 μg, n=10, p=.923). A significant difference (p=.000) was found between the iron content in the SN of the LowMag trial (Mdn=4.1 μg, IQR=17.1 μg, n=39) compared to iron content in the SN of the SentiMag trial (Mdn=39.8 μg, IQR=66.3 μg, n=31). The higher iron content found by the SentiMag trial compared to the LowMag trial corresponds to our expectation. The SN was detected with ease during the SentiMag trial, whereas it was more difficult to detect the SN during the LowMag trial.

A magnetic detection rate of 85% (n=20) was found for the analysed data of the SentiMag trial in the MST, which is lower than the average detection rate of 96% of the conventional SLNB procedure [6, 7]. However, Douek et al. analysed all for the study collected data in the MST and six other hospitals (n=160) and found a detection rate of 94.4% for the magnetic SLNB procedure, compared to a detection rate of 95.0% for the conventional SLNB procedure [12]. The difference in detection rate, 85% in our study and 94.4% in the study of Douek et al., can possibly be explained by the small number of patients analysed in our study, and due to the learning curves of the surgeons using the Sentimag magnetometer. The magnetic detection rate of the SN is unknown for the LowMag procedures. However, the overall opinion of the surgeons is that it is difficult to detect the SN. In addition, the iron content in the SN is similar for the LowMag trial and MagSNOLL trial. During the MagSNOLL trial, a low magnetic detection rate of the SN was found (10%, n=10).

The three studies have a few differences in procedures. The main differences related to the magnetic SN detection are the following: the injected Sienna+ dose, the time between injection and surgery, and the injection site.

Firstly, a high dose of 2 mL Sienna+ was used during the first magnetic SLNB procedures in breast cancer patients [12, 15–18]. It was possible to magnetically detect the SN, due to the high iron content, because of the high injection dose. However, many SPIOs remain in the breast, inducing artefacts on postoperative MRI scans [19, 69]. These artefacts could make it difficult and sometimes impossible to evaluate the breast tissue using the conventional breast MRI protocol and are therefore unwanted. One of the reasons why surgeons decided to stop using the magnetic SLNB procedure is because of these artefacts [personal conversation, see appendix E]. Other surgeons recommend not to use the SPIOs in case the patient needs follow-up using MRI. Karakatsanis et al. perform a study using a low dose of SPIOs, the Sentidose trial [70]. They use doses of 1.0 mL and 1.5 mL. In addition, they evaluate postoperative MRIs on SPIOs induced artefacts, the POSTMAG MRI trial [71]. These studies are ongoing and therefore the results are not yet published. The surgeons affiliated with these studies said that no large SPIOs artefacts were observed on postoperative MRIs [personal conversation, see appendix E]. The postoperative MRIs in the three mentioned studies [19, 69, 71] were made without the contrast agent gadolinium. A breast cancer surgeon not affiliated with the afore mentioned studies said that tumours can be detected when using this contrast agent [personal conversation, see appendix E]. If MRI artefacts will occur, despite the low dose of SPIOs, it is good to investigate if gadolinium can be used to detect the tumour.

A second difference between the procedures of the three trials is the time interval between Sienna+ injection and surgery. This time interval ranged from 20 minutes to 6 days preoperative. The time interval between injection and detection must be large enough to give the SPIOs the possibility to drain to the SN. Christenhusz et al. made a preoperative SPIOs MRI of one patient, who received a 0.04 mL Sienna+ injection [19]. They showed that using a large time interval of 5 days resulted in drainage of the SPIOs to not only the SN but also to higher-order lymph nodes. They hypothesized that it is more difficult to detect the SN due to presence of SPIOs in the higher-order lymph nodes. Therefore, they recommended using a smaller time interval between injection and surgery, to avoid SPIOs in higher-order lymph nodes. However, a clear difference in number of counts was found between the balancing location at the sternum and the axilla without SPIOs during a simulation of the LowMag procedure in healthy volunteers [appendix C]. A low dose of SPIOs, such as used in the LowMag trial, created a small hotspot in counts. This made it difficult to distinguish the counts induced by the SPIOs in the SN and the counts induced by the diamagnetism in the axilla of the human body. Besides, Karakatsanis et al. [72] and Wärnberg et al. [48] showed that the SPIOs could be injected up to 27 days preoperative, with good detection results of 95 – 98%, when using a high dose of 2 mL Sienna+. They showed that a preoperative SPIOs injection resulted in better detection of the SN compared to perioperative SPIOs injection. In addition,

they showed a significant difference in number of SNs retrieved using the preoperative injection versus the perioperative injection. They found no differences in detection rate and retrieved SNs for the preoperative time intervals of 1 – 27 days. This finding is in accordance with the interim evaluation of the LowMag trial, which showed no correlation between the retrieved SNs and the time interval between SPIOs injection and surgery (r=-.005, p=.976, n=39).

The third difference between the three procedures is the location of the injection. Sienna+ was injected periareolar during the SentiMag trial and intratumoural during the MagSNOLL trial and LowMag trial. The three injection sites are visualised in Figure 19. A periareolar SPIOs injection is unwanted, because SPIOs stay in the breast resulting in staining of the skin and remaining artefacts years after surgery [19, 69]. In case of an intratumoural SPIOs injection, the SPIOs are removed with the tumour during surgery. It is notable that the iron content in the SN is similar for the MagSNOLL trial (Mdn=3.0 μg, IQR=5.9 μg, n=10) and LowMag trial (Mdn=2.3 μg, IQR=14.3 μg, n=12, p=.923), despite the three to fourteen times higher dose of SPIOs which was injected during the MagSNOLL trial. Therefore, we think that an intratumoural injection with a higher iron concentration does not result in a higher amount of SPIOs in the SN. Several studies investigated the number of lymphatic vessels in and surround the tumour and in healthy breast tissue [37–40]. They found a significant higher lymphatic vessel density peritumoural compared to intratumoural. Furthermore, in some patients, no intratumoural lymphatic vessel was found. In case of a peritumoural injection, the SPIOs will be injected very close to the tumour. In the conventional SLNB procedure, the tumour and a part of the surrounding healthy tissue are removed during surgery. Since the SPIOs will be injected in this healthy tissue, the hypothesis is that most of the SPIOs will be removed during surgery and that therefore no clinical relevant artefact will occur during a breast MRI after surgery. A peritumoural Sienna+ injection for SLNB detection is part of the conventional care at the Uppsala University Hospital, Sweden [72]. Wärnberg et al. showed that a peritumoural SPIOs injection gives similar detection rates compared to a periareolar SPIOs injection [48].

The results of this study suggest that personal variables such as age (r=-.192, p=.241, n=39), BMI (r=-.168, p=.314, n=38), breast volume (r=-.055, p=.770, n=31), tumour size (r=-.085, p=.609, n=39), and tumour location (p=.065, n=39) did not affect the iron content in the SN. The correlations are small and not significant. In addition, no correlation was found between the iron content in the SN and the time interval between Sienna+ injection and surgery (r=.129, p=.433, n=39). This finding is comparable with the conclusion of Wärnberg et al., who showed no correlation between transcutaneous detection and the time interval between injection and surgery [48]. Therefore, we do not think that a personal adjusted SPIOs protocol results in better SN detection.

A significant correlation was found between the variable BMI and the number of removed SNs during the LowMag trial (r=-.421, p=.008, n=38). This result suggests that surgeons remove more SNs in patients with a low BMI. However, many variables influence the decision of a

surgeon to excise a lymph node. The decision is made using the preoperatively made lymphoscintigraphy, the detection with the gamma probe and/or the Sentimag probe, and the blue colouring during surgery. In addition, Percy et al. found that the decision of a surgeon can be biased by variables such as age, the size and grade of the tumour, and the receptor status of the tumour [73]. Moreover, the found correlation was determined using the small study population of the LowMag trial (n=38). A larger number of SLNB procedures, outside the study context of the LowMag trial, should be investigated to prove a significant correlation between the BMI of a patient and the number of removed SNs

A strength of this study is the similar iron content in the SN of the MagSNOLL trial (Mdn=3.0 μg, IQR=5.9 μg, n=10) and LowMag trial (Mdn=2.3 μg, IQR=14.3 μg, n=12) despite the three to fourteen times higher dose of iron which was injected during the MagSNOLL trial (p=.923). The iron content in these SNs is significantly smaller than the iron content in the SNs of the SentiMag trial (Mdn=39.8 μg, IQR=66.3 μg, n=31, p=.000). The SentiMag trial had a good detection rate of 85%. Therefore, we think that an intratumoural injection with a higher concentration Sienna+ does not result in better detection of the SN. Another strength of this study is that the three studies have a similar study population related to age (p=.053), BMI (p=.777), tumour size (p=.619) and tumour location (p=.771). Therefore, it was possible to compare the results of the studies.

The first limitation of this study is that the measured counts strongly depended on the orientation and size of the removed SN to the probe, as described in chapter III. In addition, a scaling factor was used to scale the counts of probe 1 to probe 2. This created another inaccuracy, which is however probably smaller than the inaccuracy caused by the distance. The calculated iron dose probably did not correspond to the true iron content in the SN, due to the mentioned inaccuracies. However, the large differences in iron content in the SN between the LowMag trial and SentiMag trial will probably exist. A second limitation is the small number of patients in the three studies (n=39, m=31, k=10). This makes it difficult and less reliable to draw conclusions. In addition, no significant correlations were found between personal variables and the iron content in the SN. However, due to the small number of patients, a correlation cannot completely be excluded.

In conclusion, enough SPIOs have to drain to the SN to be able to magnetically detect the SN. This study suggests that personal variables do not significantly affect the iron content in the SN, and therefore it is not necessary to make a personal SLNB procedure. In addition, no correlation was found between the iron content in the SN and the time interval between Sienna+ injection and surgery. Furthermore, an intratumoural injection with a higher dose did not result in higher iron content in the SN. Studies showed that a higher lymphatic vessel density is found peritumoural compared to intratumoural [37–40]. Therefore, it is recommended to investigate whether an adjustment of the injection site, from intratumoural to peritumoural, will increase the iron content in the SN.

V. Adjusted protocol of the LowMag trial

Introduction

The previous chapter showed that an intratumoural injection with a higher dose of SPIOs did not result in a higher iron content in the SN. Several studies found a significant higher lymphatic vessel density peritumoural compared to intratumoural [37–40]. Moreover, in some patients, no intratumoural lymphatic vessel was found. Our hypothesis is that the SPIOs will better drain to the SN after a peritumoural injection compared to an intratumoural injection, and therefore the iron content in the SN will increase. The aim of this chapter is to investigate whether a higher iron content in the SN can be found after a peritumoural injection compared to an intratumoural injection. In addition, it is investigated whether it is possible to magnetically detect the SN during the procedures.

Method

Procedure

Ten patients with breast cancer and scheduled for primary surgery including a SLNB procedure were included in the LowMag trial between August and December 2019. The patients' characteristics are shown in Table 6. Inclusion criteria were adult patients with an invasive tumour or carcinoma in situ. Patients who were pregnant or lactating and patients with a pacemaker or hypersensitivity to iron or dextran compounds were excluded. All ten patients signed an informed consent form prior to the study. The patients were scheduled for both the conventional SLNB and magnetic SLNB during one surgery.

In the conventional SLNB procedure, a ^{99m}Tc-labelled tracer injection was injected subareolar or periareolar one day preoperative or in the morning on the day of surgery. In the magnetic SLNB procedure, the patient received a SPIOs injection one to seven days preoperative. This ultrasound-guided injection was given by a radiologist specialised in breast radiology. The SPIOs were injected peritumoural at the dorsal side of the tumour. In case the tumour was located close to the musculus pectoralis major, the SPIOs were injected at the lateral side of the tumour. The first two patients, patient 1 and 2, received an injection of 0.04 mL Sienna+,

Table 6 Patients characteristics of the ten patients who participated in the peritumoural injection protocol.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Age (years)	57	48	69	52	53	48	58	47	40	61
BMI (kg/m²)	22.3	28.7	22.1	26.8	21.3	33.2	27.8	26.1	22.8	26.0
Bra size	80D	n/a	75B	80C	75E	90F	85C	90B	80F	n/a
Tumour size (T-classification)	T1b	T1c	T2	Tis	T2	T0[α]	T1b	T1c	T1c	Tis
Tumour location	MUQ	LLQ	LLQ	MLQ	MLQ	LUQ	MUQ	MUQ	LUQ	LUQ

[α] The tumour of patient 6 had a complete response to the neoadjuvant chemotherapy, and therefore the pathological tumour size was zero millimetres. LUQ = lateral upper quadrant, LLQ = lateral lower quadrant, MLQ = medial lower quadrant, MUQ = medial upper quadrant.

containing 1.1 mg iron, diluted with saline to a volume of 0.5 mL. These two procedures were evaluated for the magnetic detectability of the SN. To improve the magnetic detectability of the SN, the SPIOs concentration in the injection was increased for the other eight patients, patient 3 – 10. They received an injection of 0.16 mL Sienna+, containing 4.4 mg iron, diluted with 0.34 mL saline.

A transcutaneous hotspot was searched in the axilla using the Sentimag magnetometer and the gamma probe before the actual surgery started. Blue dye was not injected in the breast in case a gamma hotspot was found and the surgeon was comfortable with omitting the blue dye. By leaving aside the blue dye, it was possible to search for a brownish discoloured lymph node during the preparation. First, the tumour was removed from the breast during the actual surgery. Thereafter, the SN was searched using the Sentimag magnetometer, and the location was confirmed using the gamma probe. Subsequently, SNs detected by the Sentimag magnetometer or gamma probe were removed. Ex-vivo Sentimag counts and gamma counts were measured for each lymph node, which were separately placed directly at the probe. Several questions about the detectability of the SN using the magnetic and conventional procedure were asked to the surgeon after the surgery, see the questionnaire in appendix F. In addition, ex-vivo SNs of patient 5 – 10 were measured with the SPaQ magnetometer at the UT.

A postoperative MRI of the breast was made for the first five patients who were scheduled for breast-conserving surgery, respectively patient 1, 2, 4 – 6. This MRI was made approximately six weeks after surgery to evaluate the presence of residual SPIOs in the breast. Imaging was performed using a 3 Tesla Ingenia MR scanner (Phillips, Best, the Netherlands). T2-weighted Volume ISotropic Turbo spin-echo Acquisition (VISTA) images were acquired in the transversal plane. Second, T1-weighted Fast Field Echo (FFE) 3D images were acquired in the transversal plane. The image parameters are described in Table 7. The postoperative MRI sequences correspond to the diagnostic breast MRI sequences. The T2 VISTA sequence is generally used to visualise the anatomy of the breast. The T1 FFE 3D sequence is generally made to diagnose breast malignancies.

Table 7 Image parameters of the two MRI sequences used during the postoperative MRI

	T2 VISTA 3D	**T1 FFE 3D**
Echo pulse sequence	Spin Echo	Gradient Echo
Repetition time (ms)	1800	6.27
Echo time (ms)	299.06	2.74
Flip angle (°)	90	10
Field-Of-View (mm)	379 x 379 x 400	376 x 376 x 202
Reconstructed resolution (mm)	0.88 x 0.88 x 1.40	0.67 x 0.67 x 0.70
Scan time (min:s)	02:15	03:07

Analysis

Iron content in the SN – Ex-vivo counts of the SN measured with the Sentimag magnetometer were converted to an iron dose using the correlation factors calculated by the phantom studies, as described in chapter III. Subsequently, it was investigated whether a difference in iron content in the SN could be found after an intratumoural SPIOs injection (described in chapter IV) or a peritumoural SPIOs injection. Therefore, one SN per patient, the SN with the highest iron content, was used.

Ex-vivo counts of the SN measured with the SPaQ magnetometer were also converted to an iron dose using the correlation factor calculated by the phantom studies, as described in chapter III. Subsequently, the determined iron content in the SN using the Sentimag counts were compared to the determined iron content in the same SN using the SPaQ counts.

Detection rate of the SN – The detectability of the SN using the magnetic procedure was compared to the detectability of the SN using the conventional procedure, based on the surgeon's opinion. In addition, in case the SN was detectable it was investigated how easy it was to detect the SN.

Evaluation of postoperative MRIs – The postoperative MRIs were evaluated by a radiologist specialised in breast radiology to determine whether there were remaining SPIOs in the breast resulting in a void artefact. If a void artefact was seen, it was evaluated whether this artefact made it impossible to judge the breast tissue. In addition, the visible axillary and parasternal lymph nodes were evaluated for the presence of SPIOs.

Statistical analysis

All statistical analyses were performed using SPSS Statistics, version 25 (IBM corporation, Armonk, United States). The numerical variables were visually checked for normal distributions. An independent sample T-test was performed to compare the normally distributed variables and a Mann-Whitney U test was performed in the not normally distributed variables.

Results

Iron content in the SN – Table 8 shows an overview of the injected iron dose during the intratumoural protocol and the peritumoural protocol. No significant difference was found for the iron content in the SN between the peritumoural injection protocol (Mdn=10.1 μg, IQR=16.4 μg, n=10) and intratumoural injection protocol (Mdn=4.1 μg, IQR=17.1 μg, n=39, p=.723). The determined iron content in the ex-vivo SN based on the measured Sentimag counts and the determined iron content in the ex-vivo SN based on the measured SPaQ counts are shown in Table 9. No counts were detected in the SNs of one patient for both the Sentimag magnetometer and SPaQ magnetometer. For another patient, a small number of counts was detected with the Sentimag magnetometer and no counts were detected with the SPaQ magnetometer. The amount of iron in the SN based on the Sentimag counts was higher than

Table 8 An overview of the injection procedure variables for the peritumoural injection protocol and the intratumoural injection protocol.

	Peritumoural protocol (n=10)	**Intratumoural protocol (n=39)**
Injected iron dose (mg)	1.1 (n=2) 4.4 (n=8)	1.1
Injected Sienna+ volume (mL)	0.04 (n=2) 0.16 (n=8)	0.04
Injected saline volume (mL)	0.46 (n=2) 0.34 (n=8)	0.46
Total injected volume (mL)	0.5	0.5

the amount of iron in the SN based on the SPaQ counts for SNs with small iron content (smaller than 5 μg). For SNs with high iron content (higher than 10 μg), the amount of iron based on the Sentimag counts was lower than the amount of iron based on the SPaQ counts.

Detection rate of the SN – All patients received an injection with the radioactive tracer and an injection with the magnetic tracer. Four patients also received an injection with blue dye. During the procedures of these four patients, no clear transcutaneous hotspot could be detected using the gamma probe as well as the Sentimag probe, so the surgeon was not comfortable by omitting the blue dye. In all patients, it was possible to detect the SN using the conventional SLNB procedure. In 50% of the patients, it was possible to detect the SN using the magnetic SLNB procedure. The surgeons (strongly) agreed that the SN was easy to locate using the conventional SLNB procedure in 100% of the procedures. In 80% of the procedures in which it was possible to detect the SN using the magnetic procedure, the surgeons agreed that the SN was easy to locate using the magnetic SLNB procedure. The possibility to detect the SN and the score in which the surgeon agreed that it was easy to locate the SN is given in Table 10.

Table 9 The determined iron content in the ex-vivo sentinel node (SN) based on the detected number of counts using the Sentimag magnetometer and the SPaQ magnetometer.

	SN 1 (μg)		**SN 2 (μg)**		**SN 3 (μg)**	
	Sentimag	**SPaQ**	**Sentimag**	**SPaQ**	**Sentimag**	**SPaQ**
Patient 1	1.2	n/a	0	n/a	0	n/a
Patient 2	11.5	n/a				
Patient 3	8.9	n/a				
Patient 4	12.2	n/a				
Patient 5	0	0	0	0		
Patient 6	1.2	0				
Patient 7	33.9	48.6	0.5	0		
Patient 8	11.2	15.0				
Patient 9	50.8	76.0	41.9	50.7		
Patient 10	2.2	1.4				

Table 10 An overview per patient whether it was possible to detect the SN using the magnetic SLNB procedure and the conventional SLNB procedure (radioactive tracer + blue dye). In addition, the score in which the surgeon agreed that it was easy to locate the SN is given.

	Magnetic SLNB procedure		**Conventional SLNB procedure**	
	Possible to detect	Easy to detect	Possible to detect	Easy to detect
Patient 1	Yes	Agree	Yes	Agree
Patient 2	Yes	Agree	Yes	Agree
Patient 3	No	Strongly disagree	Yes	Strongly agree
Patient 4	Yes	Agree	Yes	Strongly agree
Patient 5	No	Strongly disagree	Yes	Agree
Patient 6	No	Strongly disagree	Yes	Strongly agree
Patient 7	Yes	Agree	Yes	Strongly agree
Patient 8	Yes	Disagree	Yes	Strongly agree
Patient 9	No	Disagree	Yes	Agree
Patient 10	No	Neutral	Yes	Strongly agree

Evaluation of postoperative MRIs – The evaluation of postoperative MRIs was performed by a radiologist specialised in breast radiology. In all five patients who received a postoperative MRI no void artefacts induced by remaining SPIOs in the breast were visible. In addition, no lymph nodes with iron content could be seen in the postoperative MRI images of the five patients. Figure 21 shows images of the two MRI sequences of patient 4.

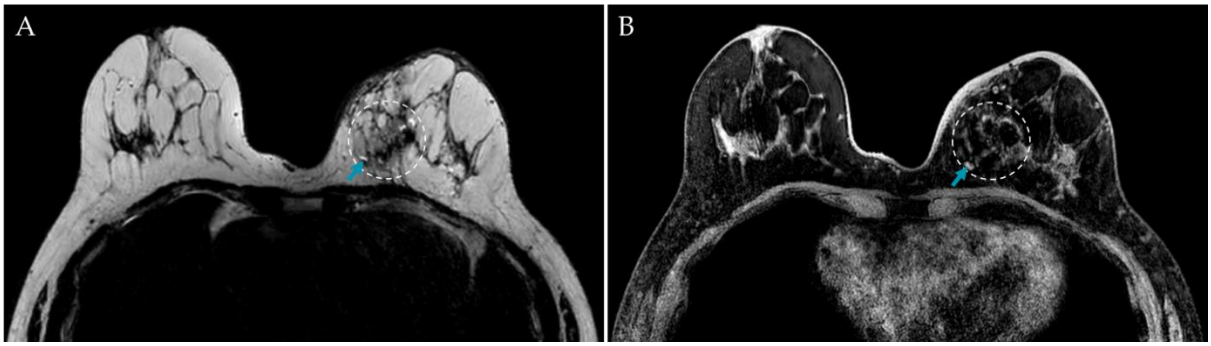

Figure 21 Postoperative MRI images of the breasts of patient 4. The dotted circle marks the postoperative location of the removed tumour. The blue arrow shows a metal marker which was placed during surgery. A) A transversal image from the T2 VISTA 3D sequence. B) A transversal image from the T1 FFE 3D sequence.

Discussion

It seems that an adjustment of the injection site does not result in a higher iron content in the SN. The median of the iron content in the SN of the peritumoural protocol (Mdn=10.1 μg, IQR=16.4 μg, n=10) is similar to the median of the iron content in the SN of the intratumoural protocol (Mdn=4.1 μg, IQR=17.1 μg, n=39, p=.723).

In 50% of the SLNB procedures was it possible to magnetically detect the SN. This detection rate is much lower than the detection rate of the conventional procedure, using a radioactive tracer and blue dye, which had a detection rate of 100%. The surgeons agreed that the SN was easy to locate using the magnetic SLNB procedure in 80% of the cases in which it was possible

to magnetically detect the SN. However, it was easy to locate the SN using the conventional SN procedure for all patients. In one patient the location of the magnetic hotspot differed from the location of the radioactive hotspot. Therefore, it seemed that the magnetic tracer drained to a different SN than the radioactive tracer. The surgeon decided not to remove the magnetic SN to avoid additional tissue damage since the magnetic SN was located in deeper tissue of the axilla. A magnetic SN other than the radioactive SN could be explained by the fact that the tracers were injected at a different site resulting in a different drainage route to the lymph nodes. In two other patients, the SN was mostly detected by the blue dye. These patients had a very small radioactive and magnetic hotspot which made it difficult to detect the SN with the gamma probe and Sentimag probe.

The SNs detected by the Sentimag magnetometer, gamma probe or blue dye were removed. A median iron content in the ex-vivo SN of 11.5 μg, based on the Sentimag counts, was found for the five procedures in which it was possible to magnetically detect the SN. However, it is hard to say whether this is the minimum iron content to detect the SN. A high number of counts, corresponding to 50.8 μg iron, was magnetically detected during an ex-vivo SN measurement of a procedure in which it was not possible to locate the SN using the magnetic SLNB procedure. In addition, in some procedures of the SentiMag trial [66] and MagSNOLL trial [67], it was not possible to magnetically detect the SN, despite an iron content higher than 11.5 μg in the SN.

There were also five SLNB procedures in which it was not possible to magnetically detect the SN. The SNs were removed using the gamma probe or blue dye during these procedures. A median iron content of 2.2 μg was found in these ex-vivo SNs, based on the counts measured with the Sentimag magnetometer. Measurements with the SPaQ magnetometer showed no iron content in two of these five SNs, which made it impossible to magnetically detect these lymph nodes during the SLNB procedure. In addition to the iron content in the SN, both the distance between the SN and the Sentimag probe and the experience of the surgeon influenced detection of the SN.

The determined iron content in the ex-vivo SN using the Sentimag counts do not exactly match the determined iron content using the SPaQ counts. In SNs with a small amount of iron the determined iron content on the Sentimag counts is higher than the iron content based on the SPaQ counts. In SNs with a higher amount of iron the determined iron content based on the Sentimag counts is lower than the iron content based on the SPaQ counts. The difference in determined iron doses could be explained by the difference in technique. The measured SPaQ counts were independent of the location of the SPIOs in the SN, while the Sentimag counts were affected by the distance of the SPIOs to the probe. In addition, the Sentimag magnetometer measured the magnetic signal from the SPIOs and the diamagnetism of the human tissue, while the SPaQ only measured the magnetic signal from the SPIOs.

The adjusted low dose magnetic procedure described in this chapter had three major changes compared to the low dose magnetic procedure described in chapter IV. First, the injection site of the SPIOs was changed from intratumoural to a peritumoural injection. Second, the injected iron dose was increased to 4.4 mg in eight of the ten procedures. The hypothesis was that a peritumoural injection with a higher dose of SPIOs resulted in higher iron content in the SN. However, no iron content was found in the SN of two patients who received an injection with 4.4 mg iron. While clear iron content was found in the SN of one patient who received an injection with 1.1 mg iron. It could be that this one patient had very good lymph drainage, while for other patients the iron dose was still too low to drain enough particles to the SN. The third change in the procedure was the expertise gained during a visit to the Uppsala University Hospital, Sweden [appendix E]. The magnetic SLNB procedure is conventional care for breast cancer patients at the Uppsala University Hospital. They inject a magnetic tracer containing 28 – 56 mg iron peritumoural up to seven days before surgery. The Swedish surgeons showed good detection rates using the magnetic SLNB procedure. After this workshop, the Dutch surgeons were more focused on searching for a small increase in counts and were less distracted by the imbalances of the Sentimag magnetometer. This new search approach could influence the possibility of locating the SN and the ease of detecting the SN.

The postoperative MRI images of the breasts of five patients showed no void artefact induced by remaining SPIOs in the breast. Therefore, it is possible to judge the breast tissue for new tumours after a peritumoural SPIOs injection. In addition, no SPIOs could be seen in the imaged lymph nodes. These results are in accordance with the results of the Uppsala University Hospital. Preliminary results showed no large artefacts on postoperative MRIs. [Personal conversation, see appendix E] The aim of the two postoperative MRI sequences during the LowMag trial was to evaluate the tissue. A different MRI sequence, specific for SPIOs, should be used to determine whether there are remaining SPIOs left in the human body.

In conclusion, it was possible to magnetically detect the SN in 50% of the procedures. In addition, no significant difference was found between the iron content in the SN after a peritumoural SPIOs injection and an intratumoural SPIOs injection. Furthermore, postoperative MRI images showed no SPIOs induced artefacts after a peritumoural SPIOs injection. These results indicate that a peritumoural SPIOs injection is a safe method without disadvantages for further imaging. Nevertheless, the SN could be detected using the conventional procedure in all SLNB procedures. Therefore, the detection rate of the magnetic SLNB procedure must increase to match the detection rate of the conventional SLNB procedure. Despite the small number of patients included in this study, which makes it hard to conclude firm statements, it is recommended to increase the injected iron dose to stimulate an increased iron content in the SN.

VI. Discussion

The aim of this thesis was to investigate how to improve the magnetic detection of the SN using the Sentimag magnetometer and a low dose magnetic tracer. This thesis confirmed the opinion of the surgeons that it is difficult to magnetically detect the SN during the LowMag trial. In 50% of the procedures it was possible to magnetically detect the SN using a peritumoural Sienna+ injection containing 1.1 – 4.4 mg iron (n=10). Whereas, it was possible to detect the SN in all procedures using the conventional SLNB procedure, using a radioactive tracer and blue dye (n=10). A promising finding is that the surgeons agreed that the SN was easy to locate using the magnetic SLNB procedure in 80% of the procedures in which it was possible to magnetically detect the SN.

Another important finding is that postoperative MRIs showed good assessable images of breast tissue, without SPIOs induced artefacts after a peritumoural SPIOs injection. These results support the hypothesis that most of SPIOs are removed with the tumour during surgery. Therefore, it seems that a peritumoural SPIOs injection is a safe method without disadvantages for further MRI imaging. This thesis found no significant difference between the iron content in SN after an intratumoural SPIOs injection containing 1.1 mg iron (Mdn=4.1 μg, IQR=17.1 μg, n=39) and the iron content in SN after a peritumoural SPIOs injection containing 1.1 – 4.4 mg iron (Mdn=10.1 μg, IQR=16.4 μg, n=10, p=.723). However, several studies showed that not all tumours contain intratumoural lymph vessels [37–40]. Therefore, we recommend a peritumoural SPIOs injection over an intratumoural SPIOs injection. In addition, this thesis also found no significant correlation between the iron content in the SN and personal factors, such as age, BMI, breast volume, tumour size, and tumour location. These findings suggest that the SPIOs injection procedure does not have to be person specific. Besides, this thesis has shown that the by Sentimag magnetometer detected number of counts strongly depends on the iron dose in the SN and on the distance between probe and SN. In addition, the magnetic aspect of the environment (diamagnetic tissue and ferromagnetic surgical instruments) affected the output, which makes it more challenging to detect the SN.

The magnetic SLNB procedure is a promising method. Some adjustments to the procedure must be made to increase the magnetic detection rate in order that the magnetic SLNB procedure can become the new conventional care. The following three main factors affect the possibility to magnetically detect the SN. First of all, the SPIOs have to drain to the SN. It is not possible to magnetically detect the SN when there are no SPIOs in the SN. In two procedures using the adjusted LowMag protocol (n=10), no or a very small number of counts were detected in the ex-vivo SN. In one of these two procedures, also a small number of radioactive counts was detected. The retrieved SN was mostly found because of its blue discolouration due to the blue dye. The particle drainage to the SN is mainly influenced by the size and the surface charge of the particle [52, 74–77]. The blue dye has an estimated diameter smaller than 2 nm [78], the radioactive tracer, has a particle size smaller or equal to 80 nm [79]

and the magnetic tracer Sienna+ has a particle size of 60 nm [22]. Small particles rapidly distribute through the lymphatic system. As a result, they do not only drain to the SN but also to higher-order lymph nodes. Large particles, larger than 100 nm, remain mainly at the injection site [76]. Besides, particles with a negative charge move faster through the interstitial matrix, because the interstitial matrix has a small net negative charge under normal physiological conditions [77]. In addition to the size and charge of the particles, the uptake in the SN is also influenced by the injection dose, injection site, and the time interval between the injection and surgery. Higher injection doses result in higher uptake in the SN [49–52, 66]. Just a small part of the injection dose, up to 5%, enters the lymph node [49–52]. Most injected particles remain at the injection site. The injection site could also influence the drainage to the lymph node. In one procedure of the adjusted LowMag protocol (n=10), it seems that the SPIOs drained to a different lymph node than the radioactive tracer. The SPIOs were injected peritumoural and the radioactive tracer was injected periareolar. In literature, there is no univocal conclusion about lymph drainage of the breast. Some studies showed a better and quicker marking of the SN for superficial injection (peri or subareolar) compared to a deep injection (peri or intratumoural) [32, 33]. Other studies showed a similar detection rate [34–36]. In addition, a significant higher lymphatic vessel density was found peritumoural compared to intratumoural [37–40]. Moreover, in some patients, no intratumoural lymphatic vessel was found. These different and sometimes conflicting findings makes it difficult to decide the best injection site for the magnetic tracer. Another variable that could influence the uptake in the SN is the time interval between injection and surgery [52, 72, 75]. Karakatsanis et al. [72] recommend a time interval of minimal one day between SPIOs injection and surgery. They found that the detection rate increased during the first day. Thereafter, the detection rate remained comparable for a time interval up to 27 days.

Second, the technique of the magnetometer affects the possibility to detect the SN. The generated magnetic field is strong when close to the probe and becomes weaker as the distance to the probe increases. Therefore, it is more difficult to detect a SN further away from the probe. The mean depth of the axillary lymph nodes is four centimetres, but the SN can be located up to eight centimetres deep [43, 44]. During the phantom study, it was possible to detect a sample containing 100 μg iron at a maximum distance of 15 mm. In one procedure of the adjusted LowMag protocol (n=10), it was not possible to magnetically detect the SN, while ex-vivo a clear number of counts could be measured. Approximately 8 μg iron was found in this SN, using the correlation factor determined in chapter III. The phantom study revealed that it is possible to detect a lymph node containing 8 μg iron at a maximum distance of approximately 5 mm. This SN was located much deeper in the axilla making it difficult or impossible to detect with the magnetometer. The iron content in the SN should be increased or the penetration depth of the magnetic field induced by the magnetometer should be expanded to be able to detect a deep SN. However, the magnetic field strength is limited because the diameter of the probe must be small enough to be able to use during surgery [61]. In addition, the ICNIRP has strict guidelines for the use of magnetic fields in patients [62].

Furthermore, a stronger magnetic field has its limitations. The magnetic field strength does not only increase in forward direction of the probe but also in lateral direction of the probe. This increases the risk that surgical instruments, the surgery table, or the Sienna+ injection site are present within the magnetic field and therefore influence the number of counts. In addition, by increasing the magnetic field, also more human tissue is located within the magnetic field. The diamagnetism of this tissue generates a magnetic field that influences the received number of counts. This could limit the benefit of increased penetration depth. The DiffMag magnetometer filters most of the signal induced by tissue and surgical instruments, as a result the detected number of counts are mostly generated by SPIOs. Studies must show whether the penetration depth of the DiffMag magnetometer is deep enough and whether it is possible to detect the SN using the DiffMag magnetometer.

The third factor which mainly affects the possibility to detect the SN is the experience of the surgeon. The Sentimag magnetometer has a different technique than the gamma probe. Therefore, a different interpretation of the detected counts is needed to locate the SN. The Sentimag magnetometer must be balanced to reduce the impact of diamagnetism of the human tissue. However, still a number of counts induced by the diamagnetism of the human tissue are detected. Therefore, the surgeon must search for a small increase in counts and follow that direction. The gamma probe is frequently used by the surgeon, while the Sentimag magnetometer is a new and not frequently used technique in the MST. This makes it challenging for the surgeon to detect the SN. The use of a low dose of SPIOs makes it even more challenging for the surgeon to detect the SN during the LowMag trial. A workshop using a phantom axilla and a phantom SN containing SPIOs could be organised to increase the experience of the surgeons on how to use the Sentimag magnetometer.

Strengths and limitations

The strengths of this thesis are, first, the determined correlation between the iron dose and number of counts using a phantom study. This is the first study that provides insight into this relationship for the three magnetometers: Sentimag, DiffMag, and SPaQ. Second, when it was possible to magnetically detect the SN, the surgeons agreed that the SN was easy to detect in 80% of these procedures. This result shows that the magnetic SLNB procedure is a promising method. Third, this thesis clearly shows that additional adjustments must be undertaken to create a low dose magnetic SLNB procedure, which can be used in the conventional care.

The limitations of this thesis are, first, the small number of patients, making it hard to define a conclusion that is true for all breast cancer patients. Second, the number of counts in the SN detected during patients' studies were converted to an iron dose using the correlation factors determined by the phantom study. However, these correlation factors do not consider the diamagnetism of the human tissue, and the distribution of the SPIOs over the SN and therefore a distance to the probe. In addition, sometimes a pathologist found more than one SN in the tissue resection. The measured ex-vivo counts were then based on the cumulative iron content of all those SNs.

Clinical perspective

We think that the magnetic SLNB procedure certainly has clinical perspective. A great advantage compared to the conventional SLNB procedure is the flexibility in planning. The magnetic tracer has a shelf-life of years, which makes it easy to store until use, while ^{99m}Tc has a half-life of six hours. In addition, the SPIOs can be injected up to 27 days before surgery with good detection rates [48, 72]. Another advantage is the possibility to evaluate lymph node metastases in preoperative MRI imaging, avoiding unnecessary SLNB with risk for arm and shoulder complaints [6, 55, 80, 81].

Some adjustments must be made before the magnetic SLNB procedure can be used in conventional care. Improvements to the current magnetic SLNB procedure must be made to increase the detection rate. Suggestions for these improvements have already been mentioned. In addition, preoperative imaging could be made to guide the surgeon.

Further recommendations

The first recommendation for the LowMag trial is to increase the injected SPIOs dose. The currently used dose containing 1.1 – 4.4 mg iron is too low to detect the SN in 50% of the magnetic SLNB procedures (n=10). Furthermore, the new magnetometer DiffMag is not approved for clinical use. In addition, it takes a long time to study and improve the SPIOs. Therefore, it is recommended to continue using the Sentimag magnetometer and Sienna+ in the LowMag trial and to increase the injection dose. Besides, the low dose of SPIOs used in the LowMag trial is much lower than the safe considered SPIOs dose, which contains 56 mg, recommended by the manufacturer.

It is recommended to consider a periareolar SPIOs injection in patients who are planned for a breast amputation. In that case, the injection site is similar to the injection site of the radioactive tracer. In addition, some studies show better and quicker lymphatic drainage after a periareolar injection [32, 33]. Besides, a periareolar injection does not have to be given ultrasound-guided. The SPIOs injection can be given during a regular appointment at the breast clinic, which also reduces the costs.

Research should be done to investigate how the SPIOs distribute over the lymph nodes. Therefore, it is recommended to perform a trial in which patients who are scheduled for an axillary lymph node dissection receive a SPIOs injection. These patients do not benefit from this trial, but they help improve further treatments. All axillary lymph nodes of these patients are removed, making it possible to measure all lymph nodes in the SPaQ magnetometer. The measured number of counts can be converted to an iron dose using the correlations determined in the phantom study, described in chapter III. Then knowledge is gained about how much iron reach the lymph nodes, and how it is distributed over all axillary lymph nodes. Subsequently, simulations of the SPIOs distribution over the lymph nodes can be made using this data. This information can be used to improve the magnetic SLNB procedure.

Appendix A – Flowchart of the LowMag trial procedure

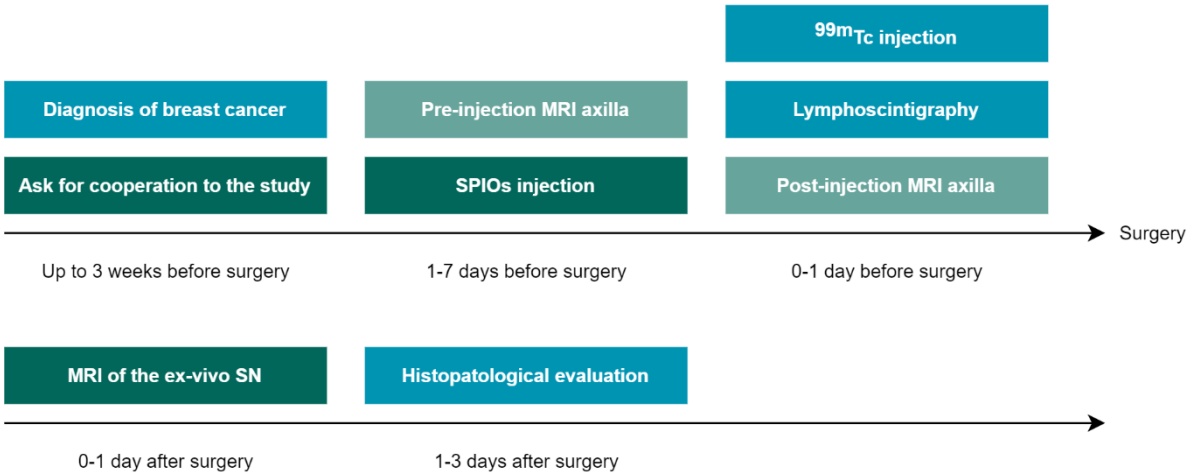

Figure 1 The procedure for patients who participated in the LowMag trial. In blue the steps which belong to the conventional sentinel lymph node biopsy (SLNB) procedure, which make use of a blue dye and 99m-Technetium (^{99m}Tc) as a radioactive tracer. In green the additional steps which belong to the LowMag trial, the magnetic SLNB procedure. The magnetic SLNB procedure uses superparamagnetic iron oxide nanoparticles (SPIOs) as a magnetic tracer. Magnetic resonance imaging (MRI) of the axilla was optional, therefore not all patients received these MRIs. An MRI was made of all ex-vivo sentinel nodes (SN).

Appendix B – Conversion table: counts to iron dose

Table 1 A conversion table to convert the detected number of counts to an iron dose (µg) for the three magnetometers

	SPaQ	Diffmag	Sentimag		
			Setting 1	Setting 2	Setting 3
Counts					
0	0	0	0	0	0
100	7	11	4	2	1
200	14	21	8	4	2
300	20	32	12	7	4
400	27	42	15	9	5
500	34	53	19	11	6
600	41	63	23	13	7
700	47	74	27	15	8
800	54	84	31	18	9
900	61	95	35	20	11
1,000	68	105	38	22	12
1,500	101	158	58	33	18
2,000	135	211	77	44	24
2,500	169	263	96	55	29
3,000	203	316	115	66	35
3,500	236	368	135	77	41
4,000	270	421	154	88	47
4,500	304	474	173	99	53
5,000	338	526	192	110	59
5,500	372	579	212	121	65
6,000	405	632	231	132	71
6,500	439	684	250	143	77
7,000	473	737	269	155	82
7,500	507	789	288	166	88
8,000	541	842	308	177	94
8,500	574	895	327	188	100
9,000	608	947	346	199	106
9,500	642	1,000	365	210	112
10,000	676	1,053	385	221	118

Appendix C – Simulation of the LowMag procedure in healthy volunteers

It is difficult to detect the SN using the Sentimag magnetometer and a low dose of SPIOs during surgery. Besides adjusting the injection protocol based on the analysis of the available data, we also wanted to know whether the performance of the procedure can be improved in order to gain better detection. Therefore the procedure was tested on four healthy volunteers with ex-vivo SPIOs.

Sentimag magnetometer start-up period before use

The Sentimag magnetometer should be switched on 15 minutes before starting the surgery for the best results, according to the instructions for use [58]. However, in practice, it happened that the first measurements were performed within those 15 minutes. Therefore, it was investigated whether there were differences between measurements performed within 15 minutes and after 15 minutes after turning on the Sentimag magnetometer.

Firstly, after switching on the Sentimag magnetometer, the Sentimag probe was balanced on the sternum and moved to the axilla without SPIOs. Subsequently, the probe was balanced again on the sternum and moved to the axilla with an ex-vivo sample containing SPIOs. After 15 minutes the probe was balanced again on the sternum and moved to the axilla without SPIOs for a measurement. Thereafter, a measurement was performed in the axilla with an ex-vivo sample containing SPIOs. No clear differences in counts were detected between the measurements performed within 15 minutes and after 15 minutes after switching on the Sentimag magnetometer for all four volunteers. In addition, no clear differences were detected in the number of balances that was needed during the measurements.

In conclusion, no clear differences in performance were found between the Sentimag magnetometer used within 15 minutes after switching on and using the magnetometer minimal 15 minutes after switching on. The manufacturer recommends to switch on the Sentimag magnetometer minimal 15 minutes before use for the best results [58]. However, similar results can be detected in cases the magnetometer is needed within first 15 minutes.

Balancing location

The Sentimag magnetometer must be balanced to create a measurement baseline [58]. For the best results, the probe should be held at least half a meter away from any metallic object. The probe can be balanced in the air or in contact with the body, depending on the preference of the user. The Sentimag probe was balanced at the sternum during the procedures in the MST. In this way, one considered the diamagnetic aspect of the body. It was investigated whether the diamagnetic aspect of the sternum is comparable with the diamagnetic aspect of the axilla.

During our measurements the Sentimag probe was balanced at the sternum and moved through the air to the axilla without any SPIOs. A higher number of counts was detected at the axilla (100 – 300 counts, setting 3) than at the sternum (0 – 10 counts, setting 3) for all four volunteers after balancing the Sentimag magnetometer.

These results suggest that the diamagnetic aspect at the sternum differs from the diamagnetic aspect at the axilla. Balancing at the sternum created a measurement baseline that did not perfectly fit the axilla. However, sterile cloths cover most of the patient during surgery. Therefore, it is not possible to balance the Sentimag probe in the healthy axilla. The other suggestion of the manufacturer is to balance the Sentimag probe in the air [58]. However, in that case, the diamagnetic aspect of the body is not considered and therefore the created measurement baseline will not perfectly fit the axilla as well.

During the LowMag trial, small numbers of counts were detected in the axilla. In that case, it was difficult to distinguish the counts induced by the SPIOs in the SN and the counts induced by the diamagnetic aspect of the axilla. Two possibilities can ensure better detection of the SN. Firstly, the impact of the diamagnetic aspect of the axilla should be reduced. The UT develops a DiffMag magnetometer which filters the signal induced by tissue [59]. Secondly, the magnetic impact of SPIOs should be increased. For this situation more SPIOs need to drain in the SN or the SPIOs should be modified in order to increase their magnetic susceptibility.

Movements with the Sentimag probe

The number of counts changed greatly during movements of the probe. According to the manufacturer, this is normal and is due to changes in conditions of the probe head [58]. For example due to a change in the thermal environment. When drift is seen in the number of counts, it is up to the personal preference of the user to choose whether to rebalance the Sentimag magnetometer. The number of rebalances during the LowMag trial at the operation room was quite high. It was investigated whether the probe can be moved differently, resulting in fewer drifts, and thereby in a smaller number of rebalances. Therefore, three factors were tested.

Firstly, it was investigated whether the speed at which the probe was moved affects drift. The probe was balanced at the sternum and moved with normal speed to the axilla without ex-vivo SPIOs. Subsequently, the probe was rebalanced at the sternum and moved with slower speed to the axilla without ex-vivo SPIOs. These measurements were repeated using an ex-vivo sample containing SPIOs in the axilla. The measurements were performed three times on four healthy volunteers. No clear differences were found in the number of drifts between the normal speed and slower speed movements of the probe. In case the probe was moved very slow, with an unnatural speed, it takes longer before the Sentimag magnetometer drifted.

Secondly, it was investigated whether the SPIOs hotspot in the breast increased the risk of the probe to drift. The probe was balanced at the sternum and moved across the ex-vivo hotspot at the breast to the axilla without ex-vivo SPIOs. These measurements were performed several

times and the distance between the probe and the hotspot was variated. The Sentimag counts drifted a little earlier when the probe was moved very close across the hotspot compared to a movement further away across the hotspot. However, this difference was not very clear.

Thirdly, it was investigated whether the Sentimag counts drift quicker when the surgeon's hand passes the black line on the probe. The probe consists of a probe head and a probe handle which are separated by a black line. The probe head creates a magnetic field and detects a change in this field. The probe handle is the place the surgeon should hold the probe. The probe was balanced in the air with one hand held on the probe handle. Subsequently, the hand was moved to the probe head and measured some counts. Then, the hand was moved back to the probe handle, and no counts were measured. After multiple times of moving the hand to the probe head and back to the probe handle, a difference in counts was measured. In addition, the probe was balanced at the sternum with the hand correctly on the probe handle, then the probe was moved to the axilla and the hand was displaced to the probe head. A small difference in counts was measured.

In conclusion, the Sentimag counts will drift despite the way the probe is moved. Slower movement of the probe, avoidance of the hotspot in the breast and correct holding of the probe can cause the counts to drift less quickly. However, the drift caused by these factors is much smaller than the difference in counts measured between the sternum and the axilla.

Influence of the operation room

There are a lot of metallic objects in the operation room. For the best results, the manufacturer recommends balancing the Sentimag probe at least half a meter away from any metallic or magnetic object [58]. In addition, plastic instruments were used to magnetically detect the SN during surgery. It was investigated whether there are currently unknown influences in the operation room on the magnetic field.

Therefore, the SN procedure was simulated using ex-vivo SPIOs in the operation room. The Sentimag probe was balanced at the sternum and moved to the axilla containing ex-vivo SPIOs. No influence of the metallic instruments was found within 10 – 15 cm to the probe head. Large negative signals were measured when the probe came close to the surgery table. At a 10 cm distance between probe and table, a number of -30 counts was measured using setting 3 of the Sentimag magnetometer. The number of counts decreased to -2000 when reducing the distance between the probe and the table to 0 cm.

In conclusion, the metallic instruments did not influence the signal when they were at least 15 cm away from the probe head. It was found that the surgery table has a strong negative influence on the Sentimag counts. While searching for the SN it is good to be aware of the possible influence of the surgery table. Therefore, it is advised to orient the probe as parallel as possible to the surgery table. Thereby, the signal from the SN is minimal obfuscated by the signal induced by the surgery table.

Appendix D – Conversion table: bra size to breast volume group

Table 1 A conversion table to translate the bra size to a breast volume group.

		Cup size							
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Band size	**65**	1	2	3	4	5	6	7	8
	70	2	3	4	5	6	7	8	9
	75	3	4	5	6	7	8	9	10
	80	4	5	6	7	8	9	10	11
	85	5	6	7	8	9	10	11	12
	90	6	7	8	9	10	11	12	13
	95	7	8	9	10	11	12	13	14
	100	8	9	10	11	12	13	14	15
	105	9	10	11	12	13	14	15	16

Appendix E – Experiences of the magnetic sentinel node procedure in other hospitals

Introduction

The Sentimag magnetometer and its magnetic tracer, Sienna+, are used as the magnetic SLNB procedure in breast cancer patients since 2012 [82]. The magnetic procedure is used in a study context in the MST since then. Experience and knowledge have been gained resulting in adjustments to the procedure. We still make adjustments to develop a magnetic procedure that can be used in the conventional care during the SLNB procedure. Therefore, we wonder how other Sentimag magnetometer users think about the following two points.

Firstly, in the opinion of the surgeons of the MST it was difficult to magnetically detect the SN using a low dose of SPIOs, according to the first 39 procedures of the LowMag trial. The Sentimag magnetometer was very sensitive to movements. In addition, in a study with healthy volunteers without SPIOs it was found that the Sentimag magnetometer measured a different number of counts at the sternum than axilla, as mentioned in appendix C. This makes it difficult to distinguish the SN. Secondly, nowadays still an injection with a high dose of iron (56 mg) is recommended by the manufacturer [58], despite large artefacts in breasts observed in MRI images taken years after surgery [19]. The manufacturer warns that the magnetic tracer can alter MRI images over a long period, and states that the surgeon should consider whether the magnetic tracer is suitable for the patient. There is very little information available in the literature about the SPIOs induced MRI artefacts in breast cancer patients. We are interested in the experiences of other Sentimag magnetometer users and how they use the Sentimag magnetometer and its magnetic tracer in their conventional care of SLNB. In addition, we wonder whether other Sentimag magnetometer users see these artefacts and how they think about the clinical impact of it.

Method

We searched for Sentimag magnetometer users by looking into published articles, posters, and ongoing studies. Subsequently, we searched the contact information of these people. The Sentimag magnetometer users were contacted by e-mail or via the research platform Researchgate.

Results

We contacted Sentimag magnetometer users from thirty hospitals and asked them to share their experiences with the magnetic SLNB procedure for breast cancer patients. Sentimag magnetometer users from seventeen hospitals responded. An overview is given in Table 1. The Sentimag magnetometer and its magnetic tracer are used in the conventional SLNB care of breast cancer patients in six of the seventeen hospitals. Two of the seventeen Sentimag magnetometer users use the magnetometer very exceptionally in the routine SLNB surgery.

They use the Sentimag magnetometer when they cannot use the radioactive tracer due to problems in the surgical schedule or in coordination with the nuclear medicine department. The other nine responders do not use the Sentimag magnetometer and its magnetic tracer in conventional care. They use a radioactive tracer to detect the SN.

The magnetic SLNB procedure slightly differs between the hospitals in which this procedure is the conventional care. An injection of 2 mL magnetic tracer is used in seven out of eight hospitals and an injection of 1 mL magnetic tracer is used in the eighth hospital. The injection is given periareolar in seven hospitals and peritumoural in one hospital. The time interval between the injection and surgery ranges from thirty days preoperative, twenty minutes before incision, up to perioperative.

The Sentimag magnetometer is mainly used in hospitals where no radioactive tracer is available. In addition, several studies proved that the magnetic SLNB procedure is non-inferior to the procedure using a radioactive tracer and blue dye, the current gold standard. An additional mentioned advantage is that the tracer can be injected up to thirty days before surgery, allowing flexible surgery planning. Another mentioned benefit is that unnecessary SLNB can be avoided in patients with DCIS. DCIS tissue is removed during primary surgery and a pathologist assesses this tissue. SLNB can be performed during a second surgery in case invasive carcinoma is found. SLNB can be avoided in case no invasive carcinoma is found, which is the case in 20% of patients with a preoperative diagnosis of DCIS [83].

The main reason why the Sentimag magnetometer is not used in conventional SLNB care is that the breast surgery department has a collaboration with the nuclear medicine department, which is near to the breast surgery department. Several other reasons were mentioned by the responders. One responder said that he does not believe that the magnetic SLNB procedure can become the standard of care in the absence of a randomised control trial. Another responder said that he cannot risk missing a local recurrence because of MRI artefacts, which do not disappear over years. A third responder said that he uses a radioactive tracer because it is reliable and less expensive.

Table 1 Overview of the hospitals of which we received a response from a Sentimag magnetometer user.

Hospital	Country	SPIOs in conventional care	Injection site	Volume	Injection time
Uppsala University Hospital	Sweden	Yes	Peritumoural	2 mL	1-30 days preoperative
Medical University of Lublin	Poland	Yes	Periareolar	2 mL	1 day preoperative
University of California San Francisco	US	Yes	Periareolar	2 mL	Intraoperative
St. Joseph Hospital Center	France	Yes	Periareolar	2 mL	Intraoperative - 7 days preoperative
Hospital Center De Vichy	France	Yes	Periareolar	2 mL	Intraoperative - 7 days preoperative
Hospital Center De Belfort Montbeliard	France	Yes	Periareolar	2 mL	Intraoperative - 7 days preoperative
Virgen de la Arrixaca University Hospital	Spain	Very exceptionally	Periareolar	2 mL	Intraoperative
University Hospital of Pisa	Italy	Very exceptionally	Periareolar	1 mL	Intraoperative
Kwong Wah Hospital	China	No, a first ongoing study	-	-	-
UCL & King's College London	UK	No	-	-	-
County Hospital of Västmanland	Sweden	No	-	-	-
Frauenarztzentrum Baden, Kantonsspital Baden	Switserland	No	-	-	-
Norwich and Norfolk University Hospitals	UK	No	-	-	-
Agaplesion Markus Hospital	Germany	No	-	-	-
Regina Elena National Tumour Institute	Italy	No	-	-	-
University Hospital of Wales at Llandough	UK	No	-	-	-
University Hospital Complex of Vigo	Spain	No	-	-	-

Almost all responders, both users and ex-users, said that they have good experiences with the Sentimag magnetometer technique. They think it is a friendly technique and it works fine. Patients seem to feel it is better than the radioactive tracer. However, the Sentimag magnetometer has a longer learning curve since the device is more sensitive than the gamma probe. Therefore the operator needs to understand the physics of the Sentimag magnetometer.

Different opinions exist about the by SPIOs induced MRI artefacts. For some responders, these MRI artefacts are one of the reasons why they do not use the magnetic SLNB procedure in their conventional care. They argue that they cannot guarantee proper follow-up care due to the MRI artefacts induced by SPIOs. Some of the responders who use the magnetic procedure in their conventional care do not use it for patients who need follow-up MRIs. One responder investigates postoperative MRI scans of patients who receive the magnetic tracer and evaluate these scans on the presence of SPIOs induced artefacts. Other responders see no relevant problems with MRI interferences. One of them said that gadolinium, an MRI contrast, can be used to detect breast cancer even in areas with remaining SPIOs.

The surgeons of the Uppsala University Hospital invited us for a workshop at their department. They balanced the probe at a rib lateral caudal from the breast. Subsequently, they searched for a hotspot in the axilla. A small increase was seen as a hotspot, for example, 50 counts using the Sentimag magnetometer in setting 1. Then, they prepared the tissue and search for a brown coloured lymph node. They recommended dropping the blue dye such that the brown colour of the SPIOs can be seen. In addition, they evaluate postoperative MRIs on SPIOs induced artefacts in an ongoing study, the POSTMAG MRI trial [71]. Preliminary results showed that no large SPIOs artefacts were observed on postoperative MRIs.

Discussion

The surgeons have very different opinions about the magnetic SLNB procedure. Most responders have not introduced the magnetic SLNB procedure in their conventional care after finishing the study. They use the radioactive tracer and a blue dye. Notable is the use of the Sentimag magnetometer and its magnetic tracer with a high dose of iron in the conventional SLNB care in six hospitals. Despite the large long-lasting artefacts on MRI images induced by remaining SPIOs, found by Christenhusz et al. [19].

The overall opinion is that the magnetic SLNB procedure is an obvious and friendly procedure to perform. However, due to the sensitivity of the Sentimag probe, the magnetic SLNB procedure has a longer learning curve than the gold standard. We think that it is even more challenging to perform the magnetic SLNB procedure in the LowMag trial because of the low dose of SPIOs. The small peaks we want to detect are more likely to disappear in the imbalances of the Sentimag counts. However, during the workshop at the Uppsala University Hospital, we learned that a small increase in counts could be enough to detect the SN. They recommended dropping the blue dye so that it is possible to search for a brown coloured lymph node during the preparation.

Appendix F – Questionnaire

A questionnaire for the surgeons about the conventional SLNB procedure, using a radioactive tracer and blue dye, and the magnetic SLNB procedure, using a magnetic tracer.

Question	Answer options				
I could detect the SN using the magnetic procedure	Yes	No			
I could detect the SN using the radioactive + blue dye procedure	Yes	No			
	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
I could easily detect the SN using the magnetic procedure	1	2	3	4	5
I could easily detect the SN using the radioactive + blue dye procedure	1	2	3	4	5

References

1. Healthwise. The lympatic system. Available at: https://www.whitecoat.com.au/blog/practitioner/the-lymphatic-system. (Accessed on: December 22, 2019)

2. Integraal Kankercentrum Nederland. Incidentie borstkanker. Available at: https://www.iknl.nl/kankersoorten/borstkanker/registratie/incidentie. (Accessed on: January 13, 2020)

3. Rijksinstituut voor Volksgezondheid en Milieu. Borstkanker cijfers & context. Available at: https://www.volksgezondheidenzorg.info/onderwerp/borstkanker/cijfers-context/huidige-situatie. (Accessed on: January 13, 2020)

4. Integraal Kankercentrum Nederland. Oncoline. Available at: https://www.oncoline.nl/borstkanker. (Accessed on: January 11, 2019)

5. Bravis Oncologie Centrum. Sentinel node localisation. Available at: https://www.bravisoncologiecentrum.nl/de-schildwachtklier-procedure. (Accessed on: January 11, 2019)

6. Goyal A, Mansel RE. Sentinel Lymph Node Biopsy in Early-stage Breast Cancer. In: Mansel R. E, Fodstad O, Jiang WG (eds) Metastasis of Breast cancer. Springer, Dordrecht, 2007; 333–353

7. Kim T, Giuliano AE, Lyman GH. Lymphatic mapping and sentinel lymph node biopsy in early-stage breast carcinoma: A metaanalysis. Cancer 2006;106:4–16.

8. National Research Council. Molybdenum-99/Technetium-99m Supply Reliability. Med. Isot. Prod. Without Highly Enriched Uranium. The National Academies Press, Washington, DC, 2009; 55–65

9. Bos AJJ, Draaisma FS, Okx WJC. Biologische gevolgen van straling. Inleid. tot stralingshygiëne. Sdu Uitgevers, Den Haag, 2007; 97–140

10. Bos AJJ, Draaisma FS, Okx WJC. Wet- en regelgeving betreffende stralingsbescherming. Inleid. tot stralingshygiëne. Sdu Uitgevers, Den Haag, 2007; 293–328

11. Ferrucci M, Franceschini G, Douek M. New techniques for sentinel node biopsy in breast cancer. Transl Cancer Res 2018;7:S405–S417.

12. Douek M, Klaase J, Monypenny I, Kothari A, Zechmeister K, Brown D, et al. Sentinel node biopsy using a magnetic tracer versus standard technique: The SentiMAG multicentre trial. Ann Surg Oncol 2014;21:1237–1245.

13. Liu J, Huang L, Wang N, Chen P. Indocyanine green detects sentinel lymph nodes in early breast cancer. J Int Med Res 2017;45:514–524.

14. Sever AR, Mills P, Jones SE, Cox K, Weeks J, Fish D, et al. Preoperative sentinel node identification with ultrasound using microbubbles in patients with breast cancer. Am J Roentgenol 2011;196:251–256.

15. Karakatsanis A, Christiansen PM, Fischer L, Hedin C, Pistioli L, Sund M, et al. The Nordic SentiMag trial: a comparison of super paramagnetic iron oxide (SPIO) nanoparticles versus Tc99 and patent blue in the detection of sentinel node (SN) in patients with breast cancer and a meta-analysis of earlier studies. Breast Cancer Res Treat 2016;157:281–294.

16. Zada A, Peek MCL, Ahmed M, Anninga B, Baker R, Kusakabe M, et al. Meta-analysis of sentinel lymph node biopsy in breast cancer using the magnetic technique. Br J Surg 2016;103:1409–1419.

17. Thill M, Kurylcio A, Welter R, van Haasteren V, Grosse B, Berclaz G, et al. The Central-European SentiMag study: Sentinel lymph node biopsy with superparamagnetic iron oxide (SPIO) vs. radioisotope. Breast 2014;23:175–179.

18. Ghilli M, Carretta E, Di Filippo F, Battaglia C, Fustaino L, Galanou I, et al. The superparamagnetic iron oxide tracer: a valid alternative in sentinel node biopsy for breast cancer treatment. Eur J Cancer Care (Engl) 2017;26:1–7.

19. Christenhusz A. Magnetic sentinel lymph node detection and metastases evaluation. 2017.

20. Mann RM, Kuhl CK, Kinkel K, Boets C. Breast MRI : guidelines from the European Society of Breast Imaging. Eur Radiol 2008;18:1307–1318.

21. Bakker MF, de Lange SV, Pijnappel RM, Mann RM, Peeters PHM, Monninkhof EM, et al. Supplemental MRI Screening for Women with Extremely Dense Breast Tissue. N Engl J Med 2019;381:2091–2102.

22. Sysmex Europe GmbH. Sentimag - Magnetic localisation of sentinel lymph nodes - easy, flexible and effective. 1–6.

23. Agur AMR, Dalley AF. Breast. Grant's Atlas Anat., Twelfth. Lippincott Williams & Wilkins, Philadelphia, 2009; 4–9

24. Moore KL, Dalley AF, Agur AMR. Breasts. Clin. Oriented Anat., sixth. Lippincott Williams & Wilkins, Philadelphia, 2010; 98–106

25. Rubin R, Strayer DS. Carcinoma of the breast. Rubin's Pathol., sixth. Lippincott Williams &Wilkins, Philadelphia, 2012; 933–944

26. Breastcancer.org. Types of Breast Cancer. Available at: https://www.breastcancer.org/symptoms/types. (Accessed on: December 21, 2019)

27. Willard-Mack CL. Normal Structure, Function, and Histology of Lymph Nodes. Toxicol Pathol 2006;34:409–424.

28. Parham P. The immune system, Third. New York: Garland Science; 2009.

29. Shayan R, Achen MG, Stacker SA. Lymphatic vessels in cancer metastasis: Bridging the gaps. Carcinogenesis 2006;27:1729–1738.

30. Borgstein PJ, Meijer S, Pijpers RJ, Van Diest PJ. Functional lymphatic anatomy for sentinel node biopsy in breast cancer: Echoes from the past and the periareolar blue method. Ann Surg 2000;232:81–89.

31. Suami H, Pan WR, Mann GB, Taylor GI. The lymphatic anatomy of the breast and its implications for sentinel lymph node biopsy: A human cadaver study. Ann Surg Oncol 2008;15:863–871.

32. Fowler JC, Solanki CK, Ballinger JR, Ravichandran D, Douglas-Jones A, Lawrence D, et al. Axillary Lymph Node Drainage Pathways from Intradermal and Intraparenchymal Breast Planes. J Surg Res 2010;161:69–75.

33. Lanchas Alfonso I, Miguel Martnez MB, CuezvaGuzmn JF, Ruprez Arribas P, Martnez Blanco S, Yartu San Mill JM, et al. Intratumoral versus subareolar injection in breast cancer sentinel lymph node biopsy. A case report. Rev. Esp. Med. Nucl.

34. Maza S, Valencia R, Geworski L, Zander A, Guski H, Winzer KJ, et al. Peritumoural versus subareolar administration of technetium-99m nanocolloid for sentinel lymph node detection in breast cancer: Preliminary results of a prospective intra-individual comparative study. Eur. J.

Nucl. Med. Mol. Imaging

35. Garcia-Manero M, Olartecoechea B, Royo P. Different injection sites of radionuclide for sentinel lymph node detection in breast cancer: Single institution experience. Eur J Obstet Gynecol Reprod Biol 2010;153:185–187.

36. Ahmed M, Purushotham AD, Horgan K, Klaase JM, Douek M. Meta-analysis of superficial versus deep injection of radioactive tracer and blue dye for lymphatic mapping and detection of sentinel lymph nodes in breast cancer. Br J Surg 2015;102:169–181.

37. Gong M, Cai Y, Zhang S, Zhang D, Zou L, Yi S. Intratumoral and peritumoral lymphatic vessel density both correlate with lymph node metastasis in breast cancer. Sci. Rep.

38. Kos M. Lymphatic and small blood vessel density in the tumor and peritumoral tissue in invasive breast carcinoma of no special type. Libr. Oncol. Croat. J. Oncol.

39. Agarwal B, Saxena R, Morimiya A, Mehrotra S, Badve S. Lymphangiogenesis does not occur in breast cancer. Am J Surg Pathol 2005;29:1449–1455.

40. El-Gohary YM, Metwally G, Saad RS, Robinson MJ, Mesko T, Poppiti RJ. Prognostic significance of intratumoral and peritumoral lymphatic density and blood vessel density in invasive breast carcinomas. Am. J. Clin. Pathol.

41. Leidenius MHK, Krogerus LA, Toivonen TS, Leppänen EA, Von Smitten KAJ. The clinical value of parasternal sentinel node biopsy in breast cancer. Ann Surg Oncol 2006;13:321–326.

42. Rahbar H, Partridge SC, Javid SH, Lehman CD. Imaging Axillary Lymph Nodes in Patients with Newly Diagnosed Breast Cancer. Curr Probl Diagn Radiol 2012;41:149–158.

43. Mathelin C, Salvador S, Huss D, Guyonnet J-L. Precise Localization of Sentinel Lymph Nodes and Estimation of Their Depth Using a Prototype Intraoperative Mini -Camera in Patients with Breast Cancer. J Nucl Med 2007;48:623–629.

44. Bentel GC, Marks LB, Hardenbergh PH, Prosnitz LR. Variability of the depth of supraclavicular and axillary lymph nodes in patients with breast cancer: Is a posterior axillary boost field necessary? Int J Radiat Oncol Biol Phys 2000;47:755–758.

45. Enriquez-Navas PM, Garcia-Martin ML. Conventional Iron-Oxide-Based NPs for MRI. In: de la Fuente JM, Grazu V (eds) Nanobiotechnology Inorg. Nanoparticles vs Org. Nanoparticles. Elsevier Science, Oxford, 2012; 233–237

46. Keselman P, Yu EY, Zhou XY, Goodwill PW, Chandrasekharan P, Ferguson RM, et al. Tracking short-term biodistribution and long-term clearance of SPIO tracers in magnetic particle imaging. Phys. Med. Biol.

47. Feng Q, Liu Y, Huang J, Chen K, Huang J, Xiao K. Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings. Sci. Rep.

48. Wärnberg F, Stigberg E, Obondo C, Olofsson H, Abdsaleh S, Wärnberg M, et al. Long-Term Outcome After Retro-Areolar Versus Peri-Tumoral Injection of Superparamagnetic Iron Oxide Nanoparticles (SPIO) for Sentinel Lymph Node Detection in Breast Cancer Surgery. Ann Surg Oncol 2019;1247–1253.

49. Waddington WA, Keshtgar MRS, Taylor I, Lakhani SR, Short MD, Ell PJ. Original article Radiation safety of the sentinel lymph node technique in breast cancer. 27:

50. Kuwahata A, Kusakabe M. Combined use of fluorescence with a magnetic tracer and dilution effect upon sentinel node localization in a murine model. 2018;2427–2433.

51. Ahmed M, Woo T, Ohashi K, Suzuki T, Kaneko A, Hoshino A, et al. Magnetic sentinel lymph node biopsy in a murine tumour model. Nanomedicine Nanotechnology, Biol Med 2016;12:1045–1052.

52. Pouw JJ, Ahmed M, Anninga B, Schuurman K, Pinder SE, Van Hemelrijck M, et al. Comparison of three magnetic nanoparticle tracers for sentinel lymph node biopsy in an in vivo porcine model. Int J Nanomedicine 2015;10:1235–1243.

53. Johnson L, Pinder SE, Douek M. Deposition of superparamagnetic iron-oxide nanoparticles in axillary sentinel lymph nodes following subcutaneous injection. Histopathology 2013;62:481–486.

54. Pouw JJ, Grootendorst MR, Bezooijen R, Klazen CAH, De Bruin WI, Klaase JM, et al. Pre-operative sentinel lymph node localization in breast cancer with superparamagnetic iron oxide MRI: The SentiMAG Multicentre Trial imaging subprotocol. Br. J. Radiol. 88:

55. Motomura K, Ishitobi M, Komoike Y, Koyama H. SPIO-Enhanced Magnetic Resonance Imaging for the Detection of Metastases in Sentinel Nodes Localized by Computed Tomography Lymphography in Patients with Breast Cancer. Ann Surg Oncol 2011;18:3422–3429.

56. Endomagnetics Limited. Sienna+ - Instructions for use.

57. Giancoli DC. Sources of Magnetic Field. Phys. Sci. Eng., 4th ed. Pearson Prentice Hall, Upper Saddle River, New Jersey, 2009; 733–757

58. Endomagnetics Limited. Sentimag - Instructions for use.

59. Waanders S, Visscher M, Wildeboer RR, Oderkerk TOB, Krooshoop HJG, Ten Haken B. A handheld SPIO-based sentinel lymph node mapping device using differential magnetometry. Phys Med Biol 2016;61:8120–8134.

60. Visscher M, Waanders S, Krooshoop HJG, Haken B. Journal of Magnetism and Magnetic Materials Selective detection of magnetic nanoparticles in biomedical applications using differential magnetometry. J Magn Magn Mater 2014;365:31–39.

61. Giancoli DC. Electromagnetic Induction and Faraday's Law. Phys. Sci. Eng., 4th ed. Pearson Prentice Hall, Upper Saddle River, New Jersey, 2009; 758–811

62. International Commission on Non-Ionizing Radiation Protection. Guidelines for limiting exposure to time-varying electric and magnetic fields (1Hz TO 100kHz). Health Phys 2010;99:818–836.

63. Selles M, Molenaar L, Lips D, ten Haken B. Ex-vivo sentinel lymph node mapping in colorectal cancer using a magnetic tracer : a pilot study. 2019.

64. Keshtgar MRS, Chicken DW, Waddington WA, Raven W, Ell PJ. A training simulator for sentinel node biopsy in breast cancer: A new standard. Eur J Surg Oncol 2005;31:134–140.

65. Pouw JJ, Bastiaan DMC, Klaase JM, ten Haken B. Phantom study quantifying the depth performance of a handheld magnetometer for sentinel lymph node biopsy. Phys Medica 2016;32:926–931.

66. SentiMag Multicentre Trial. Available at: https://www.trialregister.nl/trial/3139.

67. MagSNOLL multicenter Study. Available at: https://www.trialregister.nl/trial/4548.

68. Giuliano AE, Connolly JL, Edge SB, Mittendorf EA, Rugo HS, Solin LJ, et al. Breast Cancer-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin 2017;67:290–303.

69. Krischer B, Forte S, Niemann T, Kubik-Huch RA, Leo C. Feasibility of breast MRI after sentinel procedure for breast cancer with superparamagnetic tracers. Eur J Surg Oncol 2018;44:74–79.

70. Karakatsanis A. SentiDose Trial. Available at: https://doi.org/10.1186/ISRCTN11156955.

71. Karakatsanis A. POSTMAG MRI Trial. Available at: https://doi.org/10.1186/ISRCTN85167182.

72. Karakatsanis A, Daskalakis K, Stålberg P, Olofsson H, Andersson Y, Eriksson S, et al. Superparamagnetic iron oxide nanoparticles as the sole method for sentinel node biopsy detection in patients with breast cancer. Br J Surg 2017;104:1675–1685.

73. Percy DB, Pao JS, McKevitt E, Dingee C, Kuusk U, Warburton R. Number of nodes in sentinel lymph node biopsy for breast cancer: Are surgeons still biased? J Surg Oncol 2018;117:1487–1492.

74. Bagby TR, Cai S, Duan S, Thati S, Aires DJ, Forrest L. Impact of molecular weight on lymphatic drainage of a biopolymer-based imaging agent. Pharmaceutics 2012;4:276–295.

75. Jastrzebski T, Lass P, Świerblewski M, Wydra D, Drucis K, Kruszewski W, et al. The influence of radioisotope vehicle on breast sentinel node detection. Eur J Surg Oncol 2006;32:928–932.

76. Xie Y, Bagby TR, Cohen MS, Forrest ML. Drug delivery to the lymphatic system: Importance in future cancer diagnosis and therapies. Expert Opin Drug Deliv 2009;6:785–792.

77. Rao DA, Forrest ML, Alani AWG, Kwon GS, Robinson JR. Biodegradable PLGA based nanoparticles for sustained regional lymphatic drug delivery. J Pharm Sci 2010;99:2018–2031.

78. College ter Beoordeling van Geneesmiddelen. Bleu Patente V. 2018;1–10.

79. College ter Beoordeling van Geneesmiddelen. Nanocoll. 2018;1–11.

80. Verbelen H, Gebruers N. Shoulder and arm morbidity in sentinel node-negative breast cancer patients : a systematic review. Breast Cancer Res Treat 2014;144:21–31.

81. Verbelen H, Tjalma W, Meirte J, Gebruers N. Long - term morbidity after a negative sentinel node in breast cancer patients. Eur J Cancer Care (Engl) 2019;e13077:1–8.

82. Sysmex Europe GmbH. Magnetic SLNB for Breast Cancer: Results from 1000+ patients. 1–2.

83. Karakatsanis A, Hersi A-F, Pistiolis L, Bagge RO, Lykoudis PM, Eriksson S, et al. Effect of preoperative injection of superparamagnetic iron oxide particles on rates of sentinel lymph node dissection in women undergoing surgery for ductal carcinoma in situ (SentiNot study) . Br J Surg 2019;720–728.