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Superparamagnetic iron oxide as a tracer for sentinel node biopsy in breast cancer: A comparative non-inferiority study

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Abstract

Aims: The gold standard for detection of Sentinel Lymph Nodes (SLN) is a combined radioisotope and blue dye breast injection, using a gamma probe (GP). A new, non-radioactive method was developed, using a tracer (Sienna+®) of superparamagnetic iron oxide (SPIO) nanoparticles and a manual magnetometer (SentiMag®) (SM). The IMAGINE study was designed to show the non-inferiority of SM compared to GP, for the detection of SLN in breast cancer patients with SLN biopsy indication.
Methods: From November 2013 to June 2014, 181 patients were recruited, and 321 nodes were excised and assessed ex-vivo. Readings from both SM and GP devices were recorded during transcutaneous, intraoperative, and ex-vivo detection attempts.
Results: At the patient level, ex-vivo detection rates (primary variable) with SM and GP were 97.8% and 98.3% (concordance rate 99.4%). Transcutaneous and intraoperative detection rates were 95.5% vs 97.2%, and 97.2% vs 97.8% for SM and GP respectively (concordance rates $>$ 97%). At the node level, intraoperative and ex-vivo detection rates were 92.5% vs 89.3% and 91.0% vs 86.3% for SM and GP respectively. In all cases the non-inferiority of SM compared to SM was shown by ruling out a predefined non-inferiority margin of 5%.
Conclusions: Our study showed the non-inferiority of SM as compared to GP. Moreover, the ex-vivo and intraoperative detection rates at the node level were slightly higher with SM.

Keywords: Invasive breast cancer; Sentinel lymph node biopsy; Superparamagnetic iron oxide (SPIO); SentiMag®; Sienna+®

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Introduction

In Europe, breast cancer is the most common cancer in women, with an incidence of 429 900 new cases per year, and represents 28.9% of overall cancers.[1] Breast cancer propagates mainly through the lymphatic system and, classically, lymph node involvement is the most important prognostic factor for breast cancer management.[2] Sentinel lymph nodes (SLN) are the first nodes receiving lymphatic drainage from the tumour, and therefore those with the highest probability of affectation.[3] Currently, the SLN biopsy is a standard technique used in breast cancer patients with clinically and radiologically negative axilla for the purposes of staging and prognosis.[4] The SLNB was introduced in the 90s, and significantly reduces the morbidity associated with axillary lymphadenectomy.[5,6]

The gold standard for the detection of the SLN is a combined technique, in which both a radioisotope and blue dye are injected in the breast, and after some time to allow migration through the lymphatic chain, a gamma probe is used to detect SLNs. Lymphatic nodes that are blue or radioactive are considered SLNs and are excised. The main disadvantages of this technique are the exposure of both patients and physicians to radiation and radioisotope short half-life, availability, handling and disposal.[7,8]

A new, non-radioactive method has been developed for the identification of SLNs. This method uses a tracer (Sienna+®) composed of superparamagnetic iron oxide (SPIO) nanoparticles and a manual magnetometer (SentiMag®). When injected intravenously, SPIOs have been used as contrast agents for magnetic resonance imaging (MRI), and their characteristics are well known.[9,10] When injected subcutaneously, SPIO moves and accumulates into sentinel nodes within minutes, and iron deposition is seen predominantly within SLN sinuses and macrophages. In the event of metastatic involvement of the node, SPIOs are seen to deposit within uninvolved areas of the node only.[11] The nodes can be visualized by MRI imaging and during surgery, since the marked SLN are often coloured brown or black.[12] Sienna+® is a blackish-brown sterile aqueous suspension of SPIO carboxydextran-coated particles that is intended for use with the SentiMag device. The carboxydextran coating prevents agglomeration while maintaining biocompatibility. The particle diameter, including the organic coating, is 60 nm, ideally suited for SLNB. This diameter enables the SLNs to selectively filter out the particles and is similar to the particle size of standard radioisotope tracers.

In order to evaluate the performance of the SentiMag®/Sienna+® (SM) as compared to the gamma-probe (GP) technique (the standard practice in the Spanish healthcare system), we designed the "Isotopic vs. Magnetic Intraoperative Node Evaluation" (IMAGINE) multicentre study, to be conducted in 9 Spanish hospitals with extensive experience in SLNB. The study was designed to show the non-inferiority of SM as compared to the GP technique, for the detection of SLN in breast cancer patients in whom a SLN biopsy is indicated.

Materials and methods

Study subjects

In 9 Spanish hospitals, breast cancer patients were recruited for the study if they were 18 years of age or older, had a SLN biopsy programmed, and were preoperatively node negative, clinically and radiologically. Patients were excluded if they had received neoadjuvant therapy, were intolerant to iron or dextran compounds (present in Sienna+), the administration of a radioisotope was contraindicated, had disorders implying high iron concentration, or had a pacemaker or other metallic device implanted in the thorax wall. The study was approved by the Institutional Review Board (IRB) of the participant centres and all patients had to provide their informed consent to participate in the study.

Preoperative procedures and SLN detection methods

For the identification of the SLN, patients received an injection of radioisotope tracer, and optionally, an injection of methylene blue, according to the standard protocol of each centre. The day of the surgery, two ml of Sienna+® were diluted in saline, obtaining a final volume of 5 ml that was injected subcutaneously in the subareolar area (after anaesthesia but previous to the methylene blue injection if vital dye was used as a tracer), and followed by a 5 min massage of the injection site. Intradermal injection of the tracer must be avoided in order to prevent skin pigmentation. At least 20 min after the Sienna+® injection, transcutaneous detection was attempted with both devices (SM and GP) before incision. The existence of extraaxillary signal was also assessed transcutaneously with both methods. After incision, intraoperative detection (in the patient's armpit) was attempted with SM, and the positive spots were also measured intraoperatively using GP. All nodes with a positive reading with SM were excised as long as their reading was superior to 10% of the node with the highest SM reading. When no magnetic signal remained, the axilla was then verified using GP. All remaining nodes positive with GP were excised as long as their reading was superior to 10% of the node with the highest GP reading. Lymph nodes dyed blue or black, or suspicious by palpation were also excised. Finally, all excised nodes were measured again ex-vivo with both detection devices (SM and GP), and submitted to for either OSNA or histologic analysis, according to usual practice in each centre.

Surgeons participating in the study were trained in the use of the SM device during a single session held before the study began. In addition, support by trainers was also provided during the procedures for the first few patients recruited in each study centre.

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Data collection and outcomes

Data were recorded in an electronic case report form, including study selection criteria, demographic and anthropometric data, menopausal status, medical and surgical antecedents, the side of primary tumour (in case of bilateral tumours one side was chosen), its location and maximum diameter, and the type of surgery performed. The following data were recorded concerning the SLN detection procedures: time and location of the radioisotope, methylene blue and Sienna+® injections, start and end times of the SLN biopsy, complications, and results of readings with both the SM and GP devices during transcutaneous, intraoperative, and ex-vivo detection attempts. Brown and/or blue colouration of the detected nodes and residual readings after excision of detected nodes were also recorded. In the case of the transcutaneous detection, only the maximum reading with each device was recorded. In case of extraaxillary detection, the device showing positive signal was also recorded. We also recorded the surgeon's satisfaction with the devices and their opinion concerning their detection capacity.

Study outcomes were the results (as positive or negative) of detection attempts with each device, for all transcutaneous, intraoperative, ex-vivo and residual measurements. The (maximum) transcutaneous and residual measurements were considered positive if a positive reading was obtained. Since intraoperative and ex-vivo measurements were obtained for individual nodes (possibly, more than one per patient), nodes were considered positive if a positive reading was obtained and it was at least 10% of the maximum reading obtained for other nodes of the same patient. At the patient level, intraoperative and ex-vivo detection were considered to be positive if at least one node for that patient had been positive. The result of the ex-vivo detection at the patient level was predefined as the primary endpoint of the study.

Other outcomes of the study were the time from the Sienna+® injection to the start of the SLN biopsy, the number of nodes assessed and the number of positive nodes per patient according to the measurements obtained with each detection device, both intraoperatively and ex-vivo.

Statistical analysis

The statistical analysis was conducted according to a previous statistical analysis plan. Data were summarised as mean (SD), median (IQR) or n (%) as appropriate. The detection rates with each device were computed, and their difference estimated. For the intraoperative and the ex-vivo attempts, detection rates were computed at both the node level and the patient level. Since the objective of the study was to show non-inferiority of SM as compared to GP, one-sided 95% confidence intervals for the difference in detection rates were computed using the Wald method,[13] as implemented in function diffpropci.Wald.mp of the R package PropCIs.[14] Non-inferiority was declared if a pre-defined advantage of 5% (or higher) of the GP device over the SM device could be ruled out.

Subgroup analyses of the ex-vivo detection rates were conducted on histopathologically positive nodes (nodes with >250 copies CK19/μl by OSNA or micro-macrometastasis by traditional histological analysis), and on patients with extraaxillary detection. The association between time from Sienna+® injection and start of SLN biopsy was investigated using Spearman's rho. Patients with or without extraaxillary detection were compared for BMI values using a two-sample t-test, for the time from Sienna+® injection to the start of SLN biopsy with a Wilcoxon rank sum, and for the radioisotope injection site using a Fisher's exact test. Statistical significance was declared if $p < 0.05$. All statistical analyses were performed using the R language, version 3.0.1.[15]

Results

Patient characteristics

From November 2013 to June 2014, a total number of 181 patients meeting the study selection criteria were consecutively recruited. The age of patients ranged from 30 to 88 years, and only one patient was male. The patient and pathologic characteristics are shown in Table 1. Seventeen patients were diabetic, 50 had arterial hypertension, and three had benign breast antecedents. Twenty two patients had allergic antecedents to allergens other than those iron or dextran compounds.

Preoperative procedures and SLN detection

The radioisotope injection was peritumoral in 91 cases, and periareolar in the remaining 90 cases. In the 147 cases (81.2%) in which the SLN biopsy was performed before the breast surgery, the time from the Sienna+® injection to the start of biopsy ranged from 10 to 85 min (median 24, IQR 10), and no evidence was found of association between this time and the BMI (Spearman's rho = 0.014, p = 0.863). Also in these cases, the duration of the SLN biopsy ranged from 5 to 65 min (median 20, IQR 15).

A total number of 319 nodes were assessed intraoperatively. The number of nodes assessed per patient ranged from 1 to 5 (median 2, IQR 1), and a total number of 321 nodes were excised and assessed ex-vivo. The number of nodes excised per patient ranged from 1 to 5 (median 2, IQR 1). The mean (SD) number of sentinel nodes detected ex-vivo was 1.55 (0.77) with GP, and 1.63 (0.80) with SM. Excised sentinel lymph nodes showed brown colouration in 178 cases (61% of all SM-positive sentinel lymph nodes).

The results of the transcutaneous, intraoperative and ex-vivo detection attempts are shown in Table 2. In the case of the intraoperative and the ex-vivo detection, both the patient-level and the node level analysis are shown. In patient-level analyses, the detection rates were very similar

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Table 1
Patient and pathology data, summarized as mean (SD) or n (%) as appropriate.

	Study patients (n = 181)
Age (years)	56 (12)
BMI (Kg/m^2)	27.2 (5.6)
Thorax perimeter (cm)	94 (10)
Menopausal status[a]	
Postmenopausal	116 (64.1)
Premenopausal	64 (35.4)
Type of surgery[a]	
Mastectomy	50 (27.6)
Breast conservative	130 (71.8)
Tumour side	
Right	96 (53.0)
Left	85 (47.0)
Tumour	
Unique	155 (85.6)
Multiple	26 (14.4)
Tumour size (maximum diameter, mm)	17 (9)
Grading	
I	52 (28.7)
II	88 (48.6)
III	30 (16.6)
Not assessed	11 (6.1)
Histologic type[a]	
Invasive ductal NOS type	136 (75.1)
Intraductal	20 (11.1)
Invasive lobular	15 (8.3)
Invasive ductal special	6 (3.3)
Mixed (ductal and lobular)	3 (1.7)
Progesterone receptor status[b] (+)	131 (72.4)
Oestrogen receptor status[b] (+)	148 (81.8)
HER2 status[c] (+)	20 (11.1)
Ki67 (+)	21.2 (17.4)

[a] Data was missing in one case.
[b] Data was missing in seven cases.
[c] Data was missing in 21 cases; Positive (+) defined as more than 10% cells for progesterone and oestrogen receptor status, and more than 14% for Ki67.

in all cases (transcutaneous, intraoperative and ex-vivo attempts) and concordance rates were always above 97%. In the node level analyses of the intraoperative and ex-vivo attempts, detection rates seemed to be slightly higher with SM than with GP, and the concordance rate were about 85% or higher. In all cases, the lower bound of the one-sided 95% CI for the detection rate difference between methods allowed to rule out an advantage of 5% or higher of the GP device over SM. It is interesting to note that the 3 patients having negative results with both methods in the ex-vivo measurement (primary outcome), belong to the same centre, suggesting a possible centre effect.

After excision of the SLNs, there was residual detection in 23 (or 12.7%) patients with GP, and in 46 (or 25.4%) with SM, for a difference of 12.7% (one-sided 95% CI: $> 5.8\%$).

SLN detection of histopathologically positive nodes (subgroup analysis)

A total number of 76 out of 321 excised nodes (23.7%) showed a positive result for metastases. The results of the ex-vivo detection attempts are shown in Table 3. Note that SM had a slightly higher detection rate than GP in this subgroup, both at the node and the patient level.

Extraaxillary detection

Extraaxillary transcutaneous detection occurred in 5 cases (2.7% of the total), and all of them were cases with peritumoral injection of radiotracer. Three of them were detected by GP only, corresponding to internal mammary chain drainage cases. The remaining two were detected by both devices. One of these two was an internal mammary chain drainage case and the other one corresponded to an intramammary lymph node.

Technical complications

Complications in the procedure were reported in 11 patients (6.1%) (6 cases with SM, 2 with GP, and 3 with both devices), and were related to the failure of the initial transcutaneous detection attempt, or to a discrepancy of results

Table 2
Detection of sentinel lymph nodes.

Detection attempt	Transcutaneous	Intraoperative		Ex-vivo	
n	181 patients	181 patients	319 nodes	181 patients	321 nodes
Frequencies (n)					
GP+ SM+	172	175	272	177	260
GP+ SM−	4	2	13	1	17
GP− SM+	0	1	23	0	32
GP− SM−	5	3	11	3	12
Concordance rates	97.8%	98.3%	88.7%	99.4%	84.7%
Detection rates					
SM+	95.0%	97.2%	92.5%	97.8%	91.0%
GP+	97.2%	97.8%	89.3%	98.3%	86.3%
Rate difference	−2.2%	−0.6%	4.7%	−0.5%	4.7%
One-sided 95% CI	$> -4.0\%$	$> -2.1\%$	>1.1%	$> -1.5\%$	>1.1%

GP+: positive result with the gammaprobe; GP−: negative result with the gammaprobe.
SM+: positive result with Sentimag®; SM−: negative result with Sentimag®.
CI: confidence interval.

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Table 3
Ex-vivo detection of histologically positive sentinel lymph nodes.

Detection attempts	Ex-vivo	
n	76 nodes	60 patients
Frequencies (n)		
GP+ SM+	65	52
GP+ SM−	2	1
GP− SM+	4	3
GP− SM−	5	4
Detection rates		
SM+	90.8%	91.7%
GP+	88.2%	88.3%
Rate difference	2.6%	3.3%
One-sided 95% CI	> −2.6%	> −2.1%

GP+: positive result with the gammaprobe.
GP−: negative result with the gammaprobe.
SM+: positive result with Sentimag®.
SM−: negative result with Sentimag®.
CI: confidence interval.

between both detection devices. No differences were found in the patients' characteristics when those with or without technical complications were compared.

Discussion

Our study has shown the non-inferiority of SM as compared to the GP, since the difference (SM minus GP) in the ex-vivo detection rates at the patient level was −0.5% (one-sided 95% CI: > −1.5%), ruling out the predefined non-inferiority level of −5%. Moreover, the ex-vivo detection rates at the node level were slightly higher with SM, and similar results were observed for intraoperative detection rates (Table 2). Last, when the analysis was restricted to histologically positive nodes, the ex-vivo detection rates were also slightly higher with SM (Table 3). In all cases, the non-inferiority of SM was shown.

While our study was taking place, three studies were published evaluating the SentiMag®/Sienna+® system in hospitals from UK, Netherlands, Germany, Switzerland, Poland and Spain[16–18] (Table 4). In a trial conducted on 160 women with breast cancer scheduled for SLNB who were clinically and radiologically node negative, recruited from seven centres in the United Kingdom and The Netherlands, SLN identification rates were 94.4% and 95.0% with SM and GP respectively, and non-inferiority of SM with respect to the standard GP technique was concluded.[16] However, the results from this trial did not distinguish identification rates from transcutaneous, intraoperative and ex-vivo measurements, and their discordance rate was 6.9%, which is somewhat higher to our figures (1/181 or 0.5%, see Table 2, ex-vivo). Another multicentric and multinational study including 150 patients and 291 SLN,[17] reported detection rates of 97.3% and 98.0% with GP and SM respectively, and also concluded non-inferiority of SM with respect to GP. Their concordance rate was 99.3%, which is very similar to ours (180/181 or 99.5%, see Table 2, ex-vivo). The third study, recently published, is a Spanish single-centre experience including 120 patients (Rubio et al., 2014). They report detection rates of 95.7% and 98.3% with SM and GP respectively and a concordance rate of 98.2%, concluding that detection of SLNs with SM allows the identification of axillary nodes at a frequency not inferior to the radiotracer.

Sienna+® is injected subcutaneously into the interstitial tissue. When injected intravenously, uncommon complications for MRI tracers similar to Sienna+® have been previously described, such as pain, vasodilation, parestesia, or skin reactions in the injection site, and anaphylaxis. None of those appeared in our study, and the few reported complications were related to the failure of transcutaneous detection with both methods and transient skin pigmentation.

The Median Sienna+® migration time before surgery was 24 min, 4 min above the minimum awaiting time fixed by protocol. Different causes affecting tracer migration, such as advanced age, axillary involvement, increased BMI, previous surgery, tumour location and palpability or surgeon experience, have been proposed by several authors.[19–23] We were interested in assessing if BMI was affecting time to surgery after Sienna+® injection as a reflection of Sienna+® migration rate, but we found no correlation between these two variables. In some cases the SLNB was longer than we expected, with a maximum of 65 min (much longer than the median duration of 20 min). These cases correspond to patients with extraaxillary drainage, higher number of SLN or complex localization of the SLN, causing an extension of the SLNB procedure time.

Five out of 181 of the patients (2.7%) showed signal on extraaxillary regions, which is below the lower range of previously published data (5–24%).[24–28] Extraaxillary detection only occurred when the radiotracer injection was peritumoral, suggesting that extraaxillary tracer

Table 4
Published series comparing SPIO and isotopic methods to detect sentinel nodes in breast cancer.

Author	n	Mean number of SN with GP	Mean number of SN with SM	Identification rate with GP	Identification rate with SM	Concordance rate
Douek, M[16]	160	1.9	2.0	95.0	94.4	93.1
Thill, M[17]	150	1.8	1.9	97.3	98.0	99.3
Rubio, TI[18]	120	1.9	2.2	95.7	98.3	98.2
Current article	181	1.5	1.6	98.3	97.8	99.4

GP: gammaprobe; SM: Sentima®.

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migration is more likely to happen in this case, which is in agreement with previous findings.[28,29] In any case, besides the fact that the indication of extraaxillary nodes biopsy is controversial,[20,24] this may be unnecessary when the tracer injection was subareolar only and, anyway, a preoperative lymphoscintigraphy could rule out this possibility.

The control intervention was performed according to the standard protocol of each center. Although the lack of perfect standardization of the control intervention may be seen as a limitation, the interest of "pragmatic" trials (such as those comparing a new intervention with current practice) has beeen recognised since long, since it improves the external validity of the study results.[30] In addition, all the investigators participating in our study are members of the Senological Studies Group from the Spanish Society of Senology, and therefore follow its guideline.[4] In those cases where radiologically or echocardiographically guided incisions were not possible, intra or periareolar procedures were allowed. In any case, neither the injection technique nor the time it was done, interfered in the results, as it has been the case in other published work.[31]

Some authors have pointed out disadvantages related to the diameter of the probe, and the need of repeating the calibration of the system during the procedure, resulting in larger surgical incisions and longer times, respectively.[32] In this context, a thinner new probe has been designed by the manufacturer, and the calibration process does not imply a significant difference with the isotopic technique.[33] In fact, Sienna+® often dyes the sentinel nodes in a brownish colour allowing the surgeon an easier identification, as occurs with vital dyes.

A technical limitation that should be taken into account with this procedure is a possible interference of the surgical instrumentation with the ferromagnetic signalling. To prevent it, the use of plastic surgical material is encouraged while the measurement with SentiMag® is performed.

The fact that the SentiMag®/Sienna+® system is non-inferior to the current standard for SLNB procedure implies that it should be considered as a solid alternative. This is of special interest to hospitals without in-house nuclear medicine department, whose clinicians and patients can benefit from the logistic advantages while keeping a standard-like performance.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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