What is the future of magnetic nanoparticles in the axillary management of breast cancer?

M. Ahmed · M. Douek

Received: 21 August 2013 / Accepted: 2 December 2013 / Published online: 17 December 2013
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Abstract Magnetic nanoparticles (MNPs) possess unique properties, which make them highly attractive for medical applications. The use of MNPs in surgery has mainly been focused on their role in the identification of metastatic lymph node involvement. There have been developments within this field, including ongoing and newly conducted clinical trials. The current published evidence for the use of MNPs in assessing metastatic lymph node spread using sentinel lymph node biopsy and non-invasive imaging modalities is reviewed and future applications considered.

Keywords Magnetic nanoparticles · SPIO (superparamagnetic iron oxide) · Sentinel lymph node biopsy · Breast cancer

Introduction

Magnetic nanoparticles (MNPs) possess several unique properties, which make them highly attractive for medical applications. These properties include their small size (10^{-9} m), high surface to volume ratio and their ability to carry other compounds [1]. MNPs possess super-paramagnetic properties. This means that due to their small volume, in the absence of an externally applied magnetic field the overall magnetization of the MNPs will be zero, and the MNPs will exist in a super-paramagnetic state. However,

when an external magnetic field is applied, there is an overall alignment of individual magnetic moments of the MNPs. This property, marked by the lack of any retained magnetization after removal of the magnetic field, enables MNPs to maintain their colloidal stability and avoid agglomeration, which is important for biomedical applications [2]. The surgical application of MNPs has mainly focused on the identification of metastatic lymph node involvement using sentinel lymph node biopsy (SLNB) and in improving non-invasive imaging modalities. The current published evidence for the use of MNPs in this rapidly evolving field is reviewed and future applications considered.

The role of MNPs in sentinel lymph node biopsy

Breast cancer spreads predominantly via the lymphatic system. Cells migrate from the primary tumour and are carried away by the interstitial fluid via lymphatics [3]. Knowledge of the regional lymph node status is essential for providing information regarding staging, local control and prognostic outcome. Traditionally, the lymph node status was determined in breast cancer by axillary lymph node dissection (ALND), but this changed with the development of SLNB [4, 5]. Sentinel lymph nodes (SLNs) are defined as the first lymph nodes that receive lymphatic drainage from the primary tumour. These nodes are the most likely to possess metastatic cancer, and predict for likely involvement of higher echelon lymph nodes within the axilla. SLNB is now regarded as the standard of care in patients without clinical evidence of axillary lymph node metastases, in surgery for early-stage breast cancer [6–11].

The current SLNB technique involves injecting a technetium-sulphur colloid (^{99m}Tc) alongside blue dye (commonly Patent Blue V or isosulfan blue) interstitially around

M. Ahmed · M. Douek (✉)
Department of Research Oncology, King's College London,
Guy's Hospital Campus, Great Maze Pond, London SE1 9RT,
UK
e-mail: michael.douek@kcl.ac.uk

M. Ahmed
e-mail: muneer.ahmed@kcl.ac.uk

the tumour or peri-areolarly, during the ‘combined technique’ [13]. After allowing for the tracers to localize in the lymphatic system, the surgeon uses a handheld scintillation counter (gamma probe) to locate the nodes. The blue dye assists in the localization post-incision. Lymph nodes that are radioactive, blue, or both are judged to be ‘SLNs’. The SLNs are then removed and sent for histological examination. The detection rate of the technique has been demonstrated in a meta-analysis to be 96 % with a false negative rate of 7.3 % [7]. Despite its clinical effectiveness, this procedure has drawbacks. ^{99m}Tc has a 6 h half-life which limits its availability to proximal centres that handle its parent isotope molybdenum-99 (^{99}Mo). ^{99}Mo decays so rapidly that it must be supplied to hospital nuclear medicine departments as regularly as every 2 weeks and is produced in only a few reactors worldwide. Supply is also subject to interruption pending refurbishment or compromise of nuclear facilities, as was demonstrated in the Fukushima Daiichi nuclear disaster in Japan on March 11th 2011 [12].

The use of radioisotopes provides logistical challenges to hospitals. This includes the handling and disposal of isotopes, training of staff and legislative requirements. The 6 h half-life of the isotope restricts theatre scheduling since the injection is usually performed by nuclear medicine staff and not by surgeons themselves. In addition patients may express reluctance to radiation exposure especially in pregnancy [12]. These factors have limited the uptake of SLNB worldwide for hospitals without access to radioisotopes. Whilst the incidence of cancer is rising the performance of the SLNB procedure has remained static with only around 60 % of an estimated half a million patients in the Western world having access to the procedure [13]. This figure falls to 5 % in China and is minimal in the rest of the world [14].

MNPs exhibit potential to replace the ‘combined technique’ in SLNB due to their characteristics. Firstly, they can be externally detected in tissue pre-incision using a handheld magnetometer. Secondly, they are of similar dimensions to the radiotracer colloid used in the combined technique. Thirdly, their brown-black appearance acts as a visual stain to aid visualization and localization of nodes. The current focus has been on iron oxide coated in a biocompatible molecule such as dextran to create super-paramagnetic iron oxide (SPIO). This super-paramagnetic behaviour makes them ideal for SLNB, as they do not agglomerate whilst being transported via lymph in the absence of an external field. In the presence of a static or dynamic external field, however, their collective moment can be sensed external to the body [15].

The benefits of MNPs include their shelf life of several years allowing them to be shipped to remote locations worldwide. They do not require special handling procedures, can be injected by surgeons, removing issues of scheduling with a nuclear medicine department as well as problems with safe operating theatre waste disposal. No evidence has

demonstrated MNPs to be toxic or dangerous in clinical use. The biodistribution of SPIO has been well documented. They possess a half-life of between 1 and 36 h depending upon their coating and whether they are injected intravenously or interstitially before they are taken up by macrophages in the mononuclear phagocyte system of the liver, spleen, lymphatic system and bone marrow and then broken down to be distributed across iron stores in the body [16]. MNPs have been used as MRI contrast agents with doses of iron oxide of 25–100 mg. Such a dose is roughly equivalent to 2–5 days of normal dietary intake of iron, which results in transient changes in serum iron, ferritin and iron-binding capacity, but does not risk iron overload. Studies on the toxic effects from iron oxide MNPs at increased dosages confirmed no acute or sub-acute toxic effects in mammals receiving 150 times the standard dosage for MRI of the liver. Although the coating of the MNP could be a potential source of allergic reaction, currently most SPIO are coated in dextran or carboxydextran with no allergic reactions being reported for these materials [17, 18].

The feasibility of magnetic SLNB has been demonstrated in an *in vivo* porcine model using a subcutaneous areolar injection into the right and left 3rd inguinal mammary glands of 16 mini-pigs. In this study, the authors demonstrated successful SLNB in all 16 mini-pigs with injected volumes of between 0.1 and 0.5 mL of magnetic dye [19]. A small study assessing interstitial administration of SPIO and the use of a handheld magnetometer for SLNB in breast cancer patients has been performed [20]. Shiozawa et al. [20] injected 1.6 mL of Risovist [ferucarotran 864 mg/1.6 mL/L (44.6 mg iron)] and 3 mL patient blue subareolarly in 30 patients. They found that the combination of SPIO and blue dye resulted in SLNs being identified in 90 % of cases with the mean number of SLNs excised being 1.6 nodes. SLNs were successfully identified by SPIO alone in 77 % of cases and blue dye alone in 80 %. On confirmatory ALND, 8 patients had axillary metastases, and 7 of these were identified on SLNB. Shiozawa et al. [20] recorded a sensitivity of 86, 83 and 83 % for the combined, SPIO and blue dye alone techniques, respectively. This study was limited by its small size and the comparison of SPIO to blue dye only as opposed to the dual technique.

The current NIHR registered SentiMAG multicentre trial [21] is a phase II, non-randomized, multicentre, non-inferiority trial comparing the standard technique of SLNB in breast cancer (using ^{99m}Tc with or without blue dye) with a magnetic technique using super-paramagnetic carbodextran-coated iron oxide particles (Sienna+; Endomagetics, UK) and a handheld magnetometer (SentiMag; Endomagetics, UK). This technique was developed several years previously when an initial proof of concept study was performed at University College London by Joshi et al. [22] in which a total of 19 SLNs were resected from 9 patients with breast cancer.

Intra-operative localization was successful in 100 % of cases using either the standard dual or magnetic technique using a SentiMag prototype device. This was followed by an extended phase I/II trial of 43 patients and results suggested that the SLN detection rate using the new technique was 86 and 93 % when extended to a further 15 patients who had SPIO administered more than 1 h prior to surgery [23].

The SentiMAG Multicentre Trial recruited newly diagnosed breast cancer patients suitable for SLNB. Patients received standard radioisotope, magnetic dye and patent blue dye. Intra-operatively, sentinel node biopsy was undertaken using the magnetic technique, followed by the gamma probe. All nodes that contained magnetic tracer, were black or blue or radioactive, were removed. All lymph nodes are assessed histologically, and the node status related back to the SLNB identification rate with each technique. The primary endpoint was to compare the SLN identification rate and performance of the new technique with that of the standard technique. Secondary endpoints included morbidity from SLNB such as lymphoedema, numbness, seroma, cutaneous staining, shoulder stiffness, chronic pain and loco-regional recurrence. The primary data analysis on the first 160 patients has recently been completed. This demonstrated that the SLN identification rate was 95 per cent with the standard technique and 94.4 per cent with the magnetic technique. Of 25 patients with at least 1 macrometastasis, 23/25 were identified with the magnetic technique and 24/25 with the standard technique. On this basis it was concluded that the magnetic technique was non-inferior to the standard technique (0.62% difference; 95% upper confidence limit of 4.4%; 6.9% discordance), with a 95% confidence limit below the 5% limit set out in the trial design to define non-inferiority [24]. The trial was extended to 350 patients in order to evaluate comparative SLN identification rates, with both techniques, at each of the 7 trial sites. Analysis of the remaining patients is underway and progression to a randomized control trial of magnetic SLNB versus the current standard dual technique is scheduled.

The role of MNPs in diagnosis of metastatic lymph node spread

MNPs have been extensively used as magnetic resonance imaging (MRI) contrast agents for the purpose of visualizing lymph nodes. They are suitable contrast agents because of their ability to alter magnetic field gradients and, therefore, proton relaxation in the imaged tissue. When used as contrast agents they are usually injected intravenously, which allows them access to the draining nodes by moving into the medullary sinuses of the node via direct transcapillary passage, as well as directly accessing nodes via lymphatic flow as with interstitial injection. MNPs transported to lymph nodes act as ‘negative’ imaging contrast agents when using T2 and

T2* weighted sequences, and have been shown to identify metastases [23]. As MNPs are filtered and taken up by macrophages, healthy lymph node tissue produces a dark signal (signal drop) and if the lymph node contains a metastatic deposit, it will be identifiable as a white region. The use of MNPs as MRI contrast agents provides a non-invasive method of imaging the complete lymphatic drainage basin from the tumour. This imaging subsequently allows visualization of any clinically positive lymph nodes and precise identification of their location [25, 26].

In-vivo studies conducted on rats have identified SLNs on MRI using SPIO as contrast agents and confirmed this histologically on SLNB [27]. Studies have also looked at comparing pre-operative SPIO-enhanced MRI with histological findings on SLNB in breast cancer patients. They have demonstrated that the sensitivity, specificity and overall accuracy of SPIO-enhanced MRI make it a useful method for detecting SLN metastases. In a study on 102 patients with breast cancer the sensitivity, specificity, and accuracy of SPIO-enhanced MRI for the detection of SLN metastases were at 84.0, 90.9, and 89.2 %, respectively [26]. In a direct comparison between conventional MRI and SPIO-enhanced MRI, it was found that SPIO-enhancement was superior in terms of sensitivity, specificity and overall accuracy [25]. Conventional MRI demonstrated figures of 59.1, 86.7 and 80.4 % for sensitivity, specificity and overall accuracy, respectively, whereas post-contrast SPIO-enhanced MRI these were 100, 80 and 94 % [25]. In the study by Stadnik et al. [28] the sensitivity, specificity, positive and negative predictive values for SPIO-enhanced MRI were recorded at 100, 80, 80 and 100 %, respectively, whilst Michel et al. [29] found a sensitivity, specificity and positive predictive value of 82, 100 and 82 %, respectively. These in-vivo and clinical evidence suggests that SPIO-enhanced MRI could prove to be clinically useful as an non-invasive alternative to SLNB.

The future role of MNPs in the management of the axilla in breast cancer

Future management of the axilla in patients with breast cancer, aims to minimize the morbidity of surgery. It has now been demonstrated in prospective clinical trials, that completion ALND is unlikely to be beneficial routinely, in terms of survival outcome [30, 31]. Currently, patients with a low axillary burden are identified after an invasive SLNB and could be spared ALND. However, if they could be identified pre-operatively using reliable imaging modalities, it would be possible to spare them an unnecessary ALND as well as an invasive SLNB. Conversely, if patients with a high axillary burden could be identified accurately with pre-operative imaging, it would allow them to proceed directly to ALND and avoid a two-stage procedure of SLNB followed

by ALND. Currently, of all imaging modalities available MRI appears the most promising in this area.

A systematic review considering the use of MRI assessment of axillary lymph node status in early breast cancer was performed by Harnan et al. [15]. This looked at 9 studies, the largest of which contained 67 patients. They recorded a mean sensitivity of 90 % (95 % CI 78–96 %; range 65–100 %) and mean specificity of 95 % (95 % CI 75–96 %; range 54–100). The highest mean sensitivity and specificity was recorded for ultra-small paramagnetic iron oxide (USPIO) enhanced MRI, with values of 98 (95 % CI 61–100 %) and 96 % (95 % CI 72–100 %), respectively. Kimura et al. [32] found that MRI lymphangiography with USPIO, performed on 10 patients with breast cancer, was able to accurately predict all metastatic and normal nodes pre-operatively. However, only Motomura et al. [26] performed a breakdown of their identification of metastatic axillary lymph nodes according to micro or macrometastases. They found that whilst 100 % of patients with macrometastases were successfully identified on SPIO-enhanced MRI, this figure dropped to 40 % for patients with micrometastases. Johnson et al. [33] administered USPIO periareolarly and performed standard SLNB. The 13 nodes containing metastases had variable quantities of iron within them, but the iron was not present in the area of the node containing the metastasis. Therefore, heterogeneous enhancement of the SLNs and non-SLNs on contrast enhanced MRI may indicate a metastatic focus.

The combination of radionuclide-based imaging modalities such as PET and single photon emission computed tomography (SPECT) with MRI could represent the next generation of imaging modalities. Currently, radionuclide-based techniques (SPECT/PET) allow functional assessment of the tumour at a cellular level in vivo, but have poor spatial resolution for the detection of small tumor deposits (>10 mm). On the contrary, non-radionuclide techniques such as CT and MRI have excellent spatial resolution (<1 mm). Torres Martin de Rosales et al. [34–36] demonstrated that dual-modality imaging agents based on the conjugation of radiolabelled bisphosphonates (BP) directly to the surface of SPIO, provided excellent stability and allowed for in vivo co-localization of lymph nodes in a murine model. The application of these dual-imaging agents to future human studies provides exciting prospects to improve the sensitivity and specificity of pre-operative axillary staging.

Such studies do offer promising results for the future application of MRI in this field. MRI could potentially prevent the need for an unnecessary SLNB in all patients whether they possess a low or high axillary burden, the latter patients proceeding directly to ALND. However, the increased clinical use of breast MRI is not without limitations. Cooper et al. [37] performed a study looking at the

cost effectiveness of MRI and PET for the evaluation of axillary lymph nodes in early breast cancer. They created an individual patient discrete-event simulation model to estimate the lifetime costs and benefits of replacing SLNB with MRI or PET, or adding MRI or PET before SLNB. Their findings suggest that the most cost-effective strategy predicted was the strategy of replacing SLNB with MRI. This strategy was superior to the baseline SLNB strategy with both lower expected total costs (£19,325 vs. £20,189) and higher expected total QALYs (8.174 vs. 8.119). Under this strategy, true positive patients (39.3 % of all patients) will be correctly diagnosed by MRI and undergo ALND rather than undergo 2 sequential surgical procedures (SLNB followed by ALND). True negative patients (52.5 % of all patients) will be correctly diagnosed without the need for SLNB. The challenge of applying this model to clinical practice, is the high false positive rate for MRI of 6.3 versus 0.2 % for the current baseline strategy of SLNB. The technique of MRI lymphangiography has not yet been developed sufficiently to achieve the specificity and positive predictive value of SLNB [29].

Recent prospective studies have provided evidence that patients with low residual axillary disease do not benefit from completion ALND [30, 31]. It is, therefore, essential that we develop further imaging studies to identify standardized criteria for MRI parameters that define not only metastatic axillary lymph nodes in breast cancer, but also differentiate between micro and macrometastatic disease. The SentiMAG Multicentre Trial [22] has an MRI sub-protocol. This will evaluate pre-operative axillary MRI (with SPIO magnetic tracer) for sentinel node imaging and characterization, and ex vivo MRI with a 9.4T high resolution scanner to identify metastases. The aim of the sub-protocol is to evaluate MRI for both pre-operative and intra-operative detection of lymph node metastases. The results from such studies will allow us to move forward to a truly minimally invasive approach to the future axillary management of breast cancer. MRI lymphangiography has the promise to eliminate the need for diagnostic surgical axillary procedures such as SLNB.

Conclusion

The role of MNPs in imaging of the axilla and for SLNB in patients with breast cancer has been rapidly evolving. It opens the possibility of avoidance of radioisotopes for SLNB and, therefore, increased worldwide uptake of what is a standard procedure in the developed world. Through continued studies in pre-operative axillary imaging using magnetic tracers and MRI, they enable more individualized the axillary management of patients with breast cancer. MNPs provide an opportunity to eradicate the need for

unnecessary SLNB by allowing us to identify those patients who require axillary clearance and also those patients who do not require axillary surgery.

Conflict of interest The authors have no disclosures to make concerning financial and personal relationships with other people or organizations that could inappropriately influence their work.

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