Original Article

Optimising magnetic sentinel lymph node biopsy in an *in vivo* porcine model

Muneer Ahmed, MA, MRCS[a,1], Bauke Anninga, MSc[a,1], Joost J. Pouw, MSc[c],
Suzan Vreemann, MSc[c], Mirjam Peek, BSc[a,c], Mieke Van Hemelrijck, PhD[b], Sarah Pinder[a],
Bennie ten Haken, PhD[c], Quentin Pankhurst, PhD[d], Michael Douek, MD FRCS[a,*]

[a]*Research Oncology, Division of Cancer Studies, King's College London, Guy's Hospital, London, UK*
[b]*King's College London, School of Medicine, Cancer Epidemiology Group, Guy's Hospital, London, UK*
[c]*Institute for Biomedical Technology and Technical Medicine, Universiteit Twente, Enschede, The Netherlands*
[d]*Institute of Biomedical Engineering, University College London, London, UK*

Abstract

The magnetic technique for sentinel lymph node biopsy (SLNB) has been evaluated in several clinical trials. An *in vivo* porcine model was developed to optimise the magnetic technique by evaluating the effect of differing volume, concentration and time of injection of magnetic tracer. A total of 60 sentinel node procedures were undertaken. There was a significant correlation between magnetometer counts and iron content of excised sentinel lymph nodes (SLNs) ($r = 0.82$; $P < 0.001$). Total number of SLNs increased with increasing volumes of magnetic tracer ($P < 0.001$). Transcutaneous magnetometer counts increased with increasing time from injection of magnetic tracer ($P < 0.0001$), plateauing within 60 min. Increasing concentration resulted in higher iron content of SLNs ($P = 0.006$). Increasing magnetic tracer volume and injecting prior to surgery improve transcutaneous 'hotspot' identification but very high volumes, increase the number of nodes excised.

From the Clinical Editor: Sentinel lymph node biopsy (SLNB) is the standard of care for axillary staging of breast cancer patients. Although the current gold standard technique is the combined injection of technetium-labelled nanocolloid and blue dye into the breast, the magnetic technique, using superparamagnetic carboxydextran-coated iron oxide (SPIO), has also been demonstrated as a feasible alternative. In this article, the authors set up to study factors in order to optimize the magnetic tracers.
© 2015 Elsevier Inc. All rights reserved.

Key words: Superparamagnetic iron oxide; SPIO; Sentinel lymph node biopsy; SLNB: magnetic technique; Sentinel lymph node biopsy; Magnetic tracer

Sentinel lymph node biopsy (SLNB) is the standard of care for axillary staging of breast cancer patients with a clinically and radiologically normal axilla.[1–6] The gold-standard technique is the 'combined technique' with interstitial injection of technetium-labelled nanocolloid and blue dye into the breast. SLNB offers the benefits of minimally invasive surgery (less morbidity) and a low false negative rate.[4,7] However, the reliance upon radioisotopes has drawbacks in terms of radiation exposure, the

short (6 h) half-life of technetium [99]m (^{99m}Tc), handling and disposal of radioisotopes, the training of medical staff and legislative requirements. Perhaps for these reasons, and despite the incidence of cancer rising over the last decade, the performance of the SLNB procedure has reached a plateau with around 60% of an estimated 500,000 patients in the Western world having access to the procedure.[8] This figure drops to 5% in China and is minimal in the rest of the world.[9] This has led to interest in the development of novel techniques not reliant upon radioisotopes,[10] which are currently restricted by uptake of dye by higher echelon nodes[11–13] and high false negative rates.[14] The magnetic technique, developed by Douek et al[15] is one of the most promising alternatives. A sterile, aqueous suspension of superparamagnetic carboxydextran-coated iron oxide (SPIO) is injected interstitially into the breast and travels to the axillary lymph nodes. It is distributed within the sinuses, subcapsular space and parenchyma of the nodes.[16] High power microscopic examination has revealed iron predominantly sequestered within

macrophages. Once taken up by macrophages in the mononu-clear phagocyte system of the lymphatics, the magnetic tracer is believed to be broken down and distributed across iron stores in the body.[17] The magnetic tracer can be detected intra-operatively using a handheld magnetometer.[15]

Magnetic SLNB for breast cancer has been demonstrated as a feasible technique in 3 published clinical trials.[15,18,19] The largest and first clinical trial, the SentiMAG Multicentre Trial,[15] recruited 161 patients (170 SLNB procedures) and found that the magnetic technique was non-inferior to the standard dual technique. This was confirmed by a later study, the Central-European SentiMag Study,[19] of 150 patients. However, the false negative rate has been unacceptably high in studies of magnetic SLNB. Shiozawa et al[18] in their study of 30 patients reported a false negative rate of 17% using the magnetic technique and even the SentiMAG Multicentre Trial,[15] which was found to be non-inferior to the dual technique for SLN identification demonstrated a false negative rate of 8% and 4% for the magnetic and dual techniques respectively. The Central-European SentiMag Study[19] identified a lower false negative rate for the magnetic technique of 3% *versus* 9% for the standard radioisotope technique, inconsistent with the previous studies. The higher false negative rate in the standard technique in this trial may be explained by the omission of blue dye, which is known to improve the SLN identification rate and lower the false negative rate of the dual technique.[7,20] All studies injected the magnetic tracer periareolarly after induction of general anaesthesia, with Shiozawa et al[18] injecting 1.6 mL Resovist (Bayer Health Care Osaka, Japan; 27.9 mg iron/mL) and the other 2 trials using 2 mL Sienna + (27 mg iron/mL) diluted in 3 mL normal saline.[15,19]

In order to make the magnetic technique a feasible alternative to the current dual technique, it is essential that we understand the behaviour of magnetic tracers from the injected site to distribution within the lymphatic basin. Outstanding issues include the optimal volume of magnetic tracer to administer and timing of injection prior to surgery. By identifying these factors it would be possible to optimise the SLN identification rate of the magnetic technique, reduce the false negative rate and prevent excision of higher echelon SLNs. The identification of viable lower volumes may allow a reduction in complications including skin staining and potential artefact on magnetic resonance imaging (MRI). Our group previously developed a porcine model, which closely replicates, human size as well as vasculature and lymphatic drainage. This model was used to successfully demonstrate the feasibility of magnetic SLNB using 16 mini-pigs and 32 SLNB procedures.[21] Anninga and Ahmed et al[21] identified a significant correlation between magnetometer counts and magnetic tracer content of *ex vivo* SLNs and the significant association between the grading of *ex vivo* SLNs for their tracer content and magnetometer counts.[21] This model had therefore been validated for the purpose of magnetic SLNB using Sienna + (Endomagnetics, UK). Sienna + is a blackish-brown, sterile aqueous suspension of superparamagnetic carboxydex-tran-coated iron oxide particles. The carboxydextran coating prevents agglomeration while maintaining biocompatibility. The particle diameter, including the organic coating, is 60 nm (Z averaged diameter; <0.25 polydispersity) ideally suited for SLNB. This magnetic tracer is the only clinically licensed (CE marked) SPIO for the purpose of SLNB within Europe. On the basis of previous *in vivo* work with Sienna + and its exclusive license for clinical use, we selected it to identify the optimal volume of magnetic tracer required to ensure maximal SLN tracer uptake and therefore optimise clinical SLN identification and potentially reduce false negative rates using the magnetic technique.

Methods

This study was conducted at the IRCAD institute, Strasbourg (France), King's College London (United Kingdom) and the Universiteit Twente, Enschede (The Netherlands). Ethical permission was granted for animal experimentation, by the IRCAD Ethics Review Board, Strasbourg, France (Reference number: 38.2013.01.056). Mini-pigs used for the IRCAD laparoscopic general surgical skills course were surgically prepped and anaesthetised for the purpose of the course. Prior to commencement of the laparoscopic skills course a magnetic tracer (Sienna+, Endomagnetics UK; 27 mg iron/mL) was injected subcutaneously into the areola of the left and right 3rd inguinal mammary glands in 30 mini-pigs.

Volume-escalation study of SLNB in porcine model

Magnetic tracer was injected in escalating volumes between 0.06 mL and 2 mL neat in 24 mini-pigs. A handheld magnetometer (SentiMag, Endomagnetics UK) was then used to localise any *in vivo* signal from draining inguinal lymph nodes up to 60 min after injection using 15-min intervals and these repeated again 4 h later on completion of the laparoscopic skills course. Bilateral groin SLNB was undertaken at the site of magnetic 'hot spots' (Figure 1). All lymph nodes with a magnetometer count higher than 10% of the hottest node were considered to be SLNs and were excised with *ex-vivo* counts also recorded. Once the SLNB was completed, a groin node clearance was performed to remove all lymph nodes from each groin basin. The harvested SLNs were fixed in formalin and sent to Universiteit Twente, Enschede (The Netherlands), where the quantification of magnetic tracer in each excised node was performed using vibrating sample magnetometry (VSM) on a Physical Properties Measuring system (PPMS, Quantum Design Inc., San Diego, CA, USA). The measurements were performed using a magnetic field of $\pm$ 4.0 T, which is required to bring the magnetic iron oxide (maghemite, γ-Fe_2O_3) nanoparticles to saturation. The amount of magnetic tracer in the lymph nodes was determined by comparing the obtained amplitude of the magnetisation to known calibration samples, and was reported as an 'iron content', i.e. mass of iron (Fe) in the node, present in the form γ-Fe_2O_3.[22] The nodes from the groin clearances were also placed in formalin and sent to King's College London (United Kingdom) where they underwent fixation, thin slicing, process-ing and paraffin wax embedding and haematoxylin and eosin (H&E) staining for the presence of SPIO deposition. The grading of iron deposition within each node was recorded, using a previously validated 5-point grading scale (*0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked*) for the extent of iron deposition using H&E[21] by an experienced pathologist and a second observer (SP; BA or MA) (Figure 1, *A-D*).
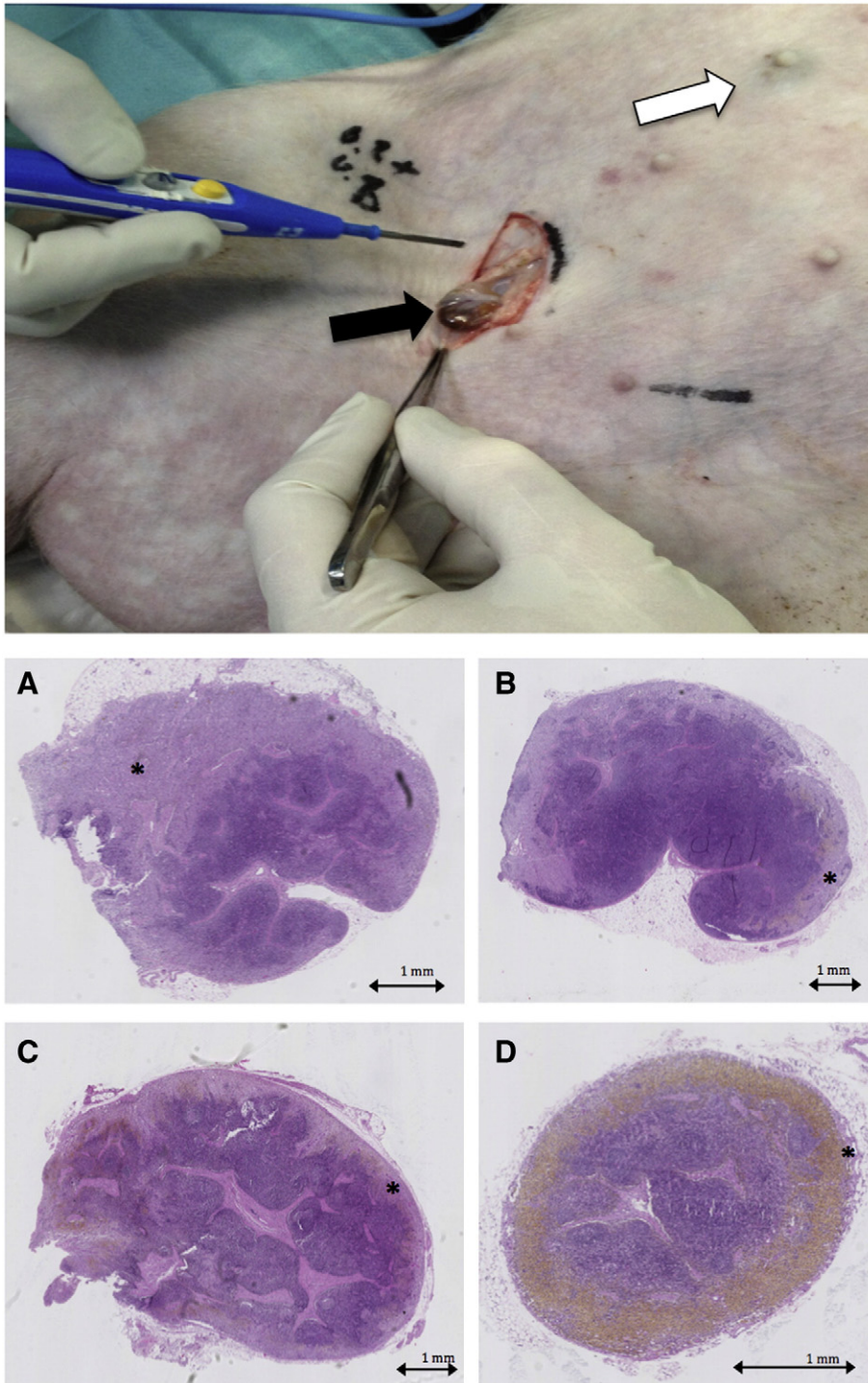
Figure 1. *In vivo* porcine SLNB (injection site indicated with the white arrow) with a black stained node *in vivo* (indicated with the black arrow) and examples of sentinel lymph nodes that were scored 1 to 4 after H&E staining **(A-D)**, clearly showing the brown discoloration in the cortex of the nodes indicated with an asterisk (*).

Concentration-comparison study of SLNB in porcine model

A volume of 0.5 mL magnetic tracer (Sienna+) was injected neat at a concentration of 27 mg iron/mL in 3 mini-pigs and the same volume of magnetic tracer (Sienna+) at a reduced concentration of 11.2 mg iron/mL (after dilution with sterile water using a pipette under aseptic techniques) injected in 3 further mini-pigs. The identical protocol for SLNB and subsequent management of specimens was performed as in the volume-escalation study (*see above*). The concentration of 11.2 mg/mL was selected to replicate the concentration of the SPIO Endorem (Guerbet, France), which was the lowest
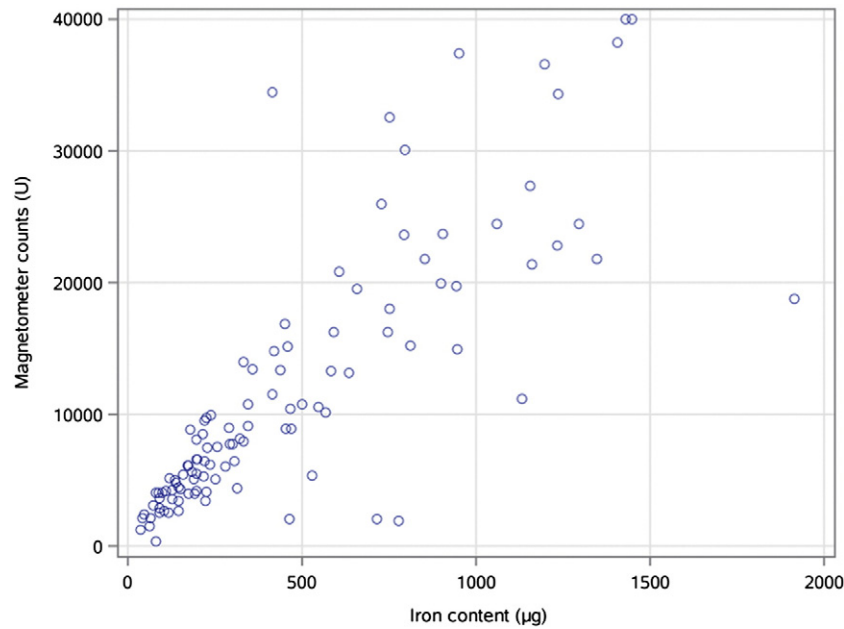
Figure 2. Scatter diagram showing the correlation between handheld magnetometer counts and iron content of excised SLNs ($r = 0.82$; $P < 0.001$).

concentration of SPIO to be used successfully in clinical studies of magnetic SLNB to date (withdrawn from production 2011).[23] It was therefore assumed that this lower concentration of Sienna + would also be functional for SLNB in the porcine model and form feasible comparison to the neat concentration.

Statistical analysis

Based upon data accrued from our previous feasibility study[21] a power calculation was performed to determine sample size. We conducted a two-sided test (alpha = 0.05) expecting a difference of 50 μg (SD: 30) in iron content readings between different volumes and concentrations of tracer. When performing a total of 6 procedures (3 mini-pigs) for each volume (0.06-2.0 mL) and concentration (27 mg/mL and 11.2 mg/mL) of magnetic tracer, these 60 procedures provided us with a power of 82% to detect this difference. The correlation between continuous variables was calculated using the Pearson's correlation coefficient (r) and associations between categorical and continuous variables using analyses of variance (ANOVA) and associations between categorical variables using Fisher's Exact Test. All statistical analyses were performed with Statistical Analysis Systems (SAS) release 9.3 (SAS Institute, Cary, NC).

Results

Volume-escalation study

A total of 48 SLNB procedures followed by 48 groin node clearances were performed on 24 mini-pigs. All 48 were successful and at least 1 'hot' node identified in each procedure. *In vivo* magnetic 'hot spots' from the draining inguinal lymph nodes were identified transcutaneously prior to surgical incision using the handheld magnetometer, with all volumes of administered magnetic tracer. A total of 423 nodes were harvested (mean 8.8 nodes (SD 2.7) per groin, range 4-14), of which 109 were SLNs (mean 2.2 nodes (SD 1.4) per groin, range 1-7). A statistically significant linear relationship was demonstrated between the handheld magnetometer counts and the iron content of excised SLNs recorded on VSM ($r = 0.82$; $P < 0.001$) (Figure 2).

The impact of the volume of magnetic tracer injected

There was a statistically significant correlation between the percentage iron-uptake (relative to injected dose) of the excised SLNs and the volume of magnetic tracer injected ($P < 0.001$) (Figure 3, *C*). This is demonstrated by a reduction in the percentage iron-uptake (amount of iron taken up by each node, relative to the amount injected) from a mean of 25% for 0.06 mL volume of magnetic tracer to less than 2% for 2 mL of magnetic tracer. Increasing the volume of magnetic tracer from 0.06 mL to 2 mL resulted in a statistically significant increase in the mean number of SLNs excised from 1 to 4 ($P < 0.001$) (Figure 3, *D*). There was a trend (not statistically significant; $P = 0.07$) observed between the volume of the magnetic tracer injected and the iron content of the excised SLNs assessed with VSM ($P = 0.07$) (Figure 3, *B*) and if only the 'hottest node' excised for each procedure was considered, there was a significant increase in nodal iron content with an increase in the volume of the magnetic tracer ($P < 0.004$). Increasing the volume of magnetic tracer injected did not result in a statistically significant difference in the magnetometer counts of the excised SLNs ($P = 0.37$) (Figure 3, *A*), but once again if only the 'hottest node' excised for each procedure was considered, this was statistically significant ($P < 0.005$).
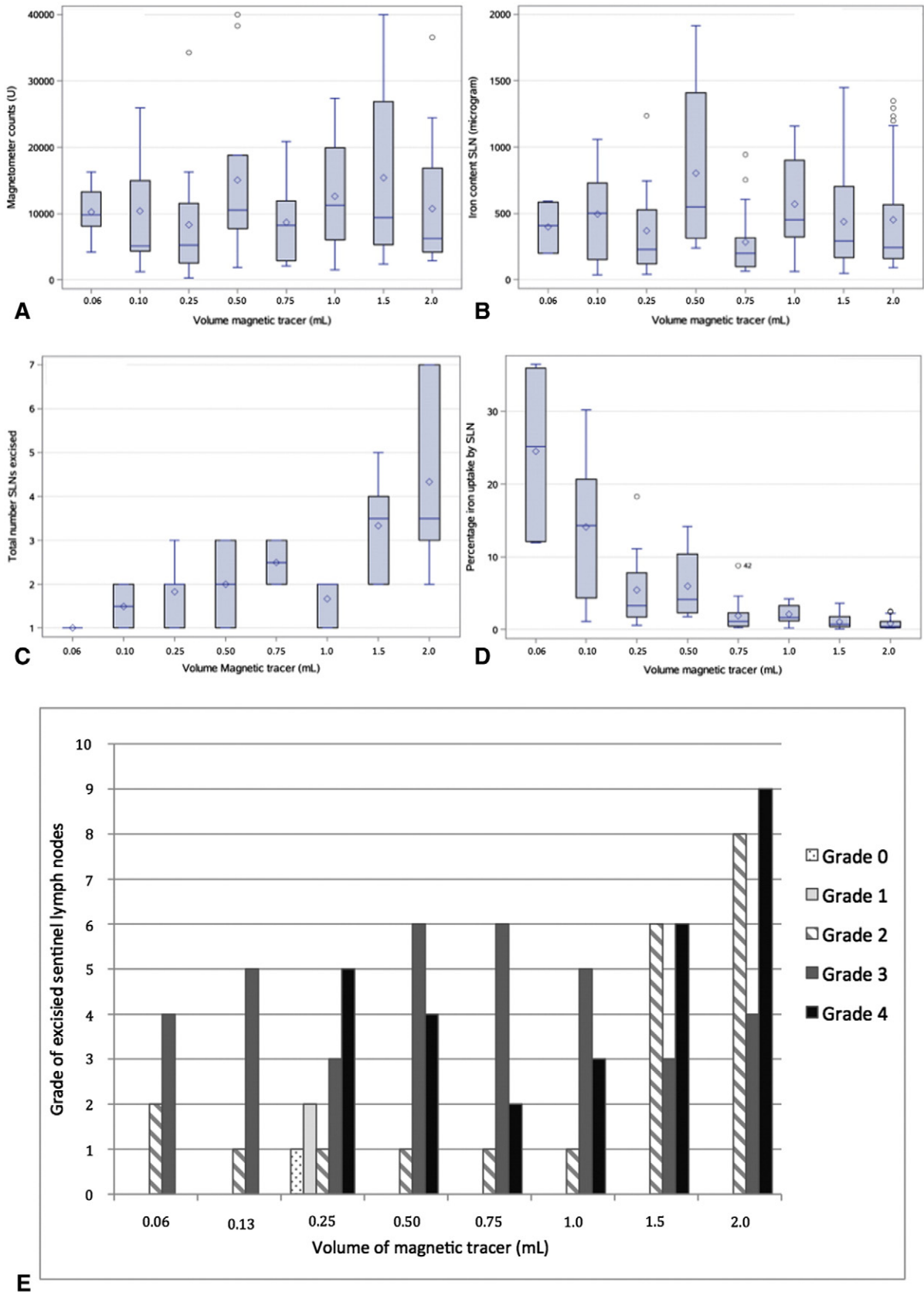
Figure 3. Boxplots demonstrating the relationship of increasing volume of magnetic tracer injected: **(A)** *versus* magnetometer counts ($P = 0.37$), **(B)** *versus* iron content ($P = 0.07$), **(C)** *versus* total number of SLNs excised ($P < 0.01$), **(D)** *versus* percentage of iron uptake by excised SLNs ($P < 0.001$), and **(E)** *versus* the grading of iron content for all excised sentinel lymph nodes ($P = 0.03$).
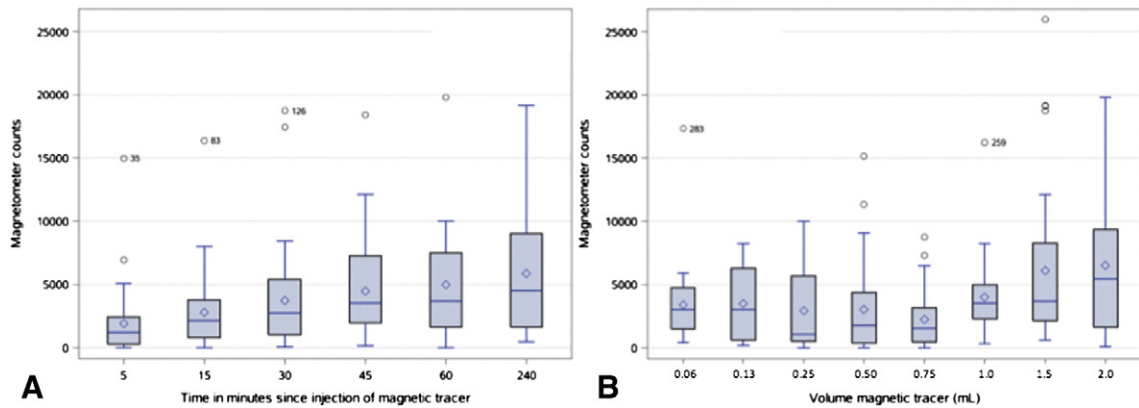
Figure 4. **(A)** Boxplot demonstrating the relationship between transcutaneous magnetometer counts ('hot spot') and increasing time elapse since injection for all volumes of magnetic tracer combined ($P < 0.0001$). **(B)** Boxplot demonstrating the relationship between increasing volume of magnetic tracer injected and transcutaneous magnetometer counts ('hot spot') 240 min post-injection ($P = 0.009$).

The relationship between the volume of magnetic tracer and time elapsed since injection

There were transcutaneous magnetometer counts recorded over the sentinel node for all volumes of magnetic tracer as early as 5 min after injection. A significant correlation was observed between transcutaneous magnetometer counts over the sentinel node and the time elapsed since injection of magnetic tracer. The longer the time since injection, the stronger was the transcutaneous magnetic hot spot ($P < 0.0001$) when all volumes of magnetic tracer injected were combined and considered at each time-point (Figure 4, *A*). The mean magnetometer count at 240 min post-injection was double that of the count at 5 min. There was no statistically significant difference between the transcutaneous magnetometer counts and different volumes of magnetic tracer injected between 5 min ($P = 0.72$), 15 min ($P = 0.84$), 30 min ($P = 0.15$), 45 min ($P = 0.36$) and 60 min ($P = 0.32$) post-injection. However, at 240 min from injection, there was a statistically significant correlation between increasing volume of magnetic tracer and higher magnetometer counts ($P < 0.009$) (Figure 4, *B*).

Iron content of excised SLNs measured by VSM (µg)

The iron content of *ex vivo* SLNs was in the range of 41 to 1431 µg (mean 463.9 µg (SD 401)). The peak distribution of iron content per excised SLN was in the range of 101 and 200 µg, representing 23% of the iron content of all nodes (Figure 5). A total of 71% of excised SLNs possessed iron contents below 600 µg.

Histopathological grading

All nodes underwent grading according to the distribution of SPIO within them using H&E staining. There was a statistically significant increase in the grade of *ex-vivo* SLNs as the volume of magnetic tracer injected was increased ($P = 0.03$) (Figure 3, *E*). No nodes excised after injecting volumes of magnetic tracer below 0.25 mL were recorded as grade 4.

Concentration-comparison study

A total of 12 SLNB procedures were performed on 6 mini-pigs. All 12 were successful and at least 1 'hot' node identified in each procedure. *In vivo* magnetic 'hot spots' from the draining inguinal lymph nodes were identified transcutaneously prior to surgical incision using the handheld magnetometer, with both concentrations (27 mg/mL and 11.2 mg/mL) of administered magnetic tracer. A total of 25 SLNs nodes were harvested (mean 2.1 nodes (SD 0.9) per groin, range 1-4).

The impact of the concentration of magnetic tracer injected

There were transcutaneous magnetometer counts recorded over the sentinel node for both concentrations of magnetic tracer as early as 5 min after injection. There was a statistically significant increase in the iron content of the excised SLNs in the 27 mg/mL *versus* the lower 11.2 mg/mL concentration ($P = 0.006$) (Figure 6, *B*). A trend towards greater magnetometer counts in excised SLNs with the higher concentration of magnetic tracer (27 mg/mL) was observed, although this did not reach statistical significance ($P = 0.09$) (Figure 6, *A*) when all excised SLNs or only the 'hottest' were considered ($P = 0.21$). There was no statistically significant difference in the number of SLNs excised between the 2 concentrations of magnetic tracer ($P = 0.76$) (Figure 6, *C*) or in the grade of *ex-vivo* SLNs ($P = 0.49$) (Figure 6, *D*).

Discussion

The magnetic technique for SLNB successfully identified at least 1 SLN in all 48 porcine groins in which it was performed in the volume-escalation study. This confirmed a significant correlation between the handheld magnetometer counts and the iron content of *ex vivo* SLNs as recorded on VSM measurements. The Pearson's Correlation (*r*) of 0.82 (Figure 2) was in keeping with our previous study in which $r = 0.86$,[21] reaffirming the ability of handheld magnetometers to quantify iron content
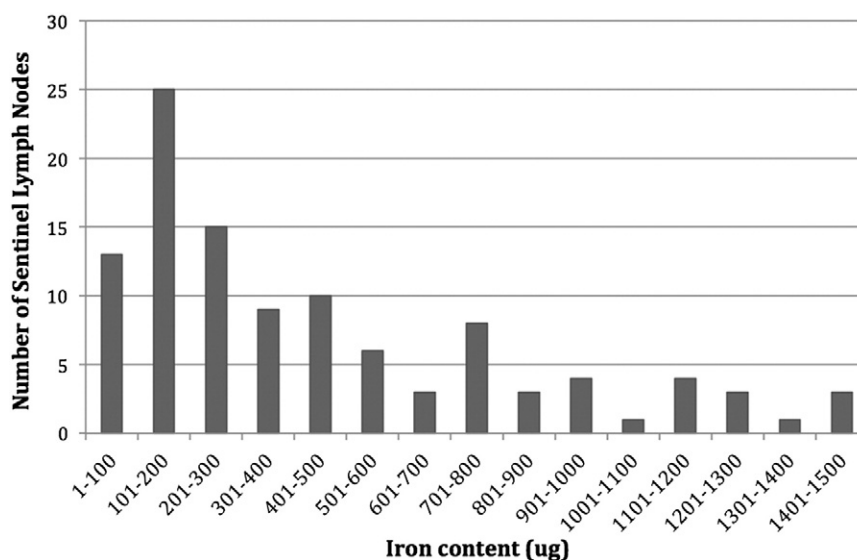
Figure 5. The distribution of iron content amongst excised sentinel lymph nodes.

in vivo and potentially provide a portable alternative to currently established iron quantification techniques.

We selected VSM for SPIO quantification over other techniques in this study due to its ability to quantify SPIO accurately (detection limit for iron in lymph nodes is 1 μg with an accuracy of 0.5 μg), in a non-destructive nature, allowing histopathological assessment of specimens, contrary to spectroscopic techniques (Inductively coupled plasma optical emission spectroscopy (ICP-OES) or ICP Mass Spectroscopy (ICP-MS)), which require digestion of the sample. MRI techniques measuring the strength of the relaxivity (R2) can be used to non-destructively assess iron content, but these procedures are sensitive to assumptions about background signals from tissue, and distribution/clustering of nanoparticles. Background measurements are therefore required to obtain accurate results with these procedures and since background measurements have to be obtained *in vivo* in our case, we chose to use a different technique. Histopathological analysis has revealed that the nanoparticles are both taken up by macrophages and reside in the sinuses of the lymph nodes.[16] The packing/clustering of the particles could change some of the magnetic properties of the tracers, however only the obtained amplitude of the magnetisation is used to determine the amount of iron present in the lymph nodes. Packing and/or clustering does not change the amount of tracer present in the lymph node, and therefore the saturation magnetisation of the tracer also does not change. Since a field of 4 T is applied, the magnetic material (maghemite, γ-Fe_2O_3) is brought to saturation. Clustering and/or packing of the particles is not of influence on the saturation magnetisation of the tracer, and therefore the tissue environment does not alter the outcome.

We did not identify any statistically significant correlation between iron content of *ex vivo* SLNs and the volume of magnetic tracer injected (Figure 3, *A* and *B*) when all excised SLNs were considered. This may be explained by the concept of the SLNs possessing a 'saturation limit' of iron, which once exceeded allows iron to be passed on to the next echelon SLN and so on and so forth. Therefore, increasing the volume resulted in an increase in the

number of SLNs excised (0.06 mL resulted in a median of 1 SLN excised, compared to 4 (range 2-7) for 2 mL ($P < 0.001$)), but not a significant increase in iron content of nodes. The distribution of the iron content of the excised SLNs demonstrated a peak between 101 and 200 μg (Figure 5). This would suggest that the majority of porcine SLNs have their saturation limit within this range. Therefore, when a subgroup analysis of only the 'hottest node' excised for each SLNB procedure was performed, a statistically significant correlation between iron content and the increasing volumes of magnetic tracer was identified, because the 'hottest nodes' likely represent those nodes within the distribution, which possess a higher saturation limit.

When histopathologically assessed during grading of the SLNs, it has been shown that the iron content within SLNs after interstitial magnetic tracer injection is predominately distributed within the cortex, subcapsular space and sinuses of the lymph nodes.[16,21] Those nodes with higher grading (3, 4) have a more uniform distribution of iron compared to lower grades. The lack of a statistically significant relationship between increasing volume and magnetometer counts is likely explained by the heterogenous distribution of iron within the nodes at lower grades, resulting in focal deposits of SPIO within nodes providing the highest magnetometer counts but not necessarily truly quantifying overall iron content.[21] Of course if subgroup analysis of the 'hottest nodes' only is performed it demonstrates statistical significance due to self-selection of nodes with higher iron saturation limits and therefore content and the known established ability of the handheld magnetometer to quantify iron content (although not as well as established techniques).

The grading of SLNs for SPIO distribution has already been demonstrated to correlate with iron content and handheld magnetometer counts.[21] This study showed that there was a statistically significant increase in grade of SLNs (as demonstrated by H&E staining for SPIO) as the volume of magnetic tracer was increased. This supports the non-statistical trend of increasing iron content of SLNs with an increasing volume of magnetic tracer on VSM measurement. It suggests that this
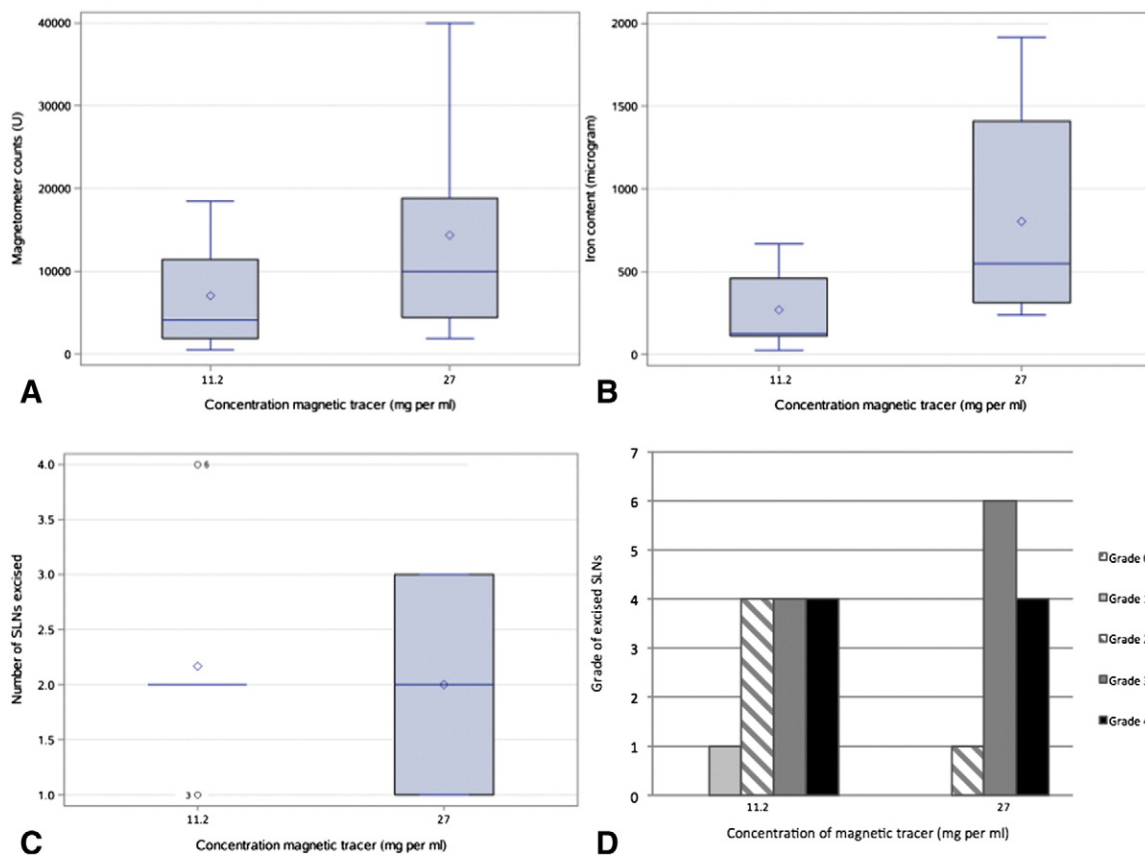
Figure 6. Boxplots demonstrating the relationships for increasing concentration of magnetic tracer injected with **(A)** magnetometer counts from all excised sentinel lymph nodes ($P = 0.09$), **(B)** iron content from all excised sentinel lymph nodes ($P = 0.06$), **(C)** the total number of excised sentinel lymph nodes ($P = 0.76$), and **(D)** grade of excised sentinel lymph nodes ($P = 0.49$).

grading system is a useful tool for the assessment of iron distribution but not as sensitive as VSM.

Our model suggests that the current practice of using 2 mL of magnetic tracer as in the SentiMAG Multicentre Trial is excessive,[15] with SLNB being possible with much lower volumes. It suggests that by varying the volume of magnetic tracer it is possible to determine the echelon level of SLNs excised and the number retrieved. Our results would suggest that by using a volume of 0.5 mL it is possible to excise a median of 2 SLNs (range 1-3), which would be ideal in most clinical scenarios. Should other clinical situations require greater numbers of SLNs to be excised – such as in the performance of SLNB after primary systemic therapy in breast cancer (where elevated false negative rates are known to be an issue)[24] – an increased volume could be administered to provide a median of 4 nodes. The use of a higher volume would ensure identification of the SLN, but at the greater risk of skin staining, artefact on MRI (should any post-operative imaging be required) and increased risk of surgical complications (haematoma, nerve injury and lymphoedema) due to the increased overall number of nodes (higher echelon) harvested and subsequent greater tissue dissection. This ability to control the level of echelon nodes excised may prove an advantage over other developing novel techniques for SLNB, such as indocyanine green (ICG) fluorescence in which studies have demonstrated the mean

number of excised SLNs ranging from 1.75 to 5.4 for the same volume of 1 mL ICG administered.[13,25,26]

This study identified that when the higher concentration (27 mg/mL) of magnetic tracer was compared to a reduced concentration (11.2 mg/mL) (which had previously been viably used within a clinical setting), there was a statistically significant increase in iron content of SLNs for the higher concentration of magnetic tracer. This suggests that concentrating the magnetic tracer does play a role in optimising delivery to reach the saturation point of the nodes. Unfortunately, we were unable to increase the concentration of the magnetic tracer beyond its neat concentration due to concerns of ultrafiltration being likely to result in damage to the shell of the particles and subsequent aggregation of particles, compromising results. It therefore remains uncertain how much further concentration of the magnetic tracer beyond 27 mg/mL would prove beneficial, before agglomeration of particles may become deleterious to lymphatic drainage. Our results would suggest that reducing the concentration below 11.2 mg/mL would prove futile, as it would almost certainly result in poorer iron uptake. Although concentrating the magnetic tracer influenced iron uptake, it did not significantly reflect upon other parameters of magnetometer counts, grade and number of SLNs excised. The lack of higher magnetometer counts demonstrates that clinical benefit from increasing the concentration is unlikely. It highlights the current

limitation of the handheld magnetometer to quantify iron content and that the distribution of iron within the nodes remained unchanged as concentration is increased.

All 3 published clinical studies[15,18,19] for the use of the magnetic technique injected the magnetic tracers after induction of general anaesthetic, periareolarly into the breast. Although the optimal timing of the magnetic tracer is not yet known, our results suggest that a pre-operative injection may be beneficial. The transcutaneous magnetometer counts were significantly greater for all volumes injected (when combined) as time was increased from injection (Figure 4, A). However, a significant increase in the transcutaneous magnetometer count between the different volumes was only observed after 240 min from injection of magnetic tracer (Figure 4, B). The greater magnetic 'hotspot' is beneficial in optimal positioning of the skin incision for technical and aesthetic outcomes in SLNB. The handheld magnetometer counts demonstrate a reducing logarithmic relationship ($r = -0.97$; $P < 0.001$) for increasing depth of injection.[17] Therefore, to assist in transcutaneous hotspot detection in the axilla, which can vary greatly upon the depth of location of the SLN, it is essential that maximal iron uptake is achieved to attain the greatest possible transcutaneous magnetometer counts. Our study demonstrates that a gap of 240 min from injection to transcutaneous magnetometer count readings will result in the highest transcutaneous magnetometer counts to be recorded for all volumes of magnetic tracer injected (Figure 4, A). However, by 60 min post-injection the mean transcutaneous magnetometer count was 5000 counts compared to just 6000 at 240 min when all volumes of tracer were compared (Figure 4, A). This suggests that a plateau in the transcutaneous magnetometer counts has been reached and that any prolonged delay between injection and surgery beyond 60 min may not be clinically relevant. However, it is important to mention that caution must be taken in attempting to optimise the transcutaneous 'hotspot' count as it can compromise other outcomes for magnetic SLNB. By increasing both the delay between injection and surgery and volume of magnetic tracer injected, there is a risk of increasing average node retrieval, by the unnecessary removal of higher echelon nodes.

There are practical issues to implementation of this delay between injection and performance of SLNB. Firstly, significantly increasing the time gap between surgery and injection would mean injecting pre-operatively, which, in the case of a periareolar injection, could cause discomfort. Increasing the volume of magnetic tracer injected (periareolar) could cause increased discolouration and discomfort. A practical alternative approach is to evaluate intra-tumoural injection of the magnetic tracer under ultrasound-guidance using local anaesthetic, for synchronous lesion localisation and sentinel node biopsy — as is currently being evaluated within the UKCRN MagSNOLL Multicentre Trial.[27]

The magnetic technique for SLNB requires further optimisation prior to evaluation within a randomised controlled trial. In a porcine model, magnetic SLNB is feasible even at very low volumes of injected magnetic tracer (0.06-2 mL). Injection of higher volumes of magnetic tracer, beyond a saturation point, results in an increase in node retrieval rate. An injected tracer volume of 0.5 mL, providing a median number of 2 SLNs (range

1-3), is ideal for most clinical situations. Pre-operative injection of magnetic tracer, improves identification of transcutaneous 'hot spot', facilitating sentinel node identification, whilst increasing the concentration is unlikely to yield clinical benefit. Further trials should evaluate pre-operative injection, higher concentration and lower volumes of injected magnetic tracer.

References

1. Gill G. Surgeons STGotRACo and Centre NCT. Sentinel-lymph-node-based management or routine axillary clearance? One-year outcomes of sentinel node biopsy versus axillary clearance (SNAC): a randomized controlled surgical trial. *Ann Surg Oncol* 2009;**16**:266-75.
2. 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.
3. Krag DN, Anderson SJ, Julian TB, Brown AM, Harlow SP, Ashikaga T, et al. Technical outcomes of sentinel lymph-node resection and conventional axillary-lymph-node dissection in patients with clinically node-negative breast cancer: results from the NSABP B-32 randomised phase III trial. *Lancet Oncol* 2007;**8**:881-8.
4. Mansel RE, Fallowfield L, Kissin M, Goyal A, Newcombe RG, Dixon JM, et al. Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC Trial. *J Natl Cancer Inst* 2006;**98**:599-609.
5. Veronesi U, Paganelli G, Viale G, Luini A, Zurrida S, Galimberti V, et al. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. *N Engl J Med* 2003;**349**:546-53.
6. Zavagno G, De Salvo GL, Scalco G, Bozza F, Barutta L, Del Bianco P, et al. A randomized clinical trial on sentinel lymph node biopsy versus axillary lymph node dissection in breast cancer: results of the Sentinella/GIVOM trial. *Ann Surg* 2008;**247**:207-13.
7. Goyal A, Newcombe RG, Chhabra A, Mansel RE, Group AT. Factors affecting failed localisation and false-negative rates of sentinel node biopsy in breast cancer—results of the ALMANAC validation phase. *Breast Cancer Res Treat* 2006;**99**:203-8.
8. Rescigno J, Zampell JC, Axelrod D. Patterns of axillary surgical care for breast cancer in the era of sentinel lymph node biopsy. *Ann Surg Oncol* 2009;**16**:687-96.
9. Leong SP, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, et al. Is breast cancer the same disease in Asian and Western countries? *World J Surg* 2010;**34**:2308-24.
10. Ahmed M, Purushotham AD, Douek M. Novel techniques for sentinel lymph node biopsy in breast cancer: a systematic review. *Lancet Oncol* 2014;**15**:e351-62.
11. Abe H, Mori T, Umeda T, Tanaka M, Kawai Y, Shimizu T, et al. Indocyanine green fluorescence imaging system for sentinel lymph node biopsies in early breast cancer patients. *Surg Today* 2011;**41**:197-202.
12. Hirche C, Mohr Z, Kneif S, Murawa D, Hunerbein M. High rate of solitary sentinel node metastases identification by fluorescence-guided lymphatic imaging in breast cancer. *J Surg Oncol* 2012;**105**:162-6.
13. Tagaya N, Yamazaki R, Nakagawa A, Abe A, Hamada K, Kubota K, et al. Intraoperative identification of sentinel lymph nodes by near-infrared fluorescence imaging in patients with breast cancer. *Am J Surg* 2008;**195**:850-3.
14. Cox K, Sever A, Jones S, Weeks J, Mills P, Devalia H, et al. Validation of a technique using microbubbles and contrast enhanced ultrasound (CEUS) to biopsy sentinel lymph nodes (SLN) in pre-operative breast cancer patients with a normal grey-scale axillary ultrasound. *Eur J Surg Oncol* 2013;**39**:760-5.
15. 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**:1237-45.

16. Johnson L, Pinder SE, Douek M. Deposition of superparamagnetic iron-oxide nanoparticles in axillary sentinel lymph nodes following subcutaneous injection. *Histopathology* 2013;**62**:481-6.

17. Ahmed M, de Rosales RT, Douek M. Preclinical studies of the role of iron oxide magnetic nanoparticles for nonpalpable lesion localization in breast cancer. *J Surg Res* 2013;**185**:27-35.

18. Shiozawa M, Lefor AT, Hozumi Y, Kurihara K, Sata N, Yasuda Y, et al. Sentinel lymph node biopsy in patients with breast cancer using superparamagnetic iron oxide and a magnetometer. *Breast Cancer* 2013;**20**:223-9.

19. 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**:175-9.

20. Straver ME, Meijnen P, van Tienhoven G, van de Velde CJ, Mansel RE, Bogaerts J, et al. Sentinel node identification rate and nodal involvement in the EORTC 10981-22023 AMAROS trial. *Ann Surg Oncol* 2010;**17**:1854-61.

21. Anninga B, Ahmed M, Van Hemelrijck M, Pouw J, Westbroek D, Pinder S, et al. Magnetic sentinel lymph node biopsy and localization properties of a magnetic tracer in an in vivo porcine model. *Breast Cancer Res Treat* 2013;**141**:33-42.

22. Visscher M, Pouw JJ, Baarlen J, Klaase J, Haken B. Quantitative analysis of superparamagnetic contrast agent in sentinel lymph nodes using ex vivo vibrating sample magnetometry. *IEEE Trans Biomed Eng* 2013;**60**:2594-602.

23. Joshi T, Pankhurst Q, Hattersley S, Brazdeikis A, Hall-Craggs M, De Vita E, et al. Magnetic nanoparticles for detecting sentinel lymph nodes. *Eur J Surg Oncol* 2007;**33**:1135.

24. Kuehn T, Bauerfeind I, Fehm T, Fleige B, Hausschild M, Helms G, et al. Sentinel-lymph-node biopsy in patients with breast cancer before and after neoadjuvant chemotherapy (SENTINA): a prospective, multicentre cohort study. *Lancet Oncol* 2013;**14**:609-18.

25. Murawa D, Hirche C, Dresel S, Hunerbein M. Sentinel lymph node biopsy in breast cancer guided by indocyanine green fluorescence. *Br J Surg* 2009;**96**:1289-94.

26. Sugie T, Sawada T, Tagaya N, Kinoshita T, Yamagami K, Suwa H, et al. Comparison of the indocyanine green fluorescence and blue dye methods in detection of sentinel lymph nodes in early-stage breast cancer. *Ann Surg Oncol* 2013;**20**:2213-8.

27. Ahmed M, Anninga B, Goyal S, Young P, Pankhurst QA, Douek M, on behalf of the MagSNOLL Trialists Group. Magnetic Sentinel Node and Occult Lesion Localization in Breast Cancer (Initial results of the MagSNOLL trial). *Br J Surg* 2015.