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The application of magnetic nanotechnology to the surgical management of non-palpable breast cancer

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The application of magnetic nanotechnology to the surgical management of non-palpable breast cancer

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PhD Submission

2015
Abstract

Background

Breast cancer is the most common cancer in the United Kingdom, with over one-third of all cases diagnosed annually being clinically occult (non-palpable). The current standard of care is surgical wide local excision using wire-guided localization and axillary staging by sentinel node biopsy. Wire-guided localization possesses limitations, which have resulted in alternative localization techniques being developed – although these have failed to gain mainstream acceptance. This thesis examined the current evidence supporting other localization techniques and aimed to develop an alternative, overcoming existing limitations.

Materials and methods

This thesis examined a handheld magnetometer and magnetic tracer for localization properties within pre-clinical phantom models, progressing to the development of an in vivo porcine model, which was also used to assess concurrent sentinel lymph node biopsy. This was followed by the establishment of the first, in-man feasibility study of a magnetic tracer for sentinel node and occult lesion localization using an intra-tumoral injection of magnetic tracer for patients with non-palpable breast cancer (MagSNOLL trial, UKCRN 14979).

Results

This thesis demonstrated the ability of a magnetic tracer to localise at a specific site within phantom models. This was replicated within an in vivo porcine model in
addition to concurrent sentinel lymph node biopsy with a single injection of the magnetic tracer. These findings were translated into a clinical trial (MagSNOLL), which demonstrated that an intra-tumoral injection of magnetic tracer allowed successful lesion localization independently of a wire. Concurrent magnetic sentinel lymph node biopsy was demonstrated to be feasible but inferior to the standard ‘dual technique’ of radioisotope and blue dye.

**Conclusion**

Magnetic lesion localization is feasible without the need for a wire or radioisotopes. Further work to assess the retention of the magnetic tracer *in vivo* and optimisation of sentinel node identification rates are required.
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1. Introduction

1.1 Breast cancer incidence

Breast cancer is the most common cancer within the UK. In 2011, there were 50,285 new cases of breast cancer in the UK with 49,936 (99%) in women and 349 (less than 1%) in men. The crude incidence rate shows that there are 155 new breast cancer cases for every 100,000 females in the UK and 1 for every 100,000 males.\(^1-^4\) Female breast cancer incidence is strongly linked to age, with the highest incidence rates overall being in older women. In the UK between 2009 and 2011 80% of breast cancers were diagnosed in women aged over 50 and 25% diagnosed in women aged over 75. Age-specific incidence rates rise steeply from age 30-34, levelling off for 50-59, rising further to 65-69. Rates decrease from 70-74 and then reach an overall peak in the 85 plus age group.\(^1-^4\)

Female breast cancer incidence rates have increased overall in Great Britain since the mid 1970s.\(^1-^3\) European age-standardised incidence rates increased by 72% between 1975 and 2011. From the mid 1970s to late 1980s the incidence rates increased steadily by 1-2% per annum.\(^1-^3,^5\) The introduction of the national breast screening programme from the late 1980s led to a transient increase in incidence. By the mid 1990s, the increase in incidence rates returned to pre-screening levels and continued like this until the mid-2000s. Over the last decade between 2001 and 2011 the European age-standardised incidence rates have increased by 7%, with the majority of this increase occurring before the mid-2000s.\(^1-^3\)
1.2 Breast cancer diagnosis

1.2.1 Triple assessment

The majority of breast cancer (approximately 70%) is detected in symptomatic patients within breast clinics using the ‘multidisciplinary triple diagnostic method’ – comprising of clinical assessment, diagnostic imaging and needle biopsy. The role of the clinical assessment is to take a thorough clinical history of the patient’s symptoms and physical examination. Appropriate imaging should be conducted with ultrasound being the modality of choice for women with focal symptoms who are aged below 40, pregnant or lactating. Ultrasound also increases the detection of small breast cancers in women who have a dense background pattern on mammography. X-ray mammography – using 2 views of craniocaudal (CC) and medio-lateral oblique (MLO) - is used in the investigation of women aged over 40 with the addition of ultrasound as needed. X-ray mammography is not indicated for the majority of patients under 40 but is applicable in those aged between 35-39 with clinically suspicious or indeterminate findings if ultrasound appears normal. All patients with proven malignancy should undergo mammography even if aged below 40. Additional mammographic views – magnification and spot compression – may be useful in characterising suspicious abnormalities. Once clinical and imaging-assessment are complete, breast needle biopsies should be performed under imaging guidance of the suspicious lesion to provide diagnostic histopathological information and oncological information. Ultrasound of the axilla should be performed in all patients with suspected malignancy and if ultrasound demonstrates abnormal morphology of lymph nodes then needle sampling should be conducted using needle core biopsy or fine needle aspiration.
Magnetic resonance imaging (MRI) is the most sensitive technique for the detection of breast cancer but has high false positive rates\textsuperscript{10,11} and is consequently not recommended in the pre-operative assessment of patients with biopsy-proven invasive or \textit{in situ} malignancy.\textsuperscript{12} MRI is suitable when a discrepancy occurs regarding the extent of disease from clinical examination, mammography and ultrasound in treatment planning, if breast density precludes accurate mammographic assessment or to assess patients considered for breast conserving surgery with invasive lobular cancer.\textsuperscript{12}

\subsection*{1.2.2 Breast cancer screening}

The UK breast cancer-screening programme was introduced in 1987 as a consequence of the Forrest Report\textsuperscript{13} commissioned by the Department of Health, which concluded that screening by mammography could lead to a prolongation of life for women aged 50 and over and formed the basis to change UK policy on the provision of mammographic screening for symptom-less women. Women between the ages of 50 and 70 are invited for regular breast screening, with women over 70 able to attend but not invited. The NHS breast-screening programme is currently in the process of phasing in breast screening for all women in the extended age range of 47-73 and should be completed by 2016. Women are invited every 3 years with mammography expected to detect breast cancer approximately 2 years before it becomes clinically apparent. The breast frequency trial completed in 1995 did not show any predicted benefit for the screened population by increasing frequency from every 3 years to annually.\textsuperscript{14} A total of 1.94 million women were screened in 2011-2012, with 15,749 cancers detected (32\% of all breast cancers diagnosed) at a rate of 8.1 per 1000 women screened. This compares with 14,725 cancers detected in 2010-2011 (a rate of 7.8 cases per 1000) and 8,545 detected in 2001-
Recent concerns with respect to the benefit of breast cancer screening resulted in the Marmot Report. This stated that on the current available evidence breast screening provides a 20% reduction in mortality in women invited to screening. The Panel identified that for every 10,000 UK women invited to screening from age 50 for 20 years, about 681 cancers will be found of which 129 will represent overdiagnosis and 43 deaths from breast cancer will be prevented. Therefore, for each breast cancer death prevented about 3 overdiagnosed cases will be identified and treated. Of the approximately 307,000 women aged 50-52 invited to screening each year, just over 1% would have an overdiagnosed cancer during the next 20 years. The Panel consequently concluded that the UK breast-screening programme confers significant benefit and should continue.

1.3 The management of screen-detected breast cancer

1.3.1 Introduction

The increased use of mammography and introduction of breast screening programmes has resulted in an increase in the diagnosis of early breast cancer. Cancers detected by screening would be expected to require less treatment because they are diagnosed at an earlier stage. The Swedish Two Counties Trial demonstrated small, node negative cancers detected by screening had a mean 10% improved survival at 15 years compared with symptomatic node negative tumours of similar size. This was reiterated by a Finnish cancer registry study, which identified that screen-detected non-palpable tumour stage 1 (T1) cancers have improved cancer-specific survival compared to symptomatic T1 cancers. It has also
been shown that screen-detected cancers have an improved overall survival and reduced breast, axillary and distant recurrence rate when compared to non-screen detected cancers. These findings suggest that there is an inherent biological difference between cancers detected by mammographic screening compared to those presenting symptomatically. Despite these differences current protocols for adjuvant therapy do not take into account the favourable prognostic outcomes associated with screen-detected breast cancers when planning their management. The evidence for adjuvant therapies in screen-detected breast cancers was reviewed to determine if they should be treated like symptomatic cancers or if the screen-detected population should be considered as a distinct subset when considering their multidisciplinary management, including surgery, adjuvant radiotherapy, chemotherapy (including herceptin) and endocrine treatment.

1.3.2 Surgery

For symptomatic cancer, the NSABP B04 trial demonstrated that surgery without adjuvant therapy can cure 57% of patients with a clinically negative axilla, at 10 years follow-up. The survival of patients with screen-detected cancer at 15 years follow-up, has been shown to exceed 90%. The NSABP B04 trial also demonstrated that routine axillary clearance does not improve survival. The lack of survival benefit for axillary clearance was also demonstrated in the ACOSOG Z0011 trial for patients with 2 involved sentinel nodes or less, so long as they received adjuvant systemic therapy. The challenge we face with respect to screen-detected cancers is to provide patients with sufficiently adequate surgery to obviate the need for systemic therapy. It is likely therefore, that the minimum requirement will remain a wide local excision with sentinel node biopsy for the majority. In the future, percutaneous excision and local ablative techniques are
likely to become a viable alternative treatment for some of these tumours, although at present, there remain areas of clinical research interest.

1.3.3 Adjuvant radiotherapy

Previous studies have demonstrated the reduction in local recurrence using breast radiotherapy after breast conserving surgery. These studies range from 4% to 76% for their proportions of screen-detected cancers. The study by Malmstrom et al. compared patients with stage I-II lymph node negative breast cancer, undergoing standardised breast conserving surgery. Patients were randomised to postoperative radiotherapy or no further local treatment after breast conserving surgery. In their study 65% of the patients included were screen-detected. They found that the risk of ipsilateral breast recurrence was lower for screen-detected compared with clinically detected breast cancers (RR=0.62, 95%CI 0.42-0.91, \( P=0.015 \)). Among non-irradiated patients, the 5-year breast recurrence cumulative incidence was 11% (95% CI, 8-16%) and 19% (95% CI, 12-30%) among screening and clinically detected women. The corresponding figures for the irradiated patients were 4% (95% CI 2-7%) and 5% (95% CI 3-10%). There was a highly significant difference comparing all 4 groups (\( P<0.001 \)). They found that the lowest risk in non-irradiated patients was found for women greater than 49 years of age whose tumours were identified on screening. Their cumulative incidence rate at 5 years was 10%. The Uppsala Breast Cancer Study randomised patients with tumours less than 20 mm, who underwent breast conserving surgery and axillary clearance, to either post-operative radiotherapy or no adjuvant treatment and was composed of 45% screen-detected patients. Point estimates for recurrence rates at 5 years were 2.9% and 10.2% for the radiotherapy and no adjuvant treatment arms respectively. Holli et al. in their
study of breast conserving surgery with and without adjuvant radiotherapy did not specify the numbers of screen-detected breast cancers. However, criteria for inclusion were lesions less than 20 mm and 40% of breast cancers were less than 10 mm. They found that the 5-year loco-regional disease free survival was 93.7% and 85.9%, for the radiotherapy and no further treatment groups respectively. Schnitt et al. 26 identified patients at low risk of recurrence who underwent breast-conserving surgery and could be spared adjuvant radiotherapy. In this study 76% of the population were composed of screen-detected breast cancers. A total of 87 patients were enrolled before the trial was prematurely closed because the pre-defined stopping boundary of the sixth local recurrence had passed. At 56 months follow-up, the recurrence rate was 16% with a mean local recurrence rate of 3.6%. No single study demonstrated a significant overall survival advantage for adjuvant radiotherapy following breast-conserving surgery. 20, 23-29. However, the benefit was demonstrated in the Early Breast Cancer Trialist’s Collaborative Group (EBCTCG) overview, 30 in which there was a significant reduction in not only local recurrence rates, but also overall mortality in patients receiving adjuvant radiotherapy after breast conserving surgery.

Adjuvant radiotherapy is essential for local control following breast-conserving surgery, even in small breast cancers, which would be considered low risk for recurrence. Even in the study by Schnitt et al. 26 which was composed of over three-quarters screen-detected breast cancers, this was the case. This suggests that in patients with screen detected breast cancer, adjuvant radiotherapy should generally be offered after breast conserving surgery. However, studies have suggested that endocrine therapy may play a role in reducing the need for radiotherapy within excellent prognostic groups. 31, 32
1.3.4 The role of endocrine therapy

Endocrine therapy has been demonstrated to increase overall and disease-free survival in patients with oestrogen-receptor positive (ER +ve) breast cancer after conserving and non-brest conserving surgery. NSABP-14 was a randomised, double blind, placebo-controlled trial of adjuvant tamoxifen in 2644 node negative and ER +ve breast cancer patients. Over 57% of patients had tumours smaller than 20 mm and 38% of patients underwent breast conserving surgery and radiotherapy, in both trial arms. NSABP-14 demonstrated that there was a significant difference (P<0.00001) between the treatment groups in disease-free survival in favour of patients receiving tamoxifen (83% vs 77%) at 4 years follow-up. Tamoxifen therapy also significantly reduced the rates of local and distant recurrence. The Early Breast Cancer Trialist's Collaborative Group (EBCTCG) performed a meta-analysis of 37,000 women from 55 randomised trials of tamoxifen versus no-tamoxifen before disease-recurrence. They identified that the proportional recurrence reductions at 1, 2 and 5 years were 21%, 29% and 47% respectively, with a significant trend towards greater effect with longer treatment. The proportional mortality reductions were 12%, 17% and 26% respectively, demonstrating a significant trend in favour of tamoxifen use. The absolute reduction in recurrence was greater during the first 5 years, whereas the improvement in survival grew steadily larger throughout the first 10 years. The proportional mortality reductions were similar for women with node-positive and node-negative disease, but the absolute mortality reductions were greater in node-positive women. In the trials of 5 years of adjuvant tamoxifen the absolute improvements in 10-year survival were 10·9% for node-positive (61·4% vs 50·5% survival, P<0·00001) and 5·6% for node-negative (78·9% vs 73·3% survival, P<0·00001). The incidence of endometrial cancer was approximately doubled in
trials of 1 or 2 years of tamoxifen and approximately quadrupled in trials of 5 years of tamoxifen.

The BASO II study \(^{31}\) evaluated the benefit of adjuvant radiotherapy and tamoxifen in node-negative, small breast cancers (<20 mm) after breast conserving surgery. All patients were over 70 years of age with clear margins, node-negative, but ER status was not identified. Randomisation of 1171 patients was by radiotherapy versus no-radiotherapy, tamoxifen versus no-tamoxifen and a 2 x 2 randomisation of no tamoxifen, no radiotherapy; radiotherapy only; tamoxifen only; radiotherapy and tamoxifen groups. BASO II \(^{31}\) demonstrated that patients receiving both tamoxifen and radiotherapy had a significantly reduced local recurrence rate \((P<0.001)\), with 15 of 95 patients receiving neither treatment developing local recurrence. However, the local recurrence in the later group was relatively low (1.9% per year) and this was reduced to 0.8% with the addition of tamoxifen. Tamoxifen had a significant protective effect after adjustment for radiotherapy \((P=0.003)\) and vice versa \((P=0.002)\). Receipt of both therapies conferred a significantly lower risk of local recurrence than radiotherapy alone \((P=0.01)\) and also a significantly lower risk than use of tamoxifen alone \((P=0.006)\). However, the authors concluded that radiotherapy could be omitted for tamoxifen alone in selected patients who fall into the Nottingham Prognostic Index Excellent Prognostic Group. \(^{31}\) This Excellent Prognostic Group accounts for 15% of all invasive breast cancers and therefore hormonal therapy in place of radiotherapy following wide local excision for primary breast cancer could provide considerable savings. \(^{31}\) Similarly, the PRIME II Trial \(^{32}\) recently reported that radiotherapy can be omitted in women over 65 years of age, so long as they receive adjuvant endocrine therapy. NSABP-21 \(^{35}\) also attempted to assess the need for breast
irradiation after breast-conserving surgery in patients with invasive breast cancers <10 mm comparing it to tamoxifen alone. In NSABP-21 35 over one-third of patients had an ER status that was unknown. After breast-conserving surgery 1009 women were randomised to tamoxifen (n=336), radiotherapy and placebo (n=336) or tamoxifen and radiotherapy (n=337). At 8 year follow-up, the ipsilateral breast recurrence rate was 13.5%, 6.9% and 2.7% for tamoxifen, radiotherapy and placebo and radiotherapy and tamoxifen groups respectively and therefore significantly favouring the latter group (P<0.0001). NSABP-21 35 also identified that the contralateral breast cancer rate was significantly lower in the tamoxifen only group (0.9%; P=0.03) compared to the radiotherapy and placebo group (4.2%) and radiotherapy and tamoxifen group (3%). There were no significant differences in adverse events potentially attributable to tamoxifen were identified between groups.

BASO II 31 did not randomise patients according to their ER status and NSABP-21 35 had over one-third of patients with unknown ER status. But both BASO II 31 and NSABP-21 35 demonstrated an improved disease-free survival in patients with small breast cancers that are ER +ve who undergo combination therapy with tamoxifen and radiotherapy as opposed to either of these treatments alone. NSABP-21 35 demonstrated the potential of tamoxifen to reduce the incidence of contralateral breast cancer when used alone and in combination with radiotherapy. The lower rate of contralateral breast cancers in the tamoxifen only group of 0.9% compared to the irradiation only group (4.2%) and combination therapy group of 3% is likely explained by the potential of radiotherapy to induce malignancy in the contralateral breast. This may suggest that prognostically good patients could benefit from a more localised administration of radiotherapy in
order to minimise this potential risk of contralateral breast malignancy and therefore optimise their therapy further. Also in view of the very low recurrence, the relative benefit of radiotherapy is low (despite the significant absolute benefit) and it can therefore be safely omitted in selected patients with very small screen-detected tumours in patients over 70 years of age, particularly if they have comorbidities.

Intra-operative radiotherapy has been used in the adjuvant treatment of breast conserving surgery. The TARGIT-A trial \(^{36}\) randomised a total of 1721 women, undergoing breast conserving surgery to intra-operative radiotherapy and 1730 to external beam radiotherapy. The 5-year risk for local recurrence was 3.3% (95% CI 2.1-5.1) for intra-operative radiotherapy and 1.3% (0.7-2.5) for external beam radiotherapy \((P=0.042)\). The ELIOT trial \(^{37}\) of 1305 patients randomised to intra-operative radiotherapy or external beam radiotherapy in breast conserving surgery found that the 5-year local recurrence rate was 4.4% (95% CI 2.7-6.1) for intra-operative radiotherapy versus 0.4% (0.0-1.0) in the external beam radiotherapy group \((\text{hazard ratio 9.3 [95% CI 3.3-26.3]})\). Both TARGIT-A and ELIOT \(^{36,37}\) did not demonstrate a significant difference in overall survival between the treatment groups. TARGIT-A and ELIOT \(^{36,37}\) identified significantly reduced skin complications within the intra-operative radiotherapy group. The ELIOT trial \(^{37}\) demonstrated, that poor prognostic factors such as pathological size >20 mm, grade 3 malignancies, ER –ve, triple negative and high proliferative index could significantly predict recurrence. Therefore, whilst intra-operative radiotherapy may not be suited for all patients, those screen-detected cancers with good prognostic outcomes could benefit from this treatment modality in order to allow
safe oncological management and also avoid the inconvenience and recognised side effects of external beam radiotherapy.

1.3.5 The role of adjuvant chemotherapy

The purpose of adjuvant systemic therapies is to improve disease-free and overall survival rates associated with treatment of breast cancer by surgery and irradiation. It has been demonstrated that screen-detected breast cancers have better overall survival and reduced recurrence rates compared to tumours of similar size and node negative symptomatic cancers. However, therapy for screen-detected breast cancers is determined by evaluating the patients likely benefit from adjuvant systemic therapy using models that are based on symptomatic breast cancer. The benefit of chemotherapy is proportionate to the risk of recurrence but complications of chemotherapy, which include thrombosis, sepsis and occasionally death occur in 1-3% of treated patients and complications increase with age. Late adverse health effects, such as leukaemia and cardiotoxicity occur in 1% of patients from 5-13 years onwards. The Early Breast Cancer Trialist’s Collaborative (EBCTCG) recommended adjuvant chemotherapy for women between 50 and 70 years of age with adverse prognostic factors based on a 10-20% relative reduction in cancer mortality. However, the reduction in absolute mortality from chemotherapy in postmenopausal women older than 50 years was 3-5%, remaining constant at 15 years. Unless an individual’s risk of death is greater than 10% at 5 years, a proportionate reduction in mortality from chemotherapy use greater than 1% is not achievable. The morbidity from its administration will equal its benefit and only breast cancers with an increased annual hazard rate for mortality of 10% or greater in the 5 years after diagnosis will benefit from chemotherapy. Bundred et al. retrospectively
assessed 875 screen-detected cancers with 600 symptomatic cases, in women aged 50-65 years of age and prognostic factors were compared with mortality. The 10-year survival was 92.1% for screen-detected breast cancers compared to 77.6% for symptomatic cases. The screen-detected breast cancers had a reduced mortality (RR=0.42 (0.31-0.57)), independent of grade, node status and tumour size. ER +ve screen-detected breast cancers had a lower annual mortality rate (0.6%) compared with symptomatic (4.3%; P<0.001) or ER -ve screen-detected breast cancer (1.8%). The Nottingham Prognostic Index (NPI) was calculated and demonstrated better survival for each NPI group in the screen-detected cohort. The screen detected breast cancers had a better prognosis despite less chemotherapy use in every NPI group (except the Excellent Prognostic Group [EPG]) compared with symptomatic cancers. Cancer survival in screen-detected breast cancer was the equivalent of one NPI group better than symptomatic cancers. In the moderate prognostic group 1, 26% of ER +ve symptomatic cancers had chemotherapy but mortality at 5 years was 8%, whereas mortality of 2% at 5 years occurred in moderate prognosis group 1 screen detected breast cancers despite only 9% receiving chemotherapy (P =0.001). Bundred et al. found that the proliferative index was lower in the screen-detected group across all NPI groups compared to symptomatic patients (P<0.001). This made them conclude that the lower mortality in screen-detected breast cancers compared to symptomatic cancers was due to this lower proliferative index and that the use of adjuvant chemotherapy was over-treatment for ER +ve screen-detected breast cancers with Good Prognostic Group (GPG) and moderate prognostic group 1 NPI scores. The study by Bucchi et al. compared the adjuvant systemic treatment of a screen-detected Italian cohort of breast cancer patients with a symptomatic one. They found that screen-detected breast cancers, which were node-negative were
significantly less often treated according to the St. Gallen Conference guidelines. This would suggest that there is a pragmatic approach amongst clinicians treating screen-detected breast cancers who are concerned about over treatment in this low risk population and are omitting adjuvant chemotherapy. Barth et al. also found that screen-detected breast cancer patients were less likely to be treated with systemic therapy compared to symptomatic patients (28% versus 56%; P<0.0001). These findings were largely attributable to their study demonstrating significantly less node positive disease and smaller median tumour size in the screen-detected group. In view of the better prognostic outcomes of matched screen detected versus symptomatic breast cancers, it is important to ensure that adjuvant systemic therapy is appropriately selected to avoid over-treatment and potential side effects. In this area the use of molecular profiling could be of assistance in determining adjuvant chemotherapy for screen-detected patients. The 21-gene Oncotype DX (Genomic Health Inc, Redwood City, CA) recurrence score assay has been shown to quantify the risk of loco-regional recurrence in ER +ve patients who are node-negative. By using molecular profiling it may be possible to optimise adjuvant therapies without overtreatment. However, the studies on which quantification of Oncotype DX assay has been performed are those consisting of symptomatic patients only. Therefore, the generalised applicability of this assay to the screen-detected population could be questioned. It would be prudent to ensure that future assay quantification takes place on a cohort comprised of screen-detected breast cancers in order to ensure further confidence in results.
1.3.6 Conclusion

The introduction of breast screening programmes has resulted in an increase in the diagnosis of clinically, occult non-palpable breast cancers. The standard surgical management consists of wide excision and sentinel node biopsy. The benefit of subsequent adjuvant treatment is largely based on studies conducted on symptomatic breast cancer patients. This has meant that despite the better prognosis of this subset of breast cancer patients, their adjuvant treatment is aligned to that of symptomatic breast cancer patients. The consequence of this management is adjuvant overtreatment of screen-detected patients with the risk of associated short and long-term side effects. Molecular profiling may offer future assistance in selecting adjuvant chemotherapy in patients, who are ER +ve and node-negative. However, validation of data against a screen-detected population is required in order to more accurately determine likely benefit of adjuvant treatments. It is therefore essential that future studies report on the proportion of screen-detected patients and take into account the different natural history of screen-detected cancer to ensure the most appropriate treatment is administered without overtreatment.

1.4 Axillary surgery for breast cancer

1.4.1 Introduction

Regional lymph node status provides information regarding staging, local control and prognostic outcomes in all cancers. This information was provided in breast cancer by axillary lymph node dissection (ALND). This changed with the development of sentinel lymph node biopsy (SLNB). Sentinel lymph nodes (SLNs) are defined as the first lymph nodes receiving lymphatic drainage from the primary tumour and therefore the most likely to harbour metastatic cancer via
lymphatic spread. SLNB is now the standard of care in patients with a clinically and radiologically clear axilla in early-stage breast cancer.

1.4.2 The role of SLNB in patients with a clinically and radiologically negative axilla

SLNB has replaced ALND in patients with a clinically and radiologically negative axilla. The SLN is the most likely node to be positive in the presence of axillary metastases and if it is positive there is a fourteen fold greater likelihood of non-sentinel lymph node (non-SLN) involvement.\(^{48}\) A systematic review of 69 trials involving 8059 patients undergoing SLNB validated by ALND demonstrated a 96% success rate for identifying the SLN and a mean false negative rate of 7.3%.\(^{49}\) This has been confirmed by five randomized controlled trials comparing SLNB followed by ALND with SLNB followed by ALND only if metastases were present in the SLNB specimens.\(^{50-54}\) The axillary recurrence rates are less that 0.5% in SLN negative patients.\(^{48,55}\) Therefore, in patients with a clinically and radiologically negative axilla SLNB is the standard for staging of the axilla. However, in 70% of patients the SLNB is negative and therefore surgery could be avoided if better axillary mapping techniques were available.

1.4.3 Patients with a positive SLNB

1.4.3.1 Patients with positive SLNs who did not undergo completion ALND

Evidence has accumulated from studies of the outcomes of patients with positive SLNB who did not proceed to completion ALND. Studies have demonstrated that the axillary recurrence rate is low.\(^{56-59}\) Naik et al.\(^ {59}\) reported 1.4% axillary recurrence rate at a median follow-up of 31 months in 210 patients with SLN positive cancer who declined ALND. Three further studies demonstrated no cases
of axillary recurrence at a range of between 27.6 and 32 month follow up.\textsuperscript{56-58} These studies demonstrate that there are patients with SLN positive cancer who have a low axillary burden and do not experience axillary recurrence with the omission of completion ALND and therefore in whom completion ALND is unnecessary.

1.4.3.2 The significance of micrometastases

Retrospective studies using serial sectioning and immunohistochemistry (IHC) suggest that micrometastases are prognostically significant.\textsuperscript{60-63} However, the ACOSOG Z0010\textsuperscript{64} and NSABP-32\textsuperscript{65} trials failed to demonstrate any reduction in overall survival in patients found to be SLN positive on IHC but not on H&E at five and eight year follow up respectively. The retrospective study of 377 patients with a single micrometastatic SLN who did not undergo completion ALND by Galimberti et al.\textsuperscript{66} found that the five year overall survival was 97.3% and the incidence of axillary recurrence only 1.6%. The report from the extensive SEER database\textsuperscript{67} suggested that the amount of completion ALND for positive SLNB, particularly micrometastases has been falling with no apparent effect upon local control or survival.

The recent update by Galimberti et al.\textsuperscript{68} of the International Breast Cancer Study Group (IBCSG) trial 23-01 demonstrated that an ALND can be omitted in patients with very low volume metastatic nodal involvement. This randomised, multicentre, phase III clinical trial compared ALND with no ALND in patients with micrometastases alone, in the sentinel lymph node (SLN). A total of 934 clinically node-negative patients were randomised, with a primary tumour of less than 50 mm, and with less than 2 mm tumour focus in one or two SLNs. The majority of
patients (67%) had tumours less than 20 mm, and 89% were oestrogen-receptor positive (ER+). The 5-year disease-free survival (DFS) and overall survival (OS) rates in the ALND versus SLNB only groups, were 87.3% versus 88.4% (P=0.48) and 97.6% versus 98% (P=0.35), respectively.

Therefore, evidence has accumulated that patients with micrometastatic disease do not need to proceed to completion ALND. This led to posing the partially answered question of which SLN positive patients actually require a completion ALND? This resulted in the concept of the development of a prospective trial in order to address this emerging trend.

**1.4.4 ACOSOG Z0011 trial**

The aim of the ACOSOG Z0011 trial was to determine whether ALND is beneficial for survival or loco-regional control in breast conserving surgery with whole breast radiotherapy and systemic therapy in patients with SLN metastases. It was a prospective phase three study which commenced in 1999, enrolling 891 women with clinically T1 or T2 invasive breast cancer without clinical lymphadenopathy and one or two SLNs containing metastases, which were identified by touch preparation, frozen section or H&E staining. Patients were ineligible if their SLN metastasis was only identifiable using IHC or if they had three or more positive SLNs. All patients were treated with breast conserving surgery and tangential whole breast radiotherapy. No third field irradiation was given to the axilla. Patients with SLN metastases on SLNB were randomised to either ALND or to no further axillary treatment.
The trial closed early in 2004 due to slow accrual and a lower than anticipated event rate. Patients were followed up for a median of 6.3 years. In the group of patients randomised to ALND, the 5-year overall survival and disease free survival was 91.8% and 82.2% respectively whereas in the SLNB alone group it was 92.5% and 83.9%. The hazard ratio for treatment related overall survival was 0.87 after adjusting for age and adjuvant therapy and it was concluded that no significant difference was observed between the two groups.

1.4.4.1 Issues with the ACOSOG Z0011 trial

The planned sample size was not achieved due to a lower than anticipated event rate, but the statistical analysis of Z0011 was able to demonstrate the non-inferiority of SLNB alone for overall survival with a p-value of 0.008. The 95% confidence intervals of the hazards ratio for survival did not cross 1.3, the predefined point at which the treatments would be considered unequal, suggesting the results would not change with a larger sample size. 70

The study has a relatively short-term follow-up. In NSABP B04, the median time to axillary recurrence was 12 months in clinically node negative women not undergoing ALND. 71 However, the NSABP B04 group did not receive any radiotherapy to the axilla compared to the Z0011 study in which all patients underwent whole breast radiotherapy, where standard opposing tangential fields will irradiate much of level I and II axillary lymph nodes, meaning that the two groups are not truly comparable. In studies by Martelli et al. 72 and Greco et al. 73 with populations of largely ER-positive patients comparable to those in Z0011, the median times to axillary recurrence were 33.0 and 30.6 months, respectively. Therefore, the Z0011 authors would argue that the median follow-up period of 6.3
years is sufficient to capture the majority of axillary recurrences. However, if we look at the results of the Guy's breast conservation trial consisting of 629 patients with early breast cancer, randomised to either radical mastectomy and postoperative radiotherapy or wide local excision and postoperative radiotherapy, we may want to reconsider this viewpoint. At 10 years follow up, whilst there was a significant difference between both local and distant recurrence for the radical mastectomy and wide local excision groups (7% vs 25% respectively), there was no difference in overall survival (80% in both groups). It was only at 25 years follow up that the divergence developed and there was a statistically significant increase in breast cancer deaths in the breast conservation group compared to the mastectomy group (48% vs 38%, $p=0.0016$). The EBCTCG overview of randomised controlled trials (RCTs) considering the effects of local treatments upon local recurrence rates and 15-year survival found that whilst at 5 years the absolute risk reduction in breast cancer mortality was 0.6%, at 15 years it reached 5% which was statistically significant ($p<0.00001$). This extensive study of 42,000 women highlights the benefit of adequate local control for reduced long-term breast cancer mortality. This does raise the point that the median 6.3 year follow up in the Z0011 trial may be inadequate to demonstrate differences in overall survival, which will only become prominent with longer term follow up.

All patients with heavy axillary burden were excluded from Z0011. However, no attempt was made to determine the axillary burden preoperatively using radiological assessment of the axilla, contrary to the standard practice of the modern management of the axilla. There was a very high proportion of patients who possessed micrometastases, 120 out of 436 in the ALND group and 160 out of 420 in the SLNB only group. We know that large-scale studies have shown in
patients with micrometastases there are no significant differences in axillary recurrence or overall survival for patients who undergo SLNB only versus completion ALND.\(^{64-68}\) We also know that for patients with low axillary burden fewer ALNDs were being performed before Z0011, suggesting a more surgically conservative approach to the management of the axilla prior to publication of Z0011.\(^{76}\)

Adjuvant systemic therapy was delivered to 423 out of 436 in the SLNB only arm and 403 out of 420 in the ALND arm. Adjuvant systemic therapy is known to diminish loco-regional recurrence in breast cancer patients.\(^{77}\) All patients in Z0011 underwent whole breast radiotherapy. It is known that standard opposing tangential fields will irradiate the SLNB operative field and by placing the deep field edge 2 cm below the chest wall/lung interface, the entire axillary dissection field site (levels I and II) can be included in nearly all patients.\(^{78}\) It is therefore likely that significant portions of the axilla were treated in patients in both arms of the Z0011 trial.

1.4.4.2 Conclusions to be drawn from the Z0011 trial

Z0011 demonstrates the existence of a group of patients with low axillary burden who do not benefit from completion ALND when combined with whole breast radiotherapy and systemic therapy. When put in this context the conclusion does not appear radically remarkable, especially considering the high numbers of patients with micrometastatic disease in both arms. We can conclude that Z0011 does support the already existing evidence on the treatment of patients with low axillary burden in the form of micrometastases. It reaffirms that such patients do not benefit from completion ALND as was already supported by current evidence.
Therefore, one can safely conclude that this group when treated in combination with whole breast radiotherapy and systemic therapy can be avoided proceeding to completion ALND. However, we have to consider whether the numbers of patients with macrometastatic disease (283 in the ALND and 218 in the SLNB alone arm) are large enough to gain any insight into their safe long-term management. We would suggest that the findings are promising. However, full confidence of the implementation of the Z0011 findings will only come with the longer term follow up of a larger cohort of patients with macrometastatic disease to ensure there is no late divergence in recurrence and overall survival in these patients. Where does this leave us for managing patients with macrometastatic SLN disease right now? Our opinion is that those patients fitting the Z0011 criteria with macrometastatic SLN disease would benefit from being made fully aware of the findings of the study and the fact that conclusions have been drawn from a small cohort over a relatively short follow-up period who all received whole breast radiotherapy and systemic therapy. If they are happy to undergo these adjuvant therapies and take on the uncertainty we would be happy to proceed with omitting completion ALND from their management. The Association of Breast Surgeons of the United Kingdom amended their guidelines following a consensus meeting on January 26th 2015, making further axillary treatment no longer mandatory in patients who are receiving breast conservation with whole breast radiotherapy, that are post menopausal and have T1, grade 1 or 2, ER positive and HER2 negative tumours. These patients could also be included into clinical trials.

1.4.4.3 New trials beyond Z0011

Beyond Z0011 trials are now necessary to focus upon patients with a clinically and radiologically clear axilla who were excluded from the Z0011 trial. This includes
those not suitable for breast conservational surgery. This would allow us to determine if the findings of Z0011 could be reproduced in this group of patients. Currently, Z0011 data would have to be applied with great caution to patients undergoing mastectomy since they may not receive adjuvant radiotherapy, as was the case in all Z0011 patients. Patients undergoing primary systemic therapy (PST) could also be assessed. All patients undergoing PST would undergo sentinel lymph node biopsy (SLNB) prior to commencing chemotherapy. Those that demonstrated low axillary burden would then be eligible for the study. Subgroups would have to be established for those patients who would undergo mastectomy and those who responded adequately to allow breast conservative surgery. These patients would be randomised to either observation alone or ALND. The third group to be assessed could include patients undergoing breast conservative surgery without whole breast radiotherapy. This study could involve women over the age of 70 years of age suitable for breast conservative surgery. All patients would undergo breast conservative surgery and SLNB and those with a low axillary burden would be eligible for the study. These patients would then be randomised between observation alone and ALND. Such trials would build on the information accrued from the Z0011 data set rather than simply replicating it and allow us to determine which other patients with a low axillary burden could benefit from the omission of ALND.

The issue of slow accrual rate highlighted in Z0011 may mean that the performance of such studies would be difficult at best and impossible at worst. We would therefore propose the case for a prospective register of patients who fit the Z0011 criteria and do not undergo completion ALND. This would allow the accumulation of greater patient numbers and long-term follow up. The analysis of
such collected data at key milestone intervals would allow rapid determination of any divergence between the two groups and the possibility of rapid implementation of appropriate action and thus ensuring patient safety and clinician confidence. Although such prospective registers would be ideal, they would require the support and funding of professional and governmental organisations and more importantly the support of healthcare professionals managing breast cancer to function. We believe that whilst funding may not be easily forthcoming, the desire from healthcare professionals to have additional, robust evidence to support the omission of ALND in patients fitting the Z0011 criteria does exist. In the absence of funding retrospective collection of data from breast units at the time of relapse could be an alternative to determine trends in these patients, whilst minimizing resource requirements. For patients outside of the Z0011 criteria it would be essential that any data collected upon them should be performed within the confines of a clinical trial to ensure standardisation of treatment regimes and patient safety. Currently the POSNOC trial (Positive sentinel node: adjuvant therapy alone versus adjuvant therapy plus clearance or axillary radiotherapy. A randomised controlled trial of axillary treatment in women with early breast cancer who have metastases in 1 or 2 sentinel nodes), which is open to recruitment is randomising patients with 1 or 2 sentinel node macrometastases to adjuvant therapy alone or adjuvant therapy plus axillary treatment (including axillary radiotherapy only) and will assess the 5 year axillary recurrence rate. This randomised, multicentre, non-inferiority trial recruiting 1900 patients, will include those undergoing breast conservation as well as mastectomy. Therefore, in addition to providing a greater cohort size to Z0011, this trial will be composed entirely of macrometastatic disease and will provide information about omission of ALND in mastectomy patients. All patients in addition to being clinically node
negative are also required to be radiologically node negative – in contrast to Z0011. This will make the trial more representative of current UK practice, but the significance of excluding the radiologically only involved group is an area of ongoing debate with respect to depriving patients of avoiding further axillary surgery who may have had only 1 or 2 positive nodes on SLNB – should they have received it. 79

1.5 Localized wide local excision - using wire-guided localization

The widespread initiation of screening programmes for breast cancer, combined with increasing utilisation of advanced imaging modalities such as magnetic resonance imaging (MRI) for diagnostic purposes has resulted in an increase in the identification of non-palpable breast cancers. It is estimated that over one-third of all breast cancers diagnosed annually are non-palpable. 80 Wide local excision (WLE) of primary breast cancer – in those amenable to breast conservation – is the standard of care for early breast cancer, 81, 82 with those clinically non-palpable or ‘occult lesions’ requiring an additional localization procedure using wire-guidance. The use of a wire was first described by Dodd et al. 83 in 1965 and the technique modified with the addition of a hooked tip by Frank et al. 84 in 1976. Wire-guided localization (WGL) is the standard of care for non-palpable breast cancer and is performed under local anaesthesia (LA) by a radiologist using ultrasound (US) - in greater than 95% of cases - 85 or mammographic guidance when lesions are not visible on US. After insertion of the tip of the wire into the lesion, a position confirmation mammogram is performed in 2 planes (craniocaudal (CC) and mediolateral (MLO)) to help the surgeon visually orientate the wire relative to the lesion.
In the operating theatre, the surgeon assesses where the tip of the wire lies - using the post-insertion mammograms - before placing the incision to remove the wire and associated lesion.  

Despite being the current standard of surgical care for non-palpable breast cancer, WGL suffers from significant drawbacks. WGL is performed on the day of surgery - to avoid the risk of wire migration – negatively impacting upon theatre scheduling through the requirement of radiology support on the day of surgery.  Complications related to wire insertion include cases of wires becoming dislodged, transected, migrating from the site of original placement and rarely thoracic injuries. Technical issues arising intra-operatively include potential needle stick injuries, diathermy burns, limitations in incision placement - by the way the wire is inserted - with adverse impact upon cosmetic outcome and high re-operation rates for incomplete tumour excision - as high as 50% with WGL surgery. This has led to the development of alternative localization techniques in the surgical management of breast cancer.

1.6 Aims

The aim of this thesis was to commence with evaluating the outcomes of ‘established alternative’ localization techniques against the gold standard of wire-guided localization - in the treatment of non-palpable breast cancer. The was to be followed by an assessment of the outcomes for the current, clinically available, novel techniques for sentinel lymph node biopsy and also the different sites of injection of the standard technique (radioactive tracer ± blue dye) in breast cancer. Consequently, progressing from the evidence accrued to develop a new innovative
Localization procedure, which superseded the limitations of existing techniques. This innovative technique was to be formally evaluated and optimised within phantom and pre-clinical models before proceeding to confirming proof of principle in the clinical setting on breast cancer patients within a clinical trial.

1.7 Originality of work

I performed all systematic reviews incorporated within this thesis with statistical assistance from Review Manager 5.0 (Cochrane Collaborative) for the performance of meta-analyses. All pre-clinical work using phantom models was performed by myself at the Research Oncology Laboratories at King’s College London. Dr RTM de Rosales at the Division of Imaging Sciences and Biomedical Engineering (King’s College London) performed radiolabelling of the magnetic tracer and nano-SPECT-CT on phantom models. The in vivo porcine model was developed by myself, with assistance from Mr Bauke Anninga (Research Oncology, King’s College London) at the IRCAD Institute (Strasbourg, France) and all animal experimentation was performed after ethical permission was granted from the IRCAD ethics review board. The surgical procedures performed at the IRCAD Institute, data collection and analysis, were performed by myself with assistance from Mr Bauke Anninga. Statistical assistance for analysis of porcine data was attained from Dr Mieke van Hemelrijick (Department of Epidemiology, King’s College London). The performance of quantitative magnetometry of ex vivo sentinel lymph nodes was performed at the University of Enschede, Netherlands by Miss Suzan Vreeman and Mr Joost Pouw (MIRA Institute for Biomedical Technology and Technical Medicine, Enschede, Netherlands). Histopathological preparation of these nodes was performed at the Department of Research Oncology and grading of nodes by Professor Sarah Pinder (Research Oncology, King’s College London). The protocol
for the MagSNOLL trial was developed and written by myself under the supervision of Mr Michael Douek (Research Oncology, King's College London). I prepared and presented the MagSNOLL ethical application to the regional ethics committee (City Road and Hampstead; 13/LO/0636) on May 8th 2013. I coordinated the trial and performed data collection of all cases performed at Guy’s Hospital and analysed data from all involved sites.
2. Incorporated publications
3. Conclusions

3.1 Alternative localization techniques to wire-guided localization

This thesis has demonstrated that radioguided localization (RGL) and intra-operative ultrasound (IOUS) are both feasible and oncologically safe alternative techniques to wire-guided localization (WGL). The techniques have also demonstrated benefits over WGL in the surgical management of non-palpable breast cancers. Despite this, the prevention of general uptake of these techniques has been the result of inherent methodological limitations of the trials conducted so far as well as technical issues associated with the procedures themselves.

Intra-operative ultrasound benefits over both RGL and WGL by eliminating the need for further radiology input pre-operatively (after lesion characterization has been performed by a radiologist at initial diagnosis) to introduce a wire or radioisotopes, making this a surgeon independently driven procedure. This consequently means that issues of theatre scheduling with radiology on the day of surgery are eliminated - a distinct benefit over any other localization technique. Over 95% of wire-placements for non-palpable cancer are sonographically visible lesions but IOUS-guided surgery can also be performed in patients with sonographically invisible lesions (microcalcifications or cancers with a complete response to primary systemic therapies) where an ultrasound visible marker is placed at the time of the diagnostic core biopsy. In comparison to IOUS, RGL and WGL are simply additional procedures performed by the radiology department with negative economic implications. The cost of IOUS would be the initial expenditure upon a portable ultrasound device and potential maintenance only.
IOUS also possesses the benefit of allowing *ex vivo* assessment of specimen margins, which has been demonstrated to reduce re-excision rates and volumes of excised specimens compared to RGL and WGL. 95

However, the major limitation to the widespread implementation of intra-operative ultrasound has been the inability of surgeons to acquire formal training and accreditation in breast ultrasound. 96 The successful application of intra-operative ultrasound is dependent upon a combination of technique and experience to be able to utilise ultrasound during tumour excision. Such skills can only be acquired by supervised training in the operating theatre with a senior breast radiologist (supervising the first 10 cases). 97 Training can also be improved by performing IOUS-guided surgery for palpable lesions, where it has also been shown to be superior to palpation guided surgery, 96 to get used to the direct vision in the operating theatre. It is important that surgeons perform ultrasound assessment of the patient in the outpatient clinic and verify before scheduling surgery that the lesion is clearly visible on ultrasound, thus eliminating the risk of missing the lesion intra-operatively. Within this time frame surgeons are able, to easily become familiarised with the technique, rapidly mastering basic ultrasound skills and demonstrate proficiency in performing intra-operative ultrasound. This suggests that although the learning curve is short, it is also dependent upon a close working relationship with the radiology department, for their initial input within this learning phase. The greater prior experience of surgeons in the use of ultrasound, the faster their ability to become proficient during the learning phase, 97 adding support to surgeons performing outpatient-based ultrasound to enhance intra-operative ultrasound proficiency. 96 In the United States, the American Society of Breast Surgeons offers breast ultrasound certification, to surgeons who
meet the criteria in clinical experience, training and quality assurance in breast ultrasound. The American College of Radiology has also agreed to allow certification in breast ultrasound by the American Society of Breast Surgeons as one of the qualification options for physicians performing breast ultrasound in American College of Radiology accredited facilities - confirming the importance and increased use of ultrasound-guided procedures performed by breast surgeons. However, within the United Kingdom, although breast ultrasound courses requiring logbook verification and formal assessment of procedures do exist, the ability of surgeons to perform ultrasound is at the discretion of individual radiology departments within NHS trusts. Consequently, the ability of surgeons to perform ultrasound is exclusively dependent upon the support that they receive from their radiology colleagues - which can be variable. Until this limitation in breast surgeons becoming trained in ultrasound is addressed, the use of intra-operative ultrasound will not provide a future option for surgical management of non-palpable breast cancer despite its benefits over current localization-guided surgical techniques.

Radioguided localization consists of 2 techniques, (radioguided occult lesion localization (ROLL) and radioactive seed localization (RSL)) which differ from each other and can pose potential advantages and disadvantages during lesion localization. Both techniques are dependent upon the presence of a skilled radiologist to perform either ultrasound or stereotactically guided localization via administration of between 0.2-0.5mL of $^{99m}$Tc radiolabelled albumin-based colloid in ROLL and a single 8 x 0.4 mm titanium seed radiolabelled with $^{125}$I in RSL. Whilst ROLL requires localization to be performed within 24 hours of surgery due to the short 6-hour half-life of $^{99m}$Tc labelled colloid, RSL typically is performed 0-5
days prior to surgery \(^{99-103}\) and in the primary systemic therapy (PST) setting several months beforehand because the half-life of \(^{125}\)I is 60 days. \(^{104}\) This flexibility in the timing of seed insertion provides a clear advantage over ROLL and WGL by preventing scheduling conflicts between the radiology department and operating theatres and on the day of surgery – easing resource management. \(^{105}\)

Reported complications of localization techniques are minimal suggesting that these techniques are extremely safe. Concern has been raised at the issue of migration and subsequent loss of inserted seeds, but clinically relevant seed migration is rare and has been reported in less than 1% of RSL procedures. \(^{106}\) The issue of migration is similarly rare but also an issue using WGL. \(^{87, 89}\) In the 8 published studies \(^{99-104, 107}\) on RSL, 2 studies \(^{102, 108}\) have failed to demonstrate 100% successful intra-operative localization for RSL, with failure rates of 2% \(^{108}\) and 7.9%. \(^{102}\) The largest published series of RSL with 978 patients recorded a 100% successful localization rate. \(^{103}\) However, this remains an important issue requiring the establishment of a defined protocol to allow the safe handling of the seeds from intake in the nuclear medicine department, through sterilization, implantation, excision and removal. Errors at any point along this chain can convert a controlled procedure to a potentially dangerous one and result in temporary closure of RSL programmes until resolved. \(^{108}\) Twenty-nine studies have been published on ROLL \(^{109-137}\) and 9 of these studies \(^{111-114, 119, 122, 129, 136, 137}\) failed to achieve a 100% intra-operative localization rate, with failure rates being recorded between 0.4% \(^{122}\) and 4.5%, \(^{119}\) with the figure of 0.4% being from the largest published series of ROLL with 959 patients. \(^{122}\)
A potential benefit of RSL is that $^{125}$I emits 27Kev gamma rays compared to $^{99}$Tc, which emits 140KeV. This means that by altering the sensitivity on the gamma probe it is possible to differentiate between radioactive emissions from the localised lesion and the sentinel lymph nodes irrespective of the site of the primary lesion, although the benefit of this has not been confirmed in RCTs. The range for successful identification of the sentinel lymph node for RSL is between 96.8% $^{100}$ and 100% $^{99,101}$ whereas the same figure for ROLL is between 84.9% $^{116}$ and 100% $^{110,119,127}$. This would suggest a trend toward favouring RSL for successful SLNB over ROLL. In a similar fashion the administration of different radioisotopes for lesion localization and SLNB could allow for varied energy threshold settings on the probe to differentiate between radioactive emissions from the lesion itself or sentinel lymph nodes in ROLL. The possibility of using 2 radioisotopes such as $^{99m}$Tc and $^{111}$I could potentially improve surgical outcomes although it has only been applied successfully in vitro $^{138}$ and in upper limb drainage studies $^{139}$ and not been clinically applied to ROLL.

Due to financial strains in world healthcare systems it is very important to consider the economic impact of these 2 techniques. However, to date no detailed health economic assessment has been performed for RSL. The large cohort study by McGhan et al. $^{103}$ involving greater than 1000 patients demonstrated that the material costs for performing RSL was one-third of the cost for WGL (US$60 versus US$170). This is largely due to the large series of patients undergoing RSL at the institution, which can consequently order seeds in large bulk reducing costs, but the additional time required for the nuclear medicine department to process, sterilise and package the seeds is not taken into account in this calculation. This
would mean that in units performing smaller case numbers, the savings on material costs would not be as significant and costs between RSL and WGL would be similar. Postma et al. in their comprehensive cost-benefit analysis of their randomised controlled trial (RCT) of ROLL versus WGL found that there was no economic difference between ROLL and WGL when considered overall in terms of costs associated with morbidity and re-operation rates. Interestingly, they found that ROLL cost €0 in terms of localization because it allowed radioisotope injection for sentinel lymph node biopsy (SLNB) to be performed concurrently with localization as opposed to WGL which required an additional procedure to localization. In this respect ROLL would favour over RSL, which also requires a separate radioisotope injection by the nuclear medicine department in order to perform SLNB.

The issue of a learning curve associated with ROLL and RSL has never been formally assessed within the published evidence. However, the largest 2 cohort series for RSL and ROLL, each comprising nearly 1000 patients did not suggest the presence of a significant learning curve. This suggests that both techniques are intuitive and the necessary skills easily acquired. Most importantly, the resemblance of RSL and ROLL to the performance of SLNB, which is standard practice means that breast surgeons who have never performed either technique formally will still be familiar with the principles and possess the necessary skills to learn both techniques rapidly. Certainly, studies comparing RSL and ROLL to WGL have suggested that surgeons favoured the radioguided techniques over WGL for its ease of performance. This ease of performance and similarity to SLNB allows surgeons to commence performing the procedure without the need for extended training unlike intra-operative ultrasound.
The only study in the literature to compare ROLL against RSL found no significant
difference in clinical outcomes between the 2 techniques. The study by Donker et
al. 141 consisted of 154 patients (RSL; n=71, ROLL; n=83) and compared the 2
techniques in the context of patients undergoing breast-conserving surgery after
primary systemic therapy (PST). Whilst there was no significant difference in
clinical outcomes, there was a clear logistical benefit to 125I seed localization. The
125I seed was, able to be introduced radiologically into the tumour before
commencing PST and the patient did not require any further radiological
intervention. This is in contrast to the ROLL group, which required the insertion of
a radiopaque marker before commencing PST and then another radiological
procedure within 24 hours of scheduled surgery to have the injection of
radiolabelled nanocolloid. Although the economical implications of this were not
explored in the article in view of no differences in morbidity between the 2
techniques being identified, a reduction in radiology suite attendance would
inevitably lead to financial benefits favouring RSL. An issue not addressed for this
study was the issue of SLNB. All patients underwent SLNB prior to commencing
PST and therefore did not undergo repeat SLNB on completion of PST. This meant
that at the time of breast conserving surgery being performed the administration
of 99mTc for SLNB was not necessary. In the case of the ROLL group technically
SLNB could have been performed after the intra-tumoral injection of 99mTc for
lesion localization, but in the case of RSL an additional procedure would have been
necessary for 99mTc injection. Therefore, caution must be cast as the benefit of one
less radiology attendance in RSL may only be confined to PST patients and not the
more general treatment of the 35% of breast cancer patients 80 who present with
non-palpable lesions and require axillary staging in the form of SLNB at the time of
breast-conserving surgery.
However, ultimately the utilisation of radioguided localization is limited by its radioisotope dependency. This has always been the case with SLNB and due to radioisotope dependence SLNB is only available to 60% of patients in the developed world 142 and almost negligible uptake in the rest of the world. 143 Consequently, the uptake of these radioguided localization techniques has remained consistently limited to a relatively, small number of centres. This is likely due to these centres having the infrastructure established to perform these procedures on a large scale. In the absence of such infrastructure being present the case to change from the wire with the present evidence is not conclusive. The legislative and logistical issues associated with radioactive handling and disposal, are likely to be contributory to this – but inevitable with radioisotope dependency. Therefore, whilst radioguided localization possesses both technical and outcome related benefits, the issue of radioisotope dependency is likely to continue to prevent this technique, becoming mainstream in patient management.

3.2 Magnetic technique for lesion localization

This thesis has demonstrated that the magnetic localization technique is feasible for lesion localization in phantom models 144 but can also perform concurrent sentinel lymph node biopsy both in in vivo models 145 and clinically. The magnetic intra-tumoral localization procedure is straightforward and performed within the radiology suite using ultrasound-guidance. It does not require any additional precautions or safety measures - as with radioguided localization - because of its independence of radioisotopes. It does not leave any external remnant from the skin – as in the case of wire-guided localization – and therefore is not at risk of unwanted migration. The injection is minimally invasive and since proof of
principle has been demonstrated, the use of a marker coil and confinement to ultrasound visible lesions as safety measures can in future studies be removed. Therefore, in comparison to wire-guided localization and radioguided localization, the magnetic localization procedure performed within the radiology suite is advantageous.

Intra-operative localization is simplified compared to wire-guidance as there is no need to use 2 dimensional mammographic images to identify the likely position of the wire tip. Instead, the peak magnetometer count directs the operating surgeon directly to the lesion and allows them to appropriately correlate this with placement of the most appropriate skin incision. Such accuracy of incision placement would also be able to be achieved with radioguided localization and a gamma probe. Once the specimen containing the peak magnetometer counts was excised, intra-operative mammographic imaging was performed to confirm the presence of the marker coil, but this was only for safety in the proof of principle trial. This specimen required no additional safety measures for handling (inert nature of the magnetic tracer) as would be required for radioguided localization in terms of radiation handling for theatre and pathology staff. This is a distinct advantage of the magnetic technique over radioguided localization. Adequate margin determination using the magnetic technique is still limited and dependent upon excision of the area with peak magnetometer count and correlation of the size of extension dependent upon the size of the lesion on preoperative imaging. Currently, residual cavity counts have to be interpreted within this context. By reducing the volume of magnetic tracer used for localization, the cavity counts can be decreased and a more focal localization achieved. However, clinical and radiographical correlation is still required to assess what is a satisfactory margin
of excision. In this area intra-operative ultrasound would still possess a distinct advantage in terms of ability to visually assess through measurement of \textit{ex vivo} margins. \textsuperscript{146,147}

### 3.2.1 \textit{Concurrent sentinel lymph node biopsy}

A benefit of the magnetic technique was the ability of a single intra-tumoral injection to perform concurrent sentinel lymph node biopsy. This was based upon the \textit{in vivo} porcine model \textsuperscript{145} and the sentinel node and occult lesion localization (SNOLL) technique using radioisotopes. \textsuperscript{148} It was identified that the magnetic technique was a promising, novel radioisotope-independent procedure for sentinel lymph node biopsy \textsuperscript{149} and that there were no significant differences in sentinel node identification rates between deep and superficial injections of radioisotope and blue dye. \textsuperscript{150} The magnetic technique was found be inferior to the standard \textquoteleft{dual technique}\textquoteleft of radioisotope and blue dye for sentinel node identification. When the magnetic technique was combined with blue dye the identification rate was equal to the standard \textquoteleft{dual technique}\textquoteleft. This suggests that the additional periareolar injection of radioisotope or blue dye assists axillary staging compared to an intra-tumoral injection alone. This suggests a distinct difference in the outcomes of deep injections using a peri-tumoral injection \textsuperscript{150} exclusively for sentinel lymph node biopsy – as all of the included studies in the meta-analysis were – compared to an intra-tumoral injection used for additional localization. Additionally our \textit{in vivo} porcine model was not a tumour model and consequently all injections were performed periareolarly. This would also support why sentinel node identification rates were high using this model when the injection sites were excised. \textsuperscript{145}
Optimising the sentinel node identification rate using a single intra-tumoral injection would be desirable, but likely difficult to achieve within the established constraints of the intra-tumoral injection. Higher sentinel node magnetometer counts were identified in patients injected the day before surgery and therefore extending the time between localization and surgery is a potential approach. However, within our *in vivo* porcine model the clinical benefit from magnetometer counts was not significant beyond 30 minutes of injection. Again this was performed through periarolar injections in the porcine model and therefore its direct translatability to an intra-tumoral injection may be limited. Reducing the volume of magnetic tracer below 0.5 mL to achieve greater focal localization and increasing the delay of surgery from injection would be ideal. No statistically significant difference in sentinel node magnetometer counts and iron content was identified in greater volumes of magnetic tracer injected within the porcine model – making this feasible. This was performed in 20 patients who underwent a 0.1 mL intra-tumoral injection the day before surgery, but the SLN was successfully identified in 16 patients using the magnetic technique (82% SLN identification rate). This lower identification rate suggests that the only way to optimise the technique is to uncouple it and perform a ‘dual magnetic injection’, with one for localization and an additional one for sentinel node identification. The performance of a periareolar injection of magnetic tracer for sentinel lymph node biopsy has already been demonstrated to be non-inferior to the standard ‘dual technique’ of radioisotope and blue dye within the SentiMAG multicentre trial. This would be comparable to a dual-injection SNOLL procedure, as used at the European Institute of Oncology in Milan who founded the technique. This was performed successfully in 5 patients without interference in the localization procedure and successful sentinel node identification in all patients. Whilst this
requires further assessment within a larger cohort it does support a technique, which allows both magnetic lesion localization and sentinel node biopsy to be successfully performed.

3.3 Future work

The first priority of any future work should be to demonstrate the ability of the magnetic localization technique to reduce the re-excision (second operation) rate in non-palpable breast cancer from the current UK levels of 20% \(^{153}\) to 10% or less, using a suitable sample size to identify this difference with a narrow margin of error (95% CI; 5% margin of error). Once this study has been performed and pending findings, it would allow an accurate calculation of sample size to plan for a randomised controlled trial of the magnetic technique compared to wire-guided localization. This could allow for the magnetic technique to enter mainstream clinical practice.

An assessment of the ability of the magnetic tracer to retain ‘peak magnetometer’ counts at the site of injection should be explored. The findings of this would allow the possibility of a situation whereby patients recalled from the screening programme with a suspicious lesion requiring core-biopsy could concurrently undergo injection of the magnetic tracer. Once histological confirmation of malignancy is received, the patient may proceed directly to theatre, eradicating the need for an additional unnecessary localization procedure. In the event the histology excludes malignancy, there is no concern as the magnetic tracer is inert and broken down by the reticuloendothelial system without detriment to the patient.
Magnetic resonance imaging (MRI) is not used as a standard pre and post-operative assessment tool for breast imaging within the United Kingdom. However, the use of MRI varies throughout Europe and the rest of the world. The magnetic tracer - due to its constituents – will inevitably cause an artefact when visualised with MRI – although the extent of this has not been assessed in the literature. Using the intra-tumoral injection that we devised has the benefit that the area of artefact will likely be near completely excised during surgery. However, an additional peri-areolar injection of the magnetic tracer would not be excised and likely leave a large remnant artefact - interfering in post-operative MRI assessment. This limitation has to be taken into consideration and potential techniques to resolve this issue such as reducing injection volumes to absolute minimal levels and modification of standard MRI sequences to remove artefact must be identified as research priorities in this area of technology. The MRI artefact would be a significant limitation to the progression of using the magnetic tracer as a biopsy marker. However, this could be overcome in an initial trial by anticipating those patients at high risk of requiring an MRI and excluding this small number from the trial. This would be necessary until further research is able to minimise MRI associated artefacts. At the same time, SPIOs are also recognized as contrast agents for MRI and in this context, may prove useful for imaging sentinel nodes in these patients and avoiding surgery to the axilla.\textsuperscript{154}

Magnetic hyperthermia is a promising field in which magnetic nanoparticles are exposed to an alternating magnetic field and generate heat due to magnetic hysteresis loss. If cancer tissue is in contact with magnetic nanoparticles this can be therapeutic.\textsuperscript{155} The localising properties of the magnetic tracer through an
intra-tumoral injection could consequently, potentially be exploited as a means of a minimally invasive therapeutic intervention using magnetic hyperthermia. Further focal localization may be possible via conjugation of antibodies to the magnetic nanoparticles for targeted magnetic hyperthermia.\textsuperscript{156, 157}
4. Additional publications


5. Presentations


3) **M Ahmed**, M Douek. Validating the ’10 per cent Rule’ for magnetic sentinel lymph node biopsy in breast cancer. ESSO34 – BASO14 meeting, October 28th-31st 2014, Liverpool, UK.


8) **M Ahmed, M Douek:** A systematic review and meta-analysis of ultrasound-guided versus wire-guided localization in the treatment of non-palpable breast cancers. BASO, 4-5th November 2013, RCSEng, poster and oral presentation (shortlisted for Alan Edwards prize).

9) **M Ahmed, M Van Hemelrijck, M Douek:** A systematic review and meta-analysis of radio-guided versus wire-guided localization in the treatment of non-palpable breast cancers. BASO, 4-5th November 2013, RCSEng, poster and oral presentation (shortlisted for Alan Edwards prize).


11) **M Ahmed, B Anninga, M Van Hemelrijck, J Pouw, D Westbroek, S Pinder, B ten Haken, Q Pankhurst, M Douek**: Magnetic Sentinel Lymph Node Biopsy and
Localization Properties of a Magnetic Tracer in an *in vivo* Porcine Model. King’s College London PhD Symposium, best poster presentation winner, 27\textsuperscript{th} June 2013.

6. Prizes and awards


3) European Society of Surgical Oncology EYSAC Research Fellowship 2014-15 to attend the Graduate School of Engineering at the University of Tokyo, 1st March – 2nd April 2015.

4) Association of Breast Surgeon's, London Symposium, 1st prize for best oral presentation, 20th June 2014.

5) King's College London PhD Symposium, best poster presentation winner, 27th June 2013.
7. References


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