

# **Contrast-enhanced Imaging in the Biological and Functional Assessment of Breast Cancer**

**Thesis Submitted for the Degree of Doctor of Medicine (MD)  
of the University of London**



**2000**

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# Abstract

Contrast-enhanced MRI and ultrasound have emerged as additional imaging modalities in the management of breast cancer. This thesis examines the role these modalities currently play in the surgical management of breast cancer. Ways in which MRI may contribute to staging, diagnosis, treatment and prognosis are investigated.

It was demonstrated that small additional enhancing foci on MRI, away from the primary tumour, represent *in-situ* or invasive cancer foci. Although their resection may result in extended wide local excisions or even unnecessary mastectomies, it was demonstrated that MRI findings do not currently influence the amount of tissue removed during breast conservation surgery. Volumetric analysis of breast MRI was proposed as an accurate objective assessment of the extent of surgery required for a particular tumour. Breast MRI was shown to be useful in the assessment of extent of residual disease during primary medical therapy but not in the detection of axillary lymph node metastases.

In the second section of this thesis, the clinical application of pre-operative MRI in providing prognostic as well as diagnostic information was evaluated. Contrast-enhancement with both MRI and ultrasound is believed to depend on tumour angiogenesis but only a weak correlation was demonstrated between contrast-enhancement intensity and tumour angiogenesis. The detection of angiogenesis was applied to Doppler ultrasound using a novel microbubble ultrasound contrast agent (*Levovist*). Within a multicentre prospective study, Doppler ultrasound was shown to be a powerful discriminator of malignancy in suspected local recurrence. A strong correlation was found between MRI and histological assessment of tumour size but there was no correlation between enhancement intensity and other pathological prognostic variables.

This thesis has shown that breast MRI is useful in pre-operative planning of surgery and provides diagnostic as well as limited prognostic information. Future proposed studies to determine the effect of MRI on patient management and patient outcome in breast cancer are considered.

## Acknowledgements

First of all, I would like to express my gratitude to my supervisors Mr Tim Davidson and Professor Irving Taylor to whom I am greatly indebted. They provided me with an opportunity to do research, constantly supported and encouraged me, and critically discussed my results throughout this MD project. I would also like to thank Professor Michael Baum and Dr Margaret Hall-Craggs who provided me with guidance, precious expert advice and were always available when needed. In addition, Dr Sunil Lakhani and Dr Francesco Pezzella contributed significant time and gave valuable help on the pathological aspects of the project. My sincere thanks to other surgical colleagues especially Mr Mark Kissin and Miss Christobel Saunders, who supported the clinical projects in this thesis by allowing their patients to participate. I also thank Professor Bill Lees who supported the ultrasound projects.

Other members of staff assisted me in carrying out the research projects, particularly my friends and colleagues who collaborated with me on several projects and contributed to making my time at UCL a very rewarding experience. They include: Mr Jay Vaidya, Mr Jonathan Winehouse, Dr Rob Stein, Dr Andrea Schneidau, Dr Marilena Loizidou, Dr Ian Wilkinson, Dr Sean Smart, Dr Mike O'Hare, Dr Elizabeth Benjamin, Dr Tony Leatham, Elly Harrison, Jane Stumcke, Ciara McNulty, Ann Titcomb and Abdul Gafur. I also wish to thank Dr Linda Stevens and Professor Steven Senn at the Department of Epidemiology of UCL for advice with the statistical components of this thesis. Special thanks to Dr Dave Plummer, the 'computer wizard' who assisted me on many occasions and adjusted some of his software to my specifications. My sincere thanks to Maria Maloney, Julia Ternan, Diane Duncan, Sangita Naranbhai, Mathew Cunningham and other members of staff at the MRI unit of the Middlesex Hospital, who expedited willingly all requests for research scans.

Last but not least, I would like to express my gratitude to my mother and brothers for supporting me in every possible way during my research and throughout my career.

# Statement of Originality

This thesis was supervised by Mr Tim Davidson (Consultant Surgeon) and Professor Irving Taylor (Professor of Surgery and Head of Department). The clinical studies presented in this thesis were designed by me. Ethics Committee approval was obtained from both the UCL Ethics Committee and that at the Royal Surrey County Hospital in Guilford. All patients were recruited by myself from Breast Clinics at UCL and associated Hospitals as well as the Royal Surrey County Hospital, and gave informed consent. MRI protocols were planned by Dr M A Hall-Craggs (Consultant Radiologist) and myself. I was present during all MRI scans, decided on the protocol required for each patient and administered intravenous contrast agents. I assisted during surgery to patients in the study but breast surgery was performed by Professor Michael Baum, Mr Tim Davidson and Professor Irving Taylor.

Operative specimens were collected by myself and taken to the Department of Histopathology where Dr Sunil Lakhani (Consultant Breast Pathologist) was responsible for processing the specimens. Immunohistochemistry was undertaken both by myself and by the Immunohistochemistry Department at UCL. All microvessel density counts were undertaken by myself with validation by Dr Francesco Pezzella (Consultant Pathologist). Transfer of MRI images to an assessment terminal, assessment of MRI images, quantification of contrast-enhancement and correlation between imaging and histopathology, was undertaken by myself using in-house computer software developed at UCL.

Work on the radiological-histopathological correlation of MRI in the study of breast cancer multicentricity and the resulting thoughts on the management of patients with enhancing foci on MRI, are novel. Concepts regarding volumetric analysis of the breast in an attempt to objectively stage patients are original. Work on the possible use of MRI in neoadjuvant chemotherapy and prognostication has been studied in more depth in this thesis resulting in a proposed classification of breast cancer based on enhancement patterns. Work in this thesis on correlation of angiogenesis with imaging modalities, has resulted in several collaborations in multicentre trials on novel contrast-agents for MRI and ultrasound.

*In memory of my father, Dr Eliahou Douek*

*To my mother, Lisa Coen-Douek*

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# Nomenclature

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AC.....	Adriamycin and cyclophosphamide
ANC .....	Axillary node clearance
CEA .....	Carcinoembryonic antigen
CD31 .....	Endothelial marker anti-CD31 or JC70
CD34.....	Endothelial marker anti-CD34 or QBEND/10
CR.....	Complete response
CT .....	Computed tomography
2D .....	Two-dimensional
3D .....	Three-dimensional
DCIS .....	Ductal carcinoma <i>in situ</i>
ECF .....	Epirubicin, cisplatin and 5FU
ER.....	Oestrogen receptor status
FA .....	Flip angle
FLASH.....	Fast low angle shot
FNA.....	Fine needle aspiration cytology
FOV .....	Field of view
FSPGR .....	Fast radio frequency spoiled gradient echo
FVIIIrAg .....	Endothelial marker, anti-Factor VIII related antigen
Gd-DTPA .....	Gadoliniumdiethylenetriaminepentaacetic acid
Gy.....	Gray
H&E .....	Haematoxylin and eosin
IDC .....	Invasive ductal carcinoma
iv .....	Intravenous
LCIS.....	Lobular carcinoma <i>in situ</i>
MBq .....	Millibecquerel
MRI.....	Magnetic resonance imaging
MVD .....	Microvessel density
PET .....	Positron emission tomography
PMT .....	Primary medical therapy
PR .....	Progesterone receptor status

RODEO..... Rotating delivery of excitation off resonance  
ROI ..... Region of interest  
SE..... Spin echo  
SPET ..... Single photon emission tomography  
STIR..... Short-tau inversion recovery  
T ..... Tesla  
TA..... Total acquisition time  
MIBI..... Methoxyisobutylisonitrile  
TE ..... Echo time  
TI ..... Inversion time  
TR..... Repetition time  
TOPIC..... Trial of Primary Infusional Chemotherapy  
UICC..... Union international contre le cancer  
US ..... Ultrasound  
VG..... Vascularity grade  
WLE..... Wide local excision

# Aims and Structure of Thesis

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This thesis set out to:

1. Determine the current place of MRI and US in the surgical management of breast cancer.
2. Consider possible ways in which MRI could be useful in surgical management.
3. Evaluate the role of MRI and US in the pre-operative biological assessment of breast cancer.

In the first section, a literature review summarises the current knowledge on breast MRI, US and the functional assessment of breast cancer. In the second section, several projects were undertaken to evaluate MRI as a diagnostic tool and consider its role in the pre-operative assessment of breast cancer. In the third section, MRI was assessed as a prognostic tool and contrast-enhanced Doppler ultrasound was used to assess the application of functional tumour information, in a clinical setting.

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## **Section A: Introduction**

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**Chapter 1: Investigation of Breast Cancer**

**Chapter 2: Breast MRI**

**Chapter 3: Breast Ultrasound**

**Chapter 4: Prognostic Assessment of Breast Cancer**

# Chapter 1: Investigation of Breast Cancer

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- 1.1 Introduction
- 1.2 Primary Breast Cancer
  - 1.2.1 Screening
  - 1.2.2 Diagnosis
  - 1.2.3 Pre-operative staging of local disease extent
  - 1.2.4 Pre-operative staging of the axilla
  - 1.2.5 Monitoring patients undergoing primary medical therapy
- 1.3 Recurrent Disease and Follow-up
- 1.4 Advanced Breast Cancer
- 1.5 Summary

## 1.1 Introduction

Recent advances in radiological imaging have produced new tools for the diagnosis, pre-operative staging and evaluation of distant disease in patients with breast cancer. Exciting developments include imaging techniques for the evaluation of biological characteristics of breast cancer pre-operatively and in response to primary medical therapy (PMT). However, since rapid technological expansion has been associated with rising costs, all new imaging modalities should be adequately evaluated to define clinically useful and cost-effective indications prior to their integration into accepted clinical practice. The impact of each imaging modality on surgical and medical management should be considered but ultimately all imaging modalities should be assessed in terms of their impact on patient survival and quality of life.

So far, randomised studies to determine the impact of novel imaging modalities on patient outcome or surgical management, are lacking. Most studies define accuracy data for a particular patient sub-group or compare one modality against another. However, patient pre-selection is often a problem since it influences accuracy data and results may not then be applied to a different patient population. Some of these modalities have been shown to have a high sensitivity for breast cancer detection, but their low specificity may trigger further investigations or even lead to over-treatment. Comparison of published studies is also hindered by variations in techniques (table 1.1). Appropriately powered comparative studies and randomised studies to identify the optimal modality or protocol to use for a particular clinical problem, are therefore essential.

It is evident that availability, optimal accuracy and cost-effectiveness, as well as clinical usefulness guide the ideal combination of new imaging modalities for an individual patient. The imaging *armamentarium* available for the breast and axilla (table 1.2) is reviewed here from a surgical perspective. The various imaging modalities available at different stages of the disease and future developments are considered, and their place in management discussed.

**Table 1.1: Summary of efficacy data from comparative studies of breast imaging modalities:**

MRI vs scintimammography (a); MRI vs x-ray mammography (b);  
scintimammography vs x-ray mammography (c).

(a)

Study	No of patients	No of cancers	MRI				Scintimammography				Scan details and comments
			Sens (%)	Spec (%)	PPV (%)	NPV (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	
Tiling R et al. (1998)	44(44)	24(9)	64	94	-	-	63	85	-	-	Mammographic microcalcifications; <sup>99</sup> Tc <sup>m</sup> -sestamibi 740MBq; 1.5 T non-dynamic 3D FLASH contrast-enhanced MRI; both poor for detecting microcalcification
Tiling et al. (1997a)	82 (82)	29(10)	56	79	61	76	62	83	67	80	Indeterminate mammograms; <sup>99</sup> Tc <sup>m</sup> -sestamibi 740MBq; 1.5T non-dynamic 3D FLASH contrast-enhanced MRI
Tiling et al. (1997b)	56 (56)	33(2)	91	53	-	-	88	83	-	-	Suspicious of cancer (prospective); <sup>99</sup> Tc <sup>m</sup> -sestamibi 740MBq; 1.5T 3D FLASH contrast-enhanced MRI.
Fenlon et al. (1997)	44 (44)	21(0)	90	91	90	91	95	91	91	95	Palpable breast lumps (prospective); 600MBq <sup>99</sup> Tc <sup>m</sup> -tetrofosmin; 1.5T dynamic axial 3D FLASH contrast-enhanced MRI with 3.75mm slices
Sommer et al. (1997)	56	?	91	53	-	-	88	87	-	-	Suspicious of cancer; <sup>99</sup> Tc <sup>m</sup> -sestamibi; MRI ? type but non dynamic (paper in German)
Helbich et al. (1997)	66 (75)	26 (1)	96	82	74	98	83 (62)	80 (88)	67 (73)	91 (81)	Clinically or mammographic suspected malignancy; SPET vs planar; 740 MBq <sup>99</sup> Tc <sup>m</sup> -sestamibi; 1.5 T dynamic axial 3D FLASH contrast-enhanced MRI with 5mm slices; significant difference in sensitivity between MRI and planar scintimammography
Palmedo et al. (1996)	56 (56)	27(0)	93	21	52	75	85	66	70	83	Palpable lesions suspicious for cancer (prospective); planar and SPET Tc-99 MIBI with 740 MBq; 1.5 T dynamic axial 3D gradient-echo contrast-enhanced MRI with 5mm slices



(b)

Study (year)	No. of patients (lesions)	No. of cancers (DCIS)	MRI				Mammography				Scan details and comments
			Sens (%)	Spec (%)	PPV (%)	NPV (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	
Kacl et al. (1998)	50 (63)	?	92	76	-	-	82	64	-	-	Diagnosis of malignancy; dynamic 3D gradient-echo contrast-enhanced MRI; no significant difference observed
Drew et al. (1998)	105 (105)	9(0)	100	93	56	100	67	85	30	96	Screening for local recurrence; 1.5T dynamic FSPGR contrast-enhanced MRI with 4mm slices
Muller-Schimpfle et al. (1997)	89 (98)	59 (5)	95	64	80	89	83	92	94	78	Patients who had a breast lesion biopsied (retrospective); MRI vs mammography and US; 1.0 T dynamic axial 3D FLASH contrast-enhanced MRI with 4mm slices
Fenlon et al. (1997)	44 (44)	21(0)	90	91	90	91	81	82	85	78	Palpable breast lump (prospective); 1.5T dynamic axial 3D FLASH contrast-enhanced MRI with 3.75mm slices
Bone et al. (1997)	220 (238)	145 (14)	92	72	84	86	89	72	83	81	Consecutive pre-operative (prospective); axial 1.5T 3D FLASH (2 post scans) with 2mm slice thickness
Mumtaz et al. (1997a)	30 (30)	14 (14)	93	88	-	-	50	75	-	-	Suspected local recurrence; (prospective); non-dynamic 3D FLASH contrast-enhanced MRI with 5mm slices
Palmedo et al. (1996)	56 (56)	27(0)	93	21	52	75	89	14	49	57	Clinical/mammographic suspicion of malignancy, prior to surgical biopsy (prospective); 1.5 T non-dynamic 3D gradient echo contrast-enhanced MRI with 5mm slices
Fobben et al. (1995)	89(91)	21 (5)	82.5	82.5	54.2	-	74.6	79.1	48.5	-	Breast abnormalities; 3D dynamic gradient-echo contrast-enhanced MRI; difference not statistically significant
Harms et al. (1993)	288 (74)	44 (7)	94	37	-	-	89	89	-	-	High clinical or mammographic or US suspicion of cancer; RODEO MRI vs conventional imaging; 1.5 T non-dynamic unenhanced MRI with 1.4mm slices

(c)

Study	No of patients	No of cancers	Scintimammography				Mammography				Scan details and comments
			(year)	(lesions)	(DCIS)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Sens (%)	
Palmedo et al. (1998)	246 (253)		71	69			91	42			Suspicious breast mass or positive mammogram; prospective multicentric; 165 cancers; blind read; MIBI) scintimammography
Cwikla et al. (1998)	70 (74)	53 (5)	89	52	84	67	70	57	80	43	Suspicious breast lesions (prospective); 740 MBq <sup>99</sup> Tc <sup>m</sup> -sestamibi; difference not statistically significant
Fenlon et al. (1998)	44 (44)	21(0)	95.2	91.3	90.9	95.5	81	82.4	85	77.8	Palpable suspicious breast lesions (prospective); <sup>99</sup> Tc <sup>m</sup> -tetrofosmin 600MBq
Fenlon et al. (1997)	40 (40)	21(0)	95	91	91	95	81	82	85	78	Palpable breast lumps (prospective); <sup>99</sup> Tc <sup>m</sup> -tetrofosmin 600MBq
Clifford et al. (1996)	147 (147)	43(?)	84	95	88	93	86	95	88	94	Clinical or mammographic or US suspicion of cancer (prospective); 20mCi <sup>99</sup> Tc <sup>m</sup> -sestamibi

\* RODEO- rotating delivery of excitation off resonance; FLASH- fast low angle shot; T- Tesla; FSPGR- fast radio frequency spoiled gradient echo; SPET- single photon emission computer tomography; <sup>99</sup>Tc<sup>m</sup> MIBI- technetium 99 methoxyisobutylisonitrile scintimammography.

**Table 1.2: Summary of imaging modalities available for investigating patients with breast cancer: (? = future developments)**

<b>Stage of breast cancer</b>	<b>Imaging modality</b>	<b>Indications</b>
<b>Screening</b>	mammography	women 50-64y
	?MRI	women with high genetic risk
<b>Diagnosis</b>	?? <sup>99m</sup> Tc-sestamibi	} ? alternatives to mammography
	??PET	
	mammography	as part of triple assessment of breast lumps
	ultrasound	women under 40y of age
	ultrasound guided biopsy	mammographically dense breasts
	stereotactic breast biopsy	biopsy of radiologically suspicious microcalcifications
	mammotome	excision of small areas of microcalcification
	MRI	? excision of small breast tumours
		cases with equivocal results; women with breast prostheses; women who cannot tolerate mammography
		equivocal or negative mammograms
<b>Pre-operative staging of local disease extent</b>	scintimammography	
	mammography	screening for synchronous cancers / DCIS
	ultrasound	inaccurate in assessing size
	?MRI	assessing size of central or superficial masses
<b>Pre-operative staging of axilla</b>		assessing tumour size, tumour local extent, multicentricity
		nipple involvement
	Sentinel lymph node sampling by lymphoscintigraphy	reliably predicts axillary lymph node involvement
	?MRI	imaging axillary lymph nodes;
<b>Monitoring neoadjuvant chemotherapy</b>	ultrasound	better than clinical examination alone, but not reliable
	?MRI	assessing response
	mammography	} inaccurate in determining response
ultrasound		
<b>Recurrent disease</b>	?PET	under evaluation
	scintimammography	
<b>Advanced disease</b>	MRI	high sensitivity >6 months post-op
	mammography	} low sensitivity
	ultrasound	
	bone scan	} locally advanced breast cancer or symptomatic
	skeletal survey	
	ultrasound	} detect liver involvement
	isotope scans	
	CT scan	
MRI	detect bone metastasis in equivocal cases	
	chest x-ray / CT scan	} detect pulmonary metastasis
PET		

## 1.2 Primary Breast Cancer

### 1.2.1 Screening

The mainstay for breast cancer screening is mammography, which in the UK is performed at 3 yearly intervals in the 50-64 age group. The benefits of screening mammography first emerged in the Health Insurance Plan Study of New York, which showed a 30% reduction in mortality over 5 years follow-up in 62,000 women (Strax et al. 1973). Subsequent screening trials have shown similar benefits in mortality rates (Anderson et al. 1988; Tabar et al. 1992). However, recent results from the Canadian National Breast Screening Study (CNBSS) (Narod, 1997) have not shown a reduction in mortality. In women aged 40-69 years, Elmore et al. (1998) estimated the cumulative risk of a false positive result to be 49.1%, following an average of 10 mammograms over 10 years. Screening results in substantial numbers of unnecessary benign breast biopsies as well as outpatients appointments, hospital admissions and triggers a battery of additional investigations. In the UK, despite the introduction of screening mammography in 1990, the incidence of interval cancer still approaches 80% of the background incidence of breast cancer and reduction in screening intervals or possibly even a re-think of the whole concept of screening may be necessary (Baum, 1997).

The recent introduction of more expensive techniques such as digital mammography and computer aided diagnosis, although very promising, may increase running costs substantially and tip the balance of cost-effectiveness against screening, even further. However, these techniques may improve problems associated with screening dense breasts and abolish the need for x-ray filing. Debate continues on the optimal age at which screening should begin, interval of screening, number of mammographic views required (single *versus* double reporting) and their cost-effectiveness.

Other imaging modalities have been considered for screening high-risk groups. The possible role of magnetic resonance imaging (MRI) in the detection of breast cancer in women at high genetic risk is currently being evaluated in a multicentre trial (Leech et al. 1998). 500 women will be recruited and screened by MRI and mammography over 5 years. Interestingly, recent *in vitro* work suggests that cell-lines from women with increased risk of breast cancer are more susceptible to x-ray irradiation damage and it may yet transpire that in these women x-ray-induced cancer is a real risk (Vaidya and

Baum, 1997). Screening by MRI is free of ionising radiation and MRI is more sensitive for breast cancer detection than x-ray mammography.

Breast scintimammography using  $^{99m}\text{Tc}$ -sestamibi and  $^{99m}\text{Tc}$ -tetrofosmin, and Tc-99 MIBI scintimammography, have very high overall sensitivity and specificity for breast cancer detection but are not sufficiently sensitive for tumours under 1 cm compared to those over 1 cm (50% vs 96.9%) (Scopinaro et al. 1998). Ultrasonography is not currently indicated for screening due to its limited spatial resolution (Potterton et al. 1994) and its insensitivity for microcalcifications (Leucht et al. 1992). Population screening with ultrasound, would lead to an unacceptable rate of false positive and negative findings (Teh and Wilson, 1998).

### ***1.2.2 Diagnosis***

The main diagnostic protocol for symptomatic breast cancer remains triple assessment consisting of clinical examination, mammography (or ultrasonography in the dense breast) and fine-needle aspiration cytology (FNA). This approach provides a predictive value for benign disease of 99% when all 3 modalities are negative (Layfield et al. 1989). The sensitivity of mammography for breast cancer detection is between 61%-87% (Fentiman, 1990) and is lower in women less than 50 years of age compared with those over 50. This is probably due to the increased radiodensity of the breast in premenopausal women (Jackson et al. 1993), and as a result, mammography is not recommended in patients under 30 (Williams et al. 1986). In the latter, ultrasonography is a useful imaging modality for determining the size and consistency of breast lesions. Differentiation of benign from malignant lesions depends on careful analysis of sonographic features and is operator dependent. Advances in colour Doppler sonography and contrast enhancement may lead to improved sensitivity and specificity for breast cancer detection. However, recent findings suggest that spectral analysis does not contribute significantly to differentiation between malignant and benign lesions (Buadu et al. 1997a), and is discussed in further detail in Chapter 3. This is offset by the ability to obtain an ultrasound guided biopsy of clinically suspicious lesions.

Developments in stereotactic guided breast biopsy techniques, allow the supplementation of mammographic findings with tissue diagnosis thus reducing the rate of unnecessary diagnostic surgical excision biopsies. Suitable lesions, such as isolated

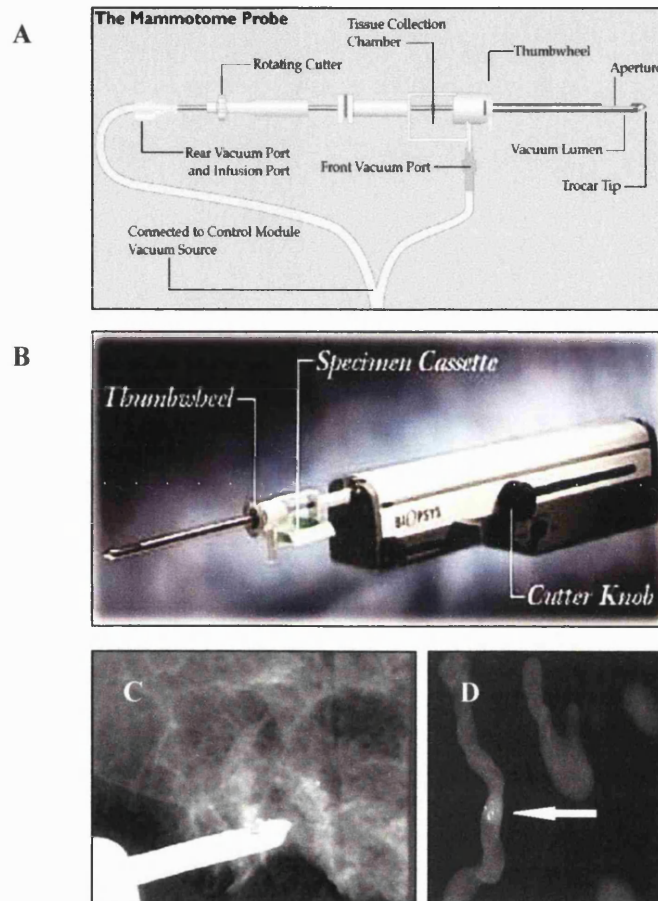
microcalcifications, may be removed by using a Tru-cut type breast biopsy device (*Mammotest* breast biopsy system, Fischer Imaging, USA) which is directed into the lesion using stereotactic mammography with computer generated co-ordinates (Head et al. 1996). A recent development is the *Mammotome* (Biopsys Medical Inc., USA), a device which allows the stereotactic removal of serial cores with the use of suction to draw tissue towards the needle bevel (figure 1.1). This enables larger amounts of breast tissue to be removed, without appreciable blood loss, and in future may become a single step diagnostic and therapeutic device.

New imaging modalities are useful to supplement conventional triple assessment in patients with equivocal results. Magnetic resonance imaging (MRI) provides the highest spatial resolution for the detection of breast cancer by greatly enhancing lesion conspicuity. Sensitivity (56%-100%) and specificity (21-91%) vary depending on the technique used (table 1.1), guidelines for interpretation of scans and patient selection (Heywang-Kobrunner et al. 1997). The highest sensitivities have been obtained using fast image acquisition (Boetes et al. 1994) and dynamic contrast-enhancement.

Comparative studies have demonstrated higher sensitivities of MRI for breast cancer detection when compared to ultrasonography or mammography. However, none of these studies demonstrated a statistically significant difference (table 1.1 a and b). Scintimammography has been demonstrated to have both high sensitivities and specificities for breast cancer detection (table 1.1 a), but only one study has shown a statistically significant difference in efficacy between MRI and scintimammography (Helbich et al. 1997). The comparative studies to date vary in patient selection, are often unblinded, relatively small and do not have the necessary statistical power to establish superiority of one or other modality. There is also a danger of over-diagnosing ductal carcinoma in situ (DCIS) which is detected in significant numbers (table 1.1 a, b and c). It is recognised that not all cases of DCIS develop into invasive disease. Thus, its over-diagnosis may cause unnecessary alarm and may lead to over-treatment. It is therefore important to make a clear distinction between invasive and non-invasive disease when determining efficacy of novel imaging modalities and this has been omitted in some studies.

**Figure 1.1: The Mammotome:**

A breast core biopsy device which enables the removal of serial core biopsies using a minimally-invasive technique. Suction draws tissue into the aperture and removes any bleeding (A). Specimens are removed from the specimen cassette and the procedure is repeated at a different angle by twisting the thumbwheel (B). Microcalcifications are localised stereotactically and removed (C). A specimen radiograph (arrow) confirms that the target has been removed (D).



\* Images supplied by Biopsys Medical Inc., 3 Morgan Irvine, CA 92618, USA.

Invasive lobular carcinoma remains a recognised diagnostic challenge for both FNA (Ciatto et al. 1993) and mammography (Hilleren et al. 1991). The best method of diagnosing these lesions remains clinical suspicion followed by biopsy.

**1.2.3 Pre-operative staging of local disease extent**

The efficacy of mammography in the primary staging of symptomatic breast cancer is unclear with problems arising mainly in the pre-menopausal breast (Kopans, 1992).

Histopathologic correlation has shown that mammography is inaccurate in assessing tumour size (Yorkshire Breast Cancer Group, 1980) and has low detection rates (Pain et al. 1992). However, mammography is useful preoperatively in screening for synchronous ipsilateral and contralateral occult cancers (Dixon and Chetty, 1991).

The ability of MRI to accurately assess tumour size, local tumour extent, nipple involvement and the axilla, makes it an important secondary imaging tool in disease staging (Mumtaz et al. 1997b). MRI is superior to mammography and ultrasound in assessing tumour size (Davis et al. 1996). Pre-operative MRI provides important information when selecting patients for breast conserving surgery but its impact on the extent of surgical resection is not known. Current limitations include detection of additional enhancing foci, away from the primary lesion, which may not be detected by any other imaging modality and which may or may not be of any significance. The only way of determining their nature would be to biopsy them using an interventional MRI, which provides direct access to the patient. However, since the spatial resolution of such scanners is lower than that of conventional scanners, these lesions may not be visible.

Ultrasound is useful in determining the size of palpable masses not visible by mammography or not completely evaluable by mammography (due to their location, such as small peripheral masses in very thin women). It is also useful in the young patient with dense breasts (Bassett et al. 1991) but not in determining multifocality or detecting intraductal disease (Boetes et al. 1995). Accuracy of ultrasound is reduced in women with very large breasts and these patients remain a challenge for most other imaging modalities.

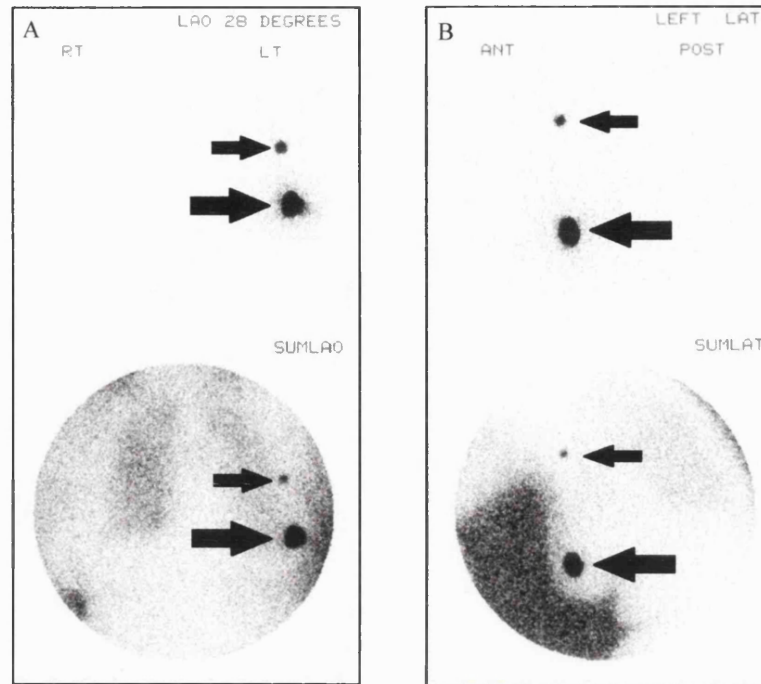
#### ***1.2.4 Pre-operative staging of the axilla***

Together with tumour size, axillary nodal status is the most important prognostic factor in breast cancer and helps determine the need for adjuvant chemotherapy. However, up to 50% of patients undergo unnecessary axillary surgery (since they are found to have negative nodes), so the risk of associated morbidity may be unacceptable. The recent emergence of sentinel node biopsy may lead to a reduction in the number of unnecessary axillary operations by reliably predicting axillary nodal involvement from the histological status of the sentinel node. When nodes are removed selectively by axillary node sampling up to 42% of axillary metastases are missed (Moffat et al. 1992).



**Figure 1.2: Sentinel node localisation using  $^{99m}\text{Tc}$  labelled albumin lymphoscintigraphy:**

Twenty minutes after a subcutaneous injection of  $^{99m}\text{Tc}$  labelled albumin (large arrow), a sentinel node was identified (small arrow) in the lower axilla. (A- antero-posterior view; B- left lateral view)



\* We thank Prof. P Ell and Mr M Keshkar for supplying these images.

Sentinel node biopsy (figure 1.2) relies on the principle that lymphatic spread of tumour cells is not a random process but a stepwise progression in which cancer cells first spread to a sentinel node and subsequently to second-order loco-regional nodes. This has been demonstrated by histopathologic correlational studies (Turner et al. 1997). By injecting blue dye intradermally (Borgstein et al. 1997) into the skin overlying the tumour following intra-mammary injection of  $^{99m}\text{Tc}$ -labelled albumin and then dissecting the sentinel node, the skin overlying the tumour has been shown to share a common lymphatic drainage with the underlying parenchyma. In a series of 163 consecutive women using  $^{99m}\text{Tc}$ -labelled albumin lymphoscintigraphy (Veronesi et al. 1997), the sentinel node accurately predicted axillary nodal involvement in 97.5% of cases and this figure rose to 100% in tumours less than 1.5 cm. Similar results were obtained with the injection of peri-tumoural isosulphan blue dye (Giuliano et al. 1997; Papa et al. 1997). These techniques are untested in patients who may have a disrupted lymphatic drainage such as patients who have had previous ipsilateral breast surgery or open biopsy, previous radiotherapy or patients with multifocal disease. A further technical problem is

the necessary intra-operative frozen section to determine tumour involvement of the sentinel node, which may prolong surgical time.

Other imaging modalities include MRI, which in one study, was shown to have a sensitivity of 90% and a specificity of 82% for axillary nodal metastasis in symptomatic breast disease (Mumtaz et al. 1997b). However, MRI does not provide histological information. Attempts to detect metastatic spread by ultrasound have been generally unsuccessful (Noguchi et al. 1996).

#### ***1.2.5 Monitoring patients undergoing primary medical therapy***

Primary medical therapy is administered to downstage the tumour and enable breast conservation surgery. It is recognised as the treatment of choice for advanced breast cancer. MRI has been shown to be useful for assessing the extent of residual disease on completion of treatment (Mumtaz et al. 1996b; Abraham et al. 1996). Abraham et al. (1996) have shown that breast MRI may accurately predict pathologically proven residual disease in 97% of patients, and that MRI is superior to both clinical examination and mammography. Ultrasound is not as accurate as MRI in assessing tumour size in such patients (Kurtz et al. 1996). Prospective comparative studies are needed in order to confirm or refute this evidence.

### 1.3 Recurrent Disease and Follow-Up

Local recurrence is a poor prognostic indicator in breast cancer (Kurtz et al. 1989). Following breast conservation surgery and radiotherapy, local scarring often results in positive clinical and mammographic findings (Sickles and Herzog, 1984) which may ultimately require open breast biopsy to exclude malignancy. However, since post-operative healing may be delayed in these patients due to previous radiotherapy, unnecessary open biopsy should be avoided. In this situation the addition of an alternative imaging modality such as MRI may reduce the benign biopsy rate without missing cases of true recurrence (Turkat et al. 1994; Muuller et al. 1998).

Magnetic resonance imaging (MRI), the best available imaging modality for the detection of breast cancer recurrence, should preferably be performed prior to interference by core-biopsy since this may affect image quality. MRI has a sensitivity of between 93-100% (Davis et al. 1996; Davis and McCarty, Jr., 1997; Mumtaz et al. 1997a) with a specificity of 88% (Mumtaz et al. 1997a). It is superior to mammography in the detection of recurrence in patients with silicone implants (Heinig et al. 1997) and has a higher sensitivity compared to triple assessment (Mumtaz et al. 1997a). However, patients within 6 months of surgery or radiotherapy have a high false positive rate. This specific clinical indication was assessed in this thesis (chapter 12).

The development of new contrast-agents for Doppler-ultrasound (Kedar et al. 1996) may enable the complementary use of ultrasound in cases of suspected recurrence, although image spatial resolution is still problematic.

### 1.4 Advanced Breast Cancer

Screening for distant metastasis is indicated in patients presenting with locally advanced disease or in whom the suspicion of metastatic spread is raised by symptoms or biochemical abnormalities (*e.g.*: raised alkaline phosphatase). There is no place for bone scans to screen patients with suspected T1 or T2 disease since the pick-up rate is so low (Yeh et al. 1995).

The diagnosis of advanced breast cancer is performed by staging the patient clinically and radiologically with both loco-regional and systemic imaging techniques. Liver and bone metastases may be screened by Doppler ultrasound, isotope scans or CT-scanning. Magnetic resonance imaging may be used in equivocal cases to increase the specificity of abnormal scans. Pulmonary metastases should initially be evaluated by chest X-ray but CT scanning may be used in equivocal cases. CT and MRI are the most effective methods for evaluating CNS disease (Dershaw and Osborne, 1989). The management of non-operable breast cancer is beyond the scope of this thesis and will not be discussed further.

## **1.5 Summary**

Recent advances in breast imaging have improved the diagnosis, staging, and detection of distant spread in breast cancer. The increasing number of available imaging modalities, require co-ordinating research efforts to determine the best methods and clinically useful indications. Initial studies should be followed by adequately powered comparative studies to determine which imaging modality is superior for a specific clinical indication, but ultimately randomised controlled studies are needed to look at the impact of each imaging modality on clinically important end-points.

# Chapter 2: Breast MRI

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- 2.1 Introduction
- 2.2 Contrast Agents for Breast MRI
- 2.3 Scanning Techniques
  - 2.3.1 High resolution imaging
  - 2.3.2 Dynamic imaging
  - 2.3.3 Image subtraction
  - 2.3.4 Other scanning techniques
  - 2.3.5 Other post-image processing techniques
- 2.4 Potential Clinical Indications for Breast MRI
  - 2.4.1 Diagnosis of primary breast cancer
  - 2.4.2 Diagnosis of DCIS
  - 2.4.3 Detection of local recurrence
  - 2.4.4 Pre-operative staging of local disease extent
  - 2.4.5 Monitoring patients undergoing primary medical therapy
  - 2.4.6 Imaging breast prostheses
  - 2.4.7 Other indications
- 2.5 Interventional Breast MRI
- 2.6 Summary

## 2.1 Introduction

When MRI was first applied to the breast in the mid 1970's (Medina et al. 1975), it was hoped that application of its high spatial resolution to breast diagnosis, would result in a useful clinical tool. However, scepticism soon prevailed since not only was it shown that there was some overlap between appearances of benign and malignant lesions (Mansfield et al. 1979), but also scanning costs were high and scanning times too long. Since then, major technological developments have led to a reduction in scanning time. The introduction of specific breast coils (Wolfman et al. 1985; Stelling et al. 1985) together with the introduction of more powerful magnetic fields, improved spatial resolution even further. A major advance was the introduction of gadolinium-chelate contrast agents (Heywang et al. 1986a; Heywang et al. 1986b), which dramatically improved sensitivity for breast cancer detection. Since then, further work has concentrated both on improving MRI equipment and developing new contrast agents to improve accuracy in breast cancer detection. Contrast agents for MRI and scanning techniques will be considered in this chapter.

Since histological correlational studies (Heywang et al. 1987; Powell and Stelling, 1988) have demonstrated a close agreement between MRI and histological appearance of breast tumours, clinical indications were sought for the new technology. However, a specific clinical problem should trigger a search for a tool to assist in its interpretation and not *vice versa*. The sheer speed of technological advance and excitement surrounding it, spiked interest in its possible applications even before specific clinical difficulties could properly be considered. Some clinical and radiological challenges in surgical management of breast cancer are listed in table 2.1 and potential qualities required of breast MRI in table 2.2.

Research work to date has provided answers to some clinical and radiological challenges (although often not in order of clinical priority) but other challenges have not even been addressed. Furthermore, some research findings have created clinical dilemmas with respect to what surgical action should be taken with some MRI findings. Potential clinical indications for breast MRI will be considered in this chapter. The possible role of MRI in providing prognostic as well as diagnostic information, will be considered in chapter 4.

**Table 2.1: Clinical and radiological challenges in the surgical management of breast cancer:**

- *Difficult cases when index of suspicion for cancer is high but other modalities have failed to reliably confirm or refute the diagnosis of malignancy: a useful imaging modality would detect small, clinically significant lesions or obviate the need for invasive tissue diagnosis of larger lesions*
- *Breast cancer diagnosis in dense breasts (young women and women on HRT) or women with fibrocystic breast disease*
- *Pre-operative acquisition of prognostic information on tumour biology (eg: lymph node status, tumour aggressiveness etc.)*
- *Detection of clinically significant multifocal or multicentric lesions in patients with primary breast cancer*
- *Differentiation of breast recurrence from post-operative scarring and post-radiation change, obviating the need for biopsy of true negatives*
- *Assessing response to primary medical therapy*
- *Determining nipple involvement by tumour when considering nipple preservation*
- *Breast cancer diagnosis and detection of leaks in patients with breast prostheses*

**Table 2.2: Potential qualities required of breast MRI, which would be of assistance in surgical practice:**

- *Aid to diagnosis - of equivocal cases or cases poorly imaged by other techniques*
- *Aid to surgical management - when considering optimal surgical procedure (mastectomy or wide local excision) or extent of surgical resection needed*
- *Aid to assessing response to primary medical therapy - assist in determining optimal timing of surgical intervention or need for alteration in cytotoxic regimen*
- *Aid to prognostic assessment – provide information on tumour biology to assist in the selection of optimal treatment modalities prior to surgical intervention*

## 2.2 Contrast Agents for Breast MRI

As the spatial resolution of breast MRI improved, it became apparent that there was a need to develop contrast agents which could selectively alter tissue relaxation times and thus improve the detection of pathology. Ideally, a para-magnetic contrast agent should be tissue-specific, should improve the detection of breast cancer and enable the investigation of physiological characteristics of both benign and malignant tumours (Orang-Khadivi et al. 1994). Mechanism of contrast agent action, include uptake by the reticuloendothelial system, distribution via the intravascular or interstitial spaces, or a combination of these. Several agents have been tested in clinical studies including olive oil, oestrogens, alcohol and glycerine (Beall, 1984). However the first paramagnetic contrast agent to gain approval for clinical use, and the most widely used, is gadolinium diethylenetriaminepentaacetic acid. The active ingredient, gadolinium ion ( $Gd^{3+}$ ), is chelated with DTPA to reduce its osmolality and toxicity. In the most common formulation, *Magnavist* (Schering, Germany), Gd-DTPA is bound with dimeglumine to form gadopentate dimeglumine. It is injected intravenously at a dose of 0.1 – 0.2 mmol/kg, a tenth of the dose used with iodinated non-ionic x-ray contrast agents. Side effects such as allergic reactions, have been observed in under 1% of cases, much less frequently than with iodinated non-ionic contrast agents (Heywang-Kobrunner and Beck, 1996). Following bolus injection, *Magnavist* is distributed both in the intravascular and interstitial spaces (Strich et al. 1985), and eliminated unchanged by glomerular filtration. Enhancement, at the relatively low dose used for breast imaging, is predominantly due to shortening of the T1 relaxation time and therefore T1-weighted sequences are commonly used to detect enhancement.

Other contrast agents that are undergoing clinical evaluation could potentially be applied to the breast. Macromolecular contrast agents are predominantly distributed in the intravascular space but leak out into the interstitial space when capillary permeability is pathologically increased. MS-325 (AngioMARK, EPIX Medical Inc., USA) is a gadolinium based blood pool contrast agent that is 80-96% albumin bound (Lauffer et al. 1998). As opposed to Gd-DTPA, it remains in the intravascular compartment. In *ex vivo* experiments (Lauffer et al. 1998), MS-325 has been shown to exhibit a relaxivity 6 – 10 times higher than that of Gd-DTPA. It has been used successfully in MR angiography (Grist et al. 1998) and may soon be applied to the breast in an attempt to improve the



specificity of breast MRI in the differentiation of malignant from benign tumours.

Attempts have also been made to image tumour lymph node drainage. Ultrasmall superparamagnetic iron oxide particles, such as AMI-227 (*Combidex*, Advanced Magnetics Inc., USA), have been used to image pelvic lymph nodes in patients with pelvic carcinoma (Bellin et al. 1998). Phase III trials on imaging breast lymphatics were completed in October 1998, but results have not yet been published in the medical literature.

### **2.3 Scanning Techniques**

Modern high-field strength superconducting magnets used in MRI, are doughnut-shaped with field strength of 1-2T (Tesla). The patient usually lies prone on a table, in order to minimise breast motion caused by respiratory movements. The breasts are placed in a specially shaped breast coil, which usually does not compress the breasts, although some authors have used breast compression to immobilise the breast and thus further reduce motion artefact (Heywang-Kobrunner et al. 1994). Since breast coils take up 10-20 cm of the diameter of the scanner, very large patients cannot be scanned. Furthermore, the size of cups within the breast coil limits the maximum breast size that can be scanned. An intravenous cannula is inserted into a cubital or dorsal vein, prior to scanning, and connected to a long line in order to administer intravenous contrast while the patient is in the scanner. The rate of contrast injection is proportional to the fourth power of the radius of a cannula, and in order to standardise the procedure, all equipment used should be the same for every patient. Contrast injectors for MRI are sometimes used to accurately control rate of infusion for dynamic MRI. However, injectors are expensive, and as a result, not widely available.

Numerous factors need to be considered when deciding on a specific scanning technique for breast imaging (Orel et al. 1996; Heywang-Kobrunner et al. 1997) including temporal and spatial resolution required, volume of breast to be covered, choice of magnetic resonance pulse sequence, imaging plane and the reduction of artefacts due to cardiac, respiratory and patient motion. Selection is predominantly influenced by the personal preference of the radiologist but also by published recommendations for specific

clinical situations as well as hardware and software availability. Since peak signal intensity of over 50% of fast enhancing malignancies occurs 1-3 minutes after contrast injection, an imaging time of at least 3 minutes is required to obtain sufficient information on spatial distribution of enhancement and to detect very small lesions (Fischer et al. 1993). In order to scan the breast for diagnostic purposes, it is of course important to run a sequence that covers the entire breast volume. If images with a very high spatial resolution are acquired, using thin contiguous slices, then image acquisition time will be prolonged and thus it may not be possible to obtain temporal information as well.

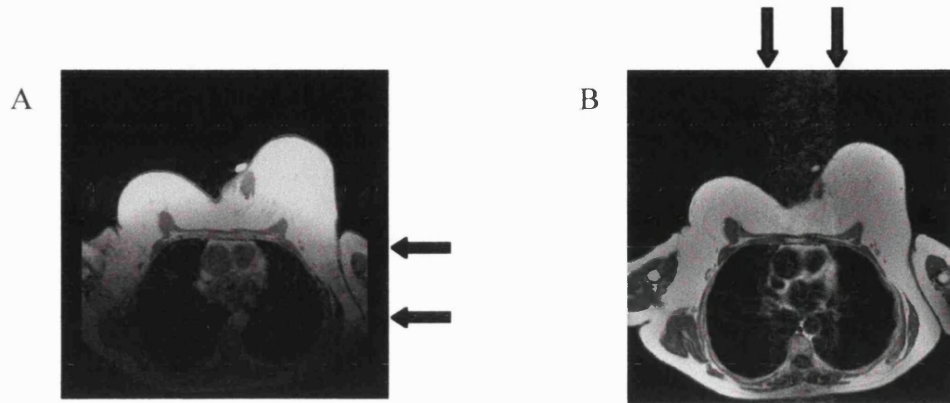
The best-suited sequences for breast diagnosis, are 3D spoiled fast gradient echo sequences (Heywang-Kobrunner and Beck, 1996). These relatively rapid sequences are very sensitive to gadolinium contrast enhancement and provide a signal intensity for fat lower than that of most breast cancers, thus facilitating the visualisation of enhancing tumours on post-contrast scans. Usually, imaging parameters are chosen so that a good T1 weighting is obtained, at the lowest possible imaging time (TA-total acquisition time; TE-echo time; TR-repetition time). Other imaging parameters (FA-flip angle; FOV-field of view) are fine-tuned by individual radiologists to provide optimal image quality.

The breast may be imaged in the transverse, coronal, sagittal, or oblique plane. The transverse (axial) plane is favoured at The Middlesex Hospital. It provides cross-sectional images similar to thoracic and abdominal CT scans and thus readily recognised by surgeons. The spatial position of breast lesions in relation to pectoral muscle, skin and nipple can easily be determined. A drawback of this technique is phase-artefact caused by misregistration of cardiac motion (figure 2.1). This band-like area, parallel to the x or y-axis, distorts signals from the axillae (in the x-axis) or the medial breast quadrants (in the y-axis). If scanning is performed in the coronal or sagittal planes, phase artefact can be positioned parallel to the spine in the z-axis thus avoiding the breast and axillae altogether. However, surgeons are less familiar with the coronal and sagittal planes and it is more difficult to relate these to a patient lying on the operating table. Throughout this thesis, scanning was limited to the transverse plane.

The most commonly used scanning techniques are high resolution and dynamic imaging. These and other imaging techniques will be considered in this section.

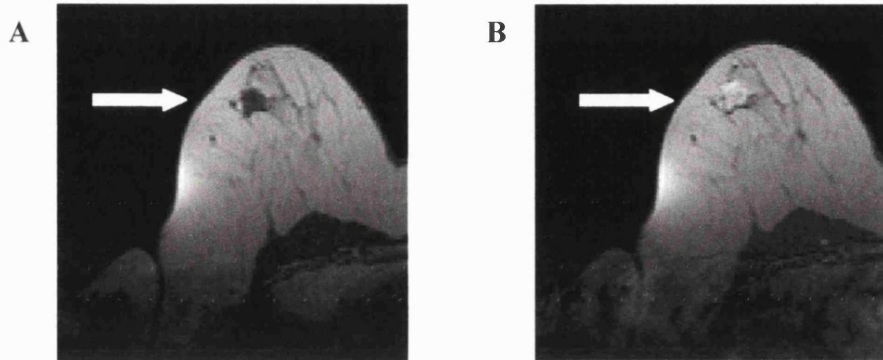
**Figure 2.1: Phase-artefact in transverse T1-weighted breast MRI:**

Cardiac motion is the most important motion artefact other than patient movement. Misregistration of signal from the moving heart and blood in heart chambers, causes artefact parallel to the phase encoding direction of the magnet. This artefact enhances following contrast injection, severely impairing the evaluation of any structures it crosses. In the transverse plane is used to image the breast, the phase-artefact (arrows) may be aligned with the x-axis to visualise both breasts (A) or with the y-axis (B) when visualising the axillae.

**2.3.1 High resolution imaging**

In high resolution contrast-enhanced MRI, two scans are carried out, one before and one after contrast injection. Image data sets are acquired and averaged out over the scanning time period (usually 1 – 5 minutes) to produce cross sectional images at the desired slice thickness. The thinner the slice thickness and the larger the field of view, the longer the scanning time. By comparing images acquired before contrast injection, with those after, any enhancement is noted and its significance assessed. High resolution imaging, provides high spatial resolution but does not provide information on rate of enhancement. It is recognised to be better suited for clinical settings that require high anatomical definition such as detecting small breast lesions (Morris et al. 1997) or imaging suspected prosthesis leaks (Orel, 1996; Harms, 1998). Three dimensional fast low angle shot (3D FLASH), a type of gradient echo sequence, can provide high spatial resolution and some temporal resolution since image acquisition time can be as low as 45seconds (Heywang-Kobrunner and Beck, 1996). On most modern equipment, in-plane resolution of 1mm and slice thickness of less than 2mm can be achieved in reasonable scanning time. Three dimensional sequences allow multiplanar reconstructions of volume data to produce both transverse and coronal projections of the breast. As a result, 3D FLASH is the sequence favoured at The Middlesex Hospital.

**Figure 2.2:** Pre-contrast (A) and post-contrast (B) MRI of a breast cancer (arrow) in a fatty breast.



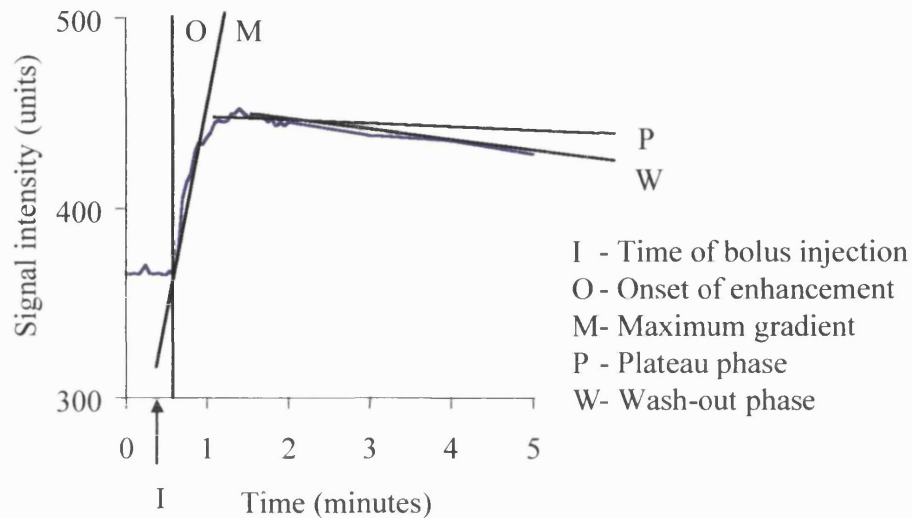
On pre-contrast T1-weighted images, all carcinomas display low signal intensity and are virtually indistinguishable from surrounding glandular tissue whereas fat has a high signal intensity. In women with very fatty breasts, breast tumours may be discernible even from the pre-contrast scan (figure 2.2). On post-contrast T1-weighted images, architectural features highly suspicious of malignancy include a spiculated margin with lesion enhancement, particularly peripheral enhancement (Nunes et al. 1997; Orel et al. 1994). Features highly suggestive of a fibroadenoma include smooth or lobulated borders, absent or reduced lesion enhancement (Nunes et al. 1997) and internal septations (Hochman et al. 1997). Compared with dynamic imaging, high resolution imaging does appear to have a lower specificity for breast cancer detection and as a result, some centres favour dynamic MRI (Piccoli, 1997).

### 2.3.2 Dynamic imaging

With dynamic contrast-enhanced MRI, information on temporal pattern of signal intensity is obtained by acquiring several sets of images after contrast-enhancement. Rapid 3D image acquisition sequences, such as 3D TurboFLASH, performed using newer scanners allow complete breast coverage in 45 –60 seconds (Kacl et al. 1998; Sherif et al. 1997; Heiberg et al. 1996) and this sequence can be repeated for several minutes after contrast injection. These newer sequences appear to offer the optimal scanning approach; a good compromise between the need for high spatial resolution (1-2 mm) and provision of temporal information on rate of enhancement.

**Figure 2.3: Contrast-enhancement profile of a breast cancer during dynamic MRI**

Signal intensity in a ROI within the tumour (y-axis) is plotted over time (x-axis). Scans were repeated every 3 seconds, until 2 minutes, and then every minute until 5 minutes. Fifteen seconds after intravenous bolus injection (I), signal intensity begins to rise (O). The maximum gradient of the curve (M), represents the rate of contrast uptake by the tumour. Between 1-2 minutes a plateau phase (P) is established, during which equilibrium has been reached between tissue contrast uptake and loss. Thereafter, during the wash-out phase (W), signal intensity is gradually lost into the venous and lymphatic circulations.



However, tumours have been shown to enhance rapidly in the first minute, and in order to study the onset and gradient of contrast enhancement, a more rapid image acquisition is required. This is usually performed by selecting slices from the pre-contrast scan. Two dimensional gradient echo or echo planar sequences (Hulka et al. 1997; Boetes et al. 1994) are then used to scan pre-selected slices at intervals of 2-6 seconds. Thus, breast volume coverage is compromised in order to obtain temporal information on signal intensity, in selected slices. To quantify enhancement over time, signal intensity can be measured in the entire tumour or in regions of interests (ROI) and plotted as a curve of signal intensity over time (figure 2.3). This enables the analysis of enhancement characteristics including rate of contrast-enhancement and washout from the lesion. Once data has been acquired for 1-2 minutes following injection of contrast, 3D gradient echo sequences such as 3D FLASH, can be used to provide full breast coverage and high spatial resolution.

Dynamic MRI has been shown to provide higher specificity for breast cancer characterisation. In a study of 2053 patients (203 cancers) Kaiser et al. (1994), showed that using rate of enhancement for breast cancer diagnosis gave a sensitivity of 98.1%

and specificity of 97.4%. However, methodologies and results from other smaller studies vary as illustrated in table 2.3. Although sensitivity for breast cancer detection is often high, in some studies specificity is low, due to overlap in time intensity curves between benign and malignant lesions.

Different parameters, derived from the signal intensity curve, have been used for the diagnosis of malignancy. Malignant tumours tend to enhance rapidly (within 20-40 seconds of injection), centripetally, predominantly peripherally (Kacl et al. 1998; Gilles et al. 1996; Boetes et al. 1994) and retain contrast for longer compared with benign lesions (Sherif et al. 1997). Scherif et al. (1997) used onset of peripheral contrast washout after 10 minutes to diagnose malignancy and achieved a specificity of 100% (table 2.3). Others have used pixel-pixel analysis by entering acquired data into complex linear mathematical models (Knopp et al. 1995; Hoffmann et al. 1995). Enhancement signal time curves are then analysed using amplitude, contrast distribution rate constant ( $k_{21}$ ) and contrast elimination rate constant ( $k_{el}$ ), but this type of analysis is time consuming. At present these techniques are research tools and it is not yet clear how useful they may be, in a clinical context.

Dynamic breast MRI with 3D gradient echo sequences, appears to be the way forwards in terms of improving specificity while at the same time retaining the high sensitivity achieved by static high-resolution MRI. However, compromising spatial resolution in favour of temporal resolution leads to some drawbacks. Firstly, selection of a tumour slice from the pre-contrast scan may lead to a non-representative assessment of the lesion (sampling error) or even missing the lesion entirely. Secondly, when rapid 3D sequences are used to scan the entire breast, a compromise is reached between thicker slices to obtain complete breast coverage and thinner slices which provide higher spatial resolution. This may lead to missing small breast lesions and it is therefore recommended that slice thickness should not exceed 3-4mm (Heywang-Kobrunner and Beck, 1996). Thirdly, tumours enhance in a heterogeneous fashion and any analysis based on regions of interest (ROI), will be prone to sampling error since measurements may not represent the entire lesion and this may result in a reduction of diagnostic accuracy. Quantitation of rate of enhancement is time consuming and is currently used for research purposes.

**Table 2.3: Summary of efficacy data for studies of dynamic contrast-enhanced breast MRI:**

Study (year)	No of patients (lesions)	No of cancers (DCIS)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Accuracy	Scan details
Kacl et al. (1998)	50 (63)	?	92	76	-	-	90.6	Consecutive breast lesions; 3D gradient-echo sequence; lesions graded benign to malignant (1-5 scale)
Sherif et al. (1997)	49 (79)	55	51	100	-	-	-	Patients with breast lesions; acquisition at 90sec intervals to 7min and repeated at 10 min intervals to 60min; peripheral washout at > 10mins used to diagnose malignancy
Huilka et al. (1997)	63 (71)	16 (12)	83	79	67	90	-	Patients with breast lesions; echo planar sequence at 1.5T; several selected slices acquired at 6sec intervals for 3 minutes post-contrast; extraction-flow product used to diagnose malignancy
Heiberg et al. (1996)	56 (83)	23	100	73	100	-	-	Patients scheduled for breast biopsy; fast 3D dynamic sequence; full breast coverage, scans repeated every 44 seconds; rate of enhancement used to diagnose malignancy
Gilles et al. (1996)	172 (172)	22(58)	95	51	-	-	-	Isolated microcalcifications; subtraction dynamic sequence; subjective early enhancement by 3 observers, used to diagnose malignancy
Stomper et al. (1995)	49 (51)	22(3)	100	65	-	-	-	Breast lesions; spoiled gradient-recalled echo (SPGR) sequence at 1.5T; absolute enhancement of more than 2 times.
Fobben et al. (1995)	89(91)	21 (5)	82.5	82.5	54.2	-	89.3	Patients scheduled for biopsy; 3D FLASH gradient-echo sequence at 1.5T; images acquired at 0, 2, 4 and 7 minutes. Lesion enhancement assessed by 3 radiologists for every scan sequence and ROC curves used for analysis
Boetes et al. (1994)	87	65	95	86	-	-	93	Patients with breast lesions; turbo fast low-angle shot sequence; selected slices imaged every 2.3 secs for 2 mins; enhancement within 2 mins regarded as malignant
Turkatt et al. (1994)	35 (39)	15	100	87.5	-	-	-	Women with mammographic abnormalities; gradient-recall sequence at 1.5T; 8 selected images acquired in 54 secs and acquisition repeated for 8 mins; >110 % change in signal intensity used to diagnose malignancy
Gilles et al. (1994b)	143 (143)	37(27)	95	53	-	-	-	Mammographic non-palpable breast lesions; subtraction dynamic sequence; early contrast enhancement considered malignant

### 2.3.3 Image subtraction

By subtracting pre-contrast from post-contrast images, it is possible to produce one set of images in which signal intensity is proportional to contrast enhancement. During the

process of image subtraction, a constant value is automatically added to each subtracted pixel or voxel value. This is necessary in order to visualise negative values on the subtracted image and to retain some background anatomical definition for orientation. Image subtraction is available on most MRI consoles but extra imaging time may be required from radiographers if extra hard copies are produced for reporting.

Although image subtraction improves lesion conspicuity, image misregistration between pre- and post-contrast scans, can potentially lead to artefactual lesions (Beier et al. 1997). Furthermore, patient movement or cardiac and respiratory motion, can also lead to artefacts which can be partially corrected by using image registration (section 2.3.5). Motion related artefacts tend to occur at the outer breast contour and in internal structures, and may be recognised by referring back to the pre- and post- contrast scans. Reduced anatomical detail, due to subtraction of breast landmarks can also be a problem.

Data on the additional benefit of image subtraction is limited although the technique is widely available commercially and is in use at many centres. In a series of 31 patients, Flanagan et al. (1995) showed that digital subtraction of 3D FLASH sequences improved diagnostic accuracy in 6 patients compared with post-contrast images. Although multicentricity was better appreciated, in 2 patients motion artefact led to blurring of tumour margins. Gilles et al.(1994b), used image subtraction of dynamic MRI to study 143 patients with non-palpable breast lesions. Image quality was satisfactory in 104 patients but respiratory and cardiac movements lead to artefacts in the remainder. In a later study by the same authors, image subtraction was used to image small, localised clusters of microcalcifications (Gilles et al. 1996) and a sensitivity of 95% was achieved although specificity was only 50% for the detection of *in-situ* or invasive disease. In order to reduce motion artefact from 11% to 2% in 157 examinations, Schorn et al. (1998) used breast compression. Although this eliminates one of the advantages of MRI over mammography, breast compression is routinely used during MRI-guided interventional procedures and may become standard technique in the future.

#### **2.3.4 Other MRI scanning techniques**

Although most centres now use gadolinium contrast agents, a major deficiency of contrast-enhancement is that the tumour may be iso-intense with fat in T1-weighted images. In this instance, lesion conspicuity may be enhanced by selectively reducing



signal intensity of surrounding tissues. The breast is rich in fatty tissue, particularly in post-menopausal women. Fat suppression can enable the appreciation of more subtle changes in breast tissue signal intensity but prolongs scanning time (unless the number of slices per acquisition is reduced). As well as improving pre-contrast lesion conspicuity, this technique can be used to improve the detection of mildly enhancing lesions. Rotating delivery of excitation off-resonance (RODEO) MRI developed by Harms et al. (1993), is a selective rapid 3D fat suppression technique. By altering the range of signal suppression, it has been shown to be useful in selectively enhancing the MRI appearance of silicon leaks (Harms et al. 1995). However, this technique is not, as yet, available elsewhere.

Short-tau inversion recovery (STIR) is another method of fat-suppression, useful for highlighting water-rich tissues such as oedematous breast lesions or lymphatic swelling, but sequence acquisition is relatively long and suffers from a low signal to noise ratio. Echo planar sequences are 2D rapid sequences which lack the spatial resolution of 3D scans but can be used to acquire a rapid sequence of images of a selected slice, during dynamic MRI. Spin echo sequences are also 2D sequences, which compensate for undesired effects of inhomogeneous magnetic fields. The latter two sequences are not widely used as yet.

### ***2.3.5 Other post-image processing techniques***

Once image data sets are acquired, further data analysis is limited by the software available at MRI work-stations. Most work-stations can subtract pre- from post-contrast images but advances in post-processing software and computer hardware, have led to a rapid expansion in the number of different forms of data analysis and display available. However, since work-stations are in constant clinical use, it is often difficult to find sufficient time to perform complex post-processing analysis. Three dimensional data sets, can be transferred to other computer work-stations where analysis can be performed and images stored on optical discs. One example of available software, is image registration software. Since the breast is fluctuant and close to the chest wall, cardiac and respiratory movements can affect individual images. Movement between pre- and post-contrast high resolution sequences or between individual dynamic sequences, can cause difficulties when attempts are made to match-up corresponding images in a sequence. Image registration software attempt to correct for movement. The simplest

approach, treats breast tissue as though it were a rigid body and corrects data sets by using a rotational and translational registration algorithm (Davey et al. 1997; Beier et al. 1997). Sophisticated 'sinc' interpolation schemes reduce the size of image data sets and thus speed up the process. Rotation and translation operators are then applied to pairs of voxels in a 3D data set, to minimise the difference of least-squares between them (Curati et al. 1996). However, since breast tissue warps rather than simply moving in 3D, more complex software is required and is currently at the experimental stage. Image registration improves the quality of subtracted images which may improve the detection of very small breast lesions.

In the interpretation of contrast enhanced breast MRI, the amount of enhancement, rate of enhancement (on dynamic MRI), morphology and distribution of enhancing lesions should all be assessed. All these parameters provide some diagnostic information although the relative importance of each parameter is not known, it is in part dependant upon the clinical indication.

When attempting to quantify enhancement intensity, it is essential to use identical scanning protocols for both the pre-contrast and post-contrast scans. If some imaging parameters are different, then the absolute signal intensity will be different. The relationship between signal intensity and magnetic resonance parameters may not be linear and therefore cannot not be simply adjusted for. If identical protocols are used for both pre- and post contrast scans, then signal intensity may be compared, and enhancement intensity determined. This may be done by either subtracting or dividing pre-contrast values, from post-contrast ones. Division offers the advantage of facilitating inter-patient comparison by partially correcting for patient related differences between pre- and post-contrast scans. However, division is not commercially available as yet since division by zero can result in computational failure. Another way to correct for patient-related tissue specific differences, is to divide tumour signal intensity in subtracted images by the signal intensity of a reference tissue (fat, breast, bone or vessel) to obtain 'normalised units' (Heywang et al. 1989).

Other post-processing techniques, include multiplanar reformatting software which enables rapid image updates on pre-acquired high resolution 3D images, using a localisation devise. This provides spatial information on patients' anatomy and location

of an instrument, during MRI guided interventional procedures.

Current exciting work includes coloured overlay of calculated and spatially registered data onto the original high resolution MRI image. Data on temperature distribution and even data acquired by PET scanning may be displayed in this way. Applications in this vein should emerge over the next 5 years.

## 2.4 Potential Clinical Indications for Breast MRI

Numerous studies have been carried out to evaluate the clinical utility of MRI in the evaluation of patients with breast cancer, at different stages of the disease, on different patient populations, with different scanning techniques and with differences in criteria for MRI evaluation. Furthermore, there have been no studies to look at the impact of breast MRI on the medical or surgical management of breast cancer. As a result, MRI does not as yet have a place in the routine investigative algorithm. However, from the body of evidence to date, potential indications for breast MRI have been suggested. These will be considered in this section.

### 2.4.1 *Diagnosis of primary breast cancer*

MRI undoubtedly provides far greater anatomical definition than any other imaging modality. However, in patients presenting with a suspicious breast lesion, triple assessment (clinical examination, mammography, and FNA) can reliably diagnose malignancy with a sensitivity of 95% and specificity of 100% (Steinberg et al. 1996). The negative predictive value of triple assessment is 99% (Layfield et al. 1989). There is therefore little scope for breast MRI to improve diagnostic accuracy for breast cancer and thus MRI does not, at present, have a place in routine breast cancer diagnosis (Steering Committee on Clinical Practice Guidelines for the Care and Treatment of Breast Cancer, 1998).

Some studies have indicated possible areas where breast MRI could aid diagnosis specifically with respect to the clinical and radiological challenges, listed in table 2.1. MRI may be useful in imaging patients with high clinical suspicion of malignancy despite equivocal or negative results on triple assessment. This would particularly be useful in

young women with dense breasts, postmenopausal women taking hormone replacement therapy or women with fibrocystic disease (table 2.1). However, false negatives do occur (Sardanelli et al. 1998; Boetes et al. 1997) and since it would be difficult to argue against obtaining tissue diagnosis in all such cases, the additional value of MRI remains unclear.

Contralateral breast cancer, not detected by other imaging modalities, has been detected by MRI. In a series of 76 pre-operative patients, Fischer et al. (1994) detected 8 (10.5%) contralateral cancers, and another 5 (6.6%) lesions ipsilaterally. In a further 4 (5.3%) cases suspicious lesions in the contralateral breast on MRI, were found to be benign. Despite the fact that these are experimental findings, the authors have altered surgical management based on the MRI findings in 15 (18.5%) patients (ipsilateral mastectomy n=5; contralateral mastectomy n=2; primary chemotherapy n=1; contralateral wide local excision n=2; contralateral open biopsy n=4). Not only does this cast a shadow over the benefit of MRI in diagnosis, it also illustrates a potential deleterious effect, that of overtreatment. Overtreatment results from the excision of additional lesions that prove to be benign as well as excision of additional malignant lesions whose natural history may be such that they may not influence a patient's outcome. The level of uncertainty here justifies further research into elucidating the clinical significance of such lesions.

Approximately 10% of malignant breast tumours are lobular carcinomas, and these are a diagnostic challenge even for MRI. Gilles et al. (1994b) studied 143 patients with non-palpable mammographic abnormalities and found that 61 of 64 invasive tumours enhanced (sensitivity 95%). However, 2 of 3 invasive tumours that did not enhance were lobular carcinomas. In a more recent retrospective study, although mammography detected 20/23 (87%) of lobular cancers compared with 19/23 (83%) by dynamic MRI, the combined sensitivity of both investigations was 100% (Sittek et al. 1998). MRI may therefore assist in the diagnosis of lobular cancers, and it may be particularly useful in younger women with dense breasts.

#### **2.4.2 Diagnosis of DCIS**

The accuracy of MRI in detecting DCIS is still unclear. Several enhancement patterns have been described including 'clumped' (Soderstrom et al. 1996), spiculated

(Soderstrom et al. 1996) or ductal (Orel et al. 1997) focal enhancement, regional enhancement (Orel et al. 1997) or widespread enhancement (Sittek et al. 1997; Fischer et al. 1996). Some studies suggest that DCIS can reliably be detected with accuracies comparable to those for detecting the invasive tumour (Soderstrom et al. 1996; Heywang-Kobrunner, 1994), whereas in other studies DCIS was not accurately detected (Fischer et al. 1996; Sittek et al. 1997; Boetes et al. 1997; Orel et al. 1997; Daniel et al. 1998b). Soderstrom et al. (1996) reported 22 cases of DCIS scanned using 3D RODEO MRI and all cases were visualised. Clumped enhancement pattern was seen in pure DCIS (n = 5) and spiculated enhancement in association with microinvasion (4 of 6, 67%) or invasive ductal carcinoma (9 of 11, 82%). In another series, Heywang-Kobrunner et al. (1994) used 3D FLASH high resolution MRI and detected all 15 cases of DCIS.

In a histological correlational study by Fischer et al. (1996), 25 of 35 cases of DCIS (sensitivity 72%) were detected based on enhancement using dynamic MRI. Rapid signal enhancement typical of malignancy, was seen in 15 cases. Interestingly, 6 false negative cases (11%) did not enhance and 4 (11%) had widespread bilateral breast enhancement interpreted as benign disease. Results from a series of 20 cases of DCIS, by Sittek et al. (1997), were very similar and 14 of 20 cases of DCIS (sensitivity 70%) were correctly diagnosed on the basis of focal enhancement on dynamic MRI. Although mammography also detected 14 of 20 cases, 4 cases of DCIS were detected only by MRI. The authors concluded that MRI was not reliable at detecting DCIS but that if it were used together with mammography, the combined sensitivity would rise to 90%. Boetes et al. (1997), detected 13 of 17 DCIS lesions, using high resolution MRI (turbo FLASH and subtraction) whereas MRI detected 96% of 145 malignant lesions in 204 patients with palpable or mammographic abnormalities. In a previous series using rapid early enhancement on dynamic MRI (11.5 seconds) to diagnose DCIS (Boetes et al. 1994), 7 of 8 lesions were identified. Orel et al. (1997), found that pre-operative MRI detected DCIS in 10 of 13 (77%) histologically proven cases of pure DCIS. The mean diameter of extent of DCIS was 10mm (2-22mm) and 3.7mm in the 3 cases missed by MRI. They also concluded that although MRI can miss mammographically detected DCIS, it may detect some lesions that are mammographically occult.

Clearly, MRI can visualise some cases of DCIS but it is not clear whether certain

subtypes of DCIS are visualised in preference to others, or whether detection of DCIS by MRI indicates a higher angiogenic activity within the lesion. Evidence so far suggests that MRI is not sufficiently sensitive for the detection of DCIS.

### ***2.4.3 Detection of local recurrence***

The diagnosis of local recurrence is currently based on clinical or mammographic suspicion. Post-surgical scarring and radiation change can mimic both clinical and mammographic signs of local recurrence. In order to conclusively refute or confirm the diagnosis, tissue diagnosis is required. Although the gold standard for tissue diagnosis is open incision or excision biopsy, tissue diagnosis is more usually performed by core biopsy and FNA, since these can be readily performed in out-patient clinics. Furthermore, open biopsy in patients who have received post-operative radiotherapy should be avoided since complications, including delayed post-operative healing or wound infection, may result. There is a clear need for imaging modalities that would reliably reduce the number of benign biopsies, while at the same time assist in selecting suspicious areas for biopsy when tissue diagnosis is unavoidable.

MRI is very accurate in the differentiation of local recurrence from scar tissue, and has the advantage of imaging local disease extent as well. One major draw back is that it cannot reliably distinguish between scarring and recurrence until 6-18 months post-operatively. Heywang et al. (1990) showed that during the initial 6 months, enhancement of scar tissue can lead to false positives on MRI. Following radiotherapy, enhancement of scar tissue occurs up to 18 months post-treatment. Heywang-Kobrunner et al. (1993) identified enhancement in a third of patients between 9 and 18 months post-treatment. They concluded that MRI could be helpful during 9-18 months so long as enhancement of scar tissue is anticipated. In another study of 67 patients who had undergone breast conservation surgery, Muuller et al. (1998) found that although dynamic MRI could detect local recurrence with a sensitivity of 100% within the first year, specificity approached 90% only after 12 months. Rieber et al. (1997a), found similar results in a series of 140 patients scanned during the initial 12 months post-surgery. MRI was diagnostic of recurrence in 19 patients (13.6%), diagnostic of scarring in 116 (82.8%) but falsely positive in 5 (3.6%). The extremely high accuracy was demonstrated in other smaller studies (Lewis-Jones et al. 1991; Sardanelli et al. 1998; Davis and McCarty, Jr., 1997; Melani et al. 1995; Gilles et al. 1993). Mumtaz et al. (1997a)

compared MRI to triple assessment in the detection of local recurrence and obtained a sensitivity of 93% and specificity of 88% for MRI.

In a study of 105 patients (Drew et al. 1998) undergoing follow-up after breast conservation surgery, the detection of recurrence (n=9) by MRI was compared with that of conventional clinical examination and mammography. Although specificity was higher for MRI (93%), compared with mammography combined with clinical examination (67%) sensitivity was 100% with both modalities. The authors suggested that MRI should be used only in those patients with clinical or mammographic findings. However, it is not clear whether biopsy can be safely avoided in those patients with a negative MRI scan. A draw-back of this study was that only 16 patients underwent tissue biopsy and the remainder assumed to be benign on clinical grounds with median follow-up of only 341 days.

Studies to date suggest that MRI is highly accurate in the detection of local recurrence 18 months post operatively. This fits in quite well with the bimodal incidence of breast local recurrence, which typically peaks at 3 and 7 years (Baum, 1996). However, if tissue diagnosis is required in all cases then the place of MRI in diagnosis of local recurrence is not clear. Advances in MRI guided biopsy techniques may enable tissue diagnosis in equivocal cases and this approach may prove to be more accurate than core biopsy or FNA without MRI.

#### ***2.4.4 Pre-operative staging of local disease extent***

MRI is emerging as the most accurate non-invasive technique for local staging of breast cancer but the additional benefit of MRI information pre-operatively has not been shown to influence clinical or surgical management.

Several studies have demonstrated that breast MRI is very accurate in assessing local disease extent (Kerslake et al. 1995; Rodenko et al. 1996; Mumtaz et al. 1997b). Mumtaz et al. (1997b) scanned 90 patients pre-operatively using high resolution MRI. Extent of contrast-enhancement on MRI, concorded with histologically confirmed residual disease at re-excision (8 of 17 patients). When compared to mammography, MRI was more accurate in determining tumour size ( $r^2 = 0.93$  versus  $r^2 = 0.59$ ), in detecting multifocality and extensive intraductal component (sensitivity, 81% versus

62%), and in detecting nipple involvement. MRI could predict axillary involvement, based on axillary asymmetry, with a sensitivity and specificity of 90% and 82% respectively.

Other smaller studies confirm that MRI is useful at assessing locoregional tumour extent but no other study has confirmed the ability of MRI to predict axillary lymph node involvement. In a histopathologic correlational study (Harms et al. 1993), RODEO high resolution MRI was undertaken prior to mastectomy in 30 patients. MRI could detect 11 cancers, 3mm to 12 cm in size, that were not detected by mammography. In another histopathologic correlational study by Boetes et al. (1995), 60 patients underwent MRI, US and mammography prior to mastectomy. MRI tumour size correlated very closely with pathologic tumour size whereas mammography and US underestimated tumour size in 14% and 18% respectively. Of the additional invasive lesions detected by pathology in 13 patients, MRI detected 100%, mammography 31% and US 38%. Orel et al. (1995) reported that MRI detected additional mammographically occult lesions in 22 of 64 patients (34%). Surgical treatment was altered in 7 patients (11%) based on additional findings detected on MRI.

With respect to lobular carcinoma it appears that MRI is less accurate at assessing tumour size, but better than mammography at assessing disease extent (Rodenko et al. 1996). Kerslake et al. (1995) compared histological with MRI tumour size, and found a lower correlation coefficient for lobular carcinomas ( $r^2 = 0.76$ ,  $n=7$ ) compared with that for invasive ductal carcinomas ( $r^2 = 0.92$ ,  $n=35$ ).

When MRI is performed for staging purposes, additional enhancing foci are often detected away from the primary tumour (Fischer et al. 1994; Kerslake et al. 1995). As was described in section 2.4.1, it is not yet clear if additional enhancing foci detected by MRI, away from a primary tumour, represent *in-situ*, invasive or benign disease. Furthermore, it is not clear what the clinical significance of these additional foci is, or even whether they require excision particularly in patients due to undergo postoperative radiotherapy. All these issues need to be addressed prior to determining the place of MRI as a staging tool. It will then be possible to assess the impact of MRI on the amount of tissue excised from the breast or on other aspects of patient management.



#### **2.4.5 Monitoring patients undergoing primary medical therapy**

The high spatial resolution of MRI has been applied to the assessment of residual disease following PMT, prior to surgery. In a study reported by Abrahams et al. (1996), MRI accurately depicted pathologically proven residual disease in 30 of 31 (97%) of patients. Interestingly, although surgical and medical oncologists agreed with their assessment of clinical response in 75% of cases, surgical and medical clinical assessment agreed with pathological assessment of response in only 52% and 55% respectively. Unfortunately, UICC criteria were used to assess response rather than comparing actual tumour measurement of cross-sectional size or volume. Furthermore, since RODEO high resolution MRI is not available elsewhere, results have not been corroborated.

Other studies using high resolution MRI also showed that MRI can detect residual disease immediately prior to surgery (Mumtaz et al. 1996b). Mumtaz et al. (1996b), showed that MRI was more accurate than mammography in determining local disease extent and suggested that MRI may help determine the extent of local excision required. However, only 8 of 15 patients underwent post-chemotherapy MRI and pre-treatment MRI was not routinely performed.

Dynamic MRI has been used in two small studies (Gilles et al. 1994a; Rieber et al. 1997b). Gilles et al. (1994a), reported that MRI detected residual disease in 17 of the 18 patients. In 2 of these 17 patients, additional malignant cells and DCIS away from the main lesion, were not detected and thus extent of residual disease correlated with histopathology in only 15 patients. Rieber et al. (1997b), observed a flattening of the enhancement intensity curve in responders to chemotherapy, after only 1 course of treatment. Following 4 cycles of treatment, a complete flattening of the enhancement curve was observed. Even though this study was small (n=13), the authors did not attempt to determine whether MRI can predict final response early on during chemotherapy.

Although MRI appears to be useful in defining extent of residual disease following PMT, studies to date are too small to adequately assess the role of MRI. MRI does not appear to detect microscopic residual disease. Furthermore, since most patients complete a series of courses of neoadjuvant chemotherapy, it is not clear whether the assessment of disease extent during treatment, accurately reflects pathological disease extent.

Chemotherapy may induce an initial inflammatory reaction or tumour necrosis, which may lead to overestimation or underestimation of tumour pathological size, respectively. The ability of MRI to detect response to chemotherapy during the initial stages of treatment, remains to be determined.

#### **2.4.6 *Imaging breast prostheses***

MRI provides a more accurate assessment of the breast in women with prosthetic implants compared with mammography or US (Brown et al. 1997). MRI can image the entire breast volume to the chest wall and thus free silicon may be detected behind or even away from the implant. Netscher et al. (1996) in a study of 160 patients, demonstrated that both MRI (sensitivity 76%; specificity 94%) and US (sensitivity 70%; Specificity 90%) were superior to clinical examination and mammography. In another larger study (Middleton, 1998), the sensitivity and specificity of MRI for the detection of implant leak were 74% and 98% respectively. Chung et al. (1998) proposed a diagnostic algorithm for the investigation of women with suspected leaks, based on a review of published studies. Patients were grouped into 3 groups: asymptomatic, symptomatic with implant age < or = to 10 years and symptomatic with an implant age of > or = to 10 years. An US was recommended as a first line investigation particularly in the later 2 groups in whom the prevalence of implant leak was higher (31% and 64%, respectively). An MRI scan was recommended as a second line investigation in asymptomatic or symptomatic patients (implant age of < or = to 10 years) with a positive US scan since MRI is more sensitive and this approach may prevent the removal of normal implants. In symptomatic women (implant age of > or = to 10 years) with a positive US scan, MRI was not recommended since the post test probability of a rupture with a positive US scan was 94%.

The MRI appearances of an implant leak or rupture have been described and depend on the type of implant used (Beekman et al. 1998; Middleton, 1998). Implant rupture can be intracapsular or extracapsular. Intracapsular rupture is more common and is defined as rupture of the elastomer shell with silicon leaking into the fibrous capsule. It is identified on MRI by the presence of curvilinear lines (linguine sign) within the implant. Extracapsular rupture results when both the elastomer shell and fibrous capsule rupture. Foci of high signal intensity silicon are seen outside the capsule within the breast substance.

#### **2.4.7 Other indications**

MRI may prove useful in detecting primary lesions in patients presenting with axillary metastasis, but in whom no primary is detected by other imaging modalities. In one study (Morris et al. 1997), 9 of 12 patients (75%) with cancer confirmed at mastectomy (n = 8) or wide local excision (n= 4), MRI detected the primary tumour.

There are several situations where MRI appears to be less helpful. MRI is currently not suitable for population screening (Potterton and Coulthard, 1997) since a clear danger exists of over-investigating women without malignant disease. However, as mentioned in chapter 1, studies are currently underway to evaluate the potential role of MRI as a screening tool in patients with an increased cancer risk (Leech et al. 1998).

### **2.5 Interventional Breast MRI**

As discussed earlier, technological advances and advances in the analysis of enhancement kinetics of MRI have not been sufficient to obviate the need for tissue diagnosis. The finding of occult focal enhancement, not apparent on other imaging modalities, stimulated the development of MRI-guided biopsy systems. However, modern high-field superconducting magnets are doughnut-shaped and patients need to be removed from the scanner for instrument placement during the procedure. Since the patient lies prone, access to the breast is limited and open access breast coils are required to enable access for instrument insertion. Manufacturers produce a range of MRI-compatible needles, localisation wires and core-biopsy guns. An important drawback is that this equipment is currently expensive and equivalent procedures performed using stereotactic techniques or ultrasound guidance, are significantly cheaper.

Interventional techniques currently under development include MRI-guided breast biopsy, MRI-guided insertion of localisation wires and MRI-guided interstitial laser photocoagulation (ILP). Breast compression (Heywang-Kobrunner et al. 1994) is often used to reduce anatomical perturbations during instrument insertion. Mediolateral compression is favoured since it maximises access to the breast from a lateral approach. MRI-guided breast biopsy (Orel et al. 1995; Fischer et al. 1998), is a technique which

does not have an established clinical indication. It is currently being developed as a research tool to determine the histology of occult enhancing lesions detected by MRI but which cannot be imaged by other modalities. These lesions may also be localised using non-magnetic hook wires, which are then excised with the primary tumour. Potential future applications include MRI-guided wire-localisation of occult breast lesions in patient who present with axillary node metastasis and no mammographic or ultrasound abnormality, or even for excision sentinel lymph nodes.

MRI-guided interstitial laser photocoagulation (ILP) therapy is at the initial stages of development. During ILP (Harries et al. 1994; Mumtaz et al. 1996a), low power laser energy is delivered directly into the tumour substance via thin optical fibres inserted percutaneously. MRI forms an integral part of the procedure and is used to plan, guide needle insertion and for follow-up. Since the size of the laser burn is limited to about 11mm, multiple fibres are needed for successful treatment of even relatively small lesions (Mumtaz et al. 1996a). Ablation of material for histological examination and confirmation of margins of excision, is an important drawback of this procedure.

## **2.6 Summary**

Contrast-enhanced breast MRI is capable of generating detailed information on the extent and character of breast lesions. When interpreting breast MRI, the amount of enhancement, rate of enhancement (on dynamic MRI), morphology and distribution of enhancing lesions should all be noted. It is essential to image the entire breast volume and thus fast T1 weighted 3D gradient echo sequences (eg: 3D FLASH) are the optimal sequences since they can provide both spatial and temporal resolution. The former is essential for detecting small breast lesions whereas the latter improves specificity for the detection of malignancy. Contrast enhancement with Gd-DTPA is now an indispensable step in the scanning procedure. Novel intravascular contrast agents may emerge and further improve discrimination of malignant from benign disease. Post-processing techniques such as subtraction imaging, may further improve detection of small lesions and morphological discrimination.

A number of clinical settings have begun to emerge in which breast MRI may be

clinically useful. MRI is useful in determining local extent of disease, both primary and recurrence. It is useful in the diagnosis of local recurrence (more than 18 months after surgery), detecting prosthetic rupture and in detecting residual disease following PMT. MRI does not appear to be useful in routine diagnosis of invasive or *in-situ* disease. It may be useful in women presenting with axillary metastasis but no obvious primary tumour and in imaging the axilla.

It is clear from the studies so far that MRI findings may potentially influence management. What is not clear is whether additional enhancing foci, often detected away from the primary tumour, represent cancer or even what their clinical significance is. It is not clear whether excision margins can or should be extended to include these. Furthermore, studies to date have not determined the impact of MRI on surgical management or even patient outcome.

Current evidence on the application of MRI to different clinical settings is very encouraging, but it would be premature to draw firm conclusions or even to produce clinical guidelines on the indications for breast MRI. MRI should not at present have an established place in algorithms for the evaluation of breast cancer patients. The jury is still out with respect to the precise clinical indications for MRI, and will remain out, until the impact of breast MRI on surgical management is determined. In the Section B of this thesis, the impact of MRI on surgical management is assessed and ways in which MRI may assist the surgeon, developed.

# Chapter 3: Breast Ultrasound

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- 3.1 Introduction
- 3.2 B-mode (Grey Scale) Ultrasound
  - 3.2.1 Differentiation of cystic from solid masses
  - 3.2.2 Evaluation of masses in radiologically dense breasts
  - 3.2.3 Guidance for interventional procedures
  - 3.2.4 Adjunctive investigation to triple assessment of breast cancer
  - 3.2.5 Assessment of residual disease following primary medical therapy
  - 3.2.6 Detection of breast cancer local recurrence
- 3.3 Colour Doppler Ultrasound
  - 3.3.1 Breast tumour characterisation
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  - 3.3.3 Detection of lymph node involvement
- 3.4 Doppler Ultrasound Contrast Agents
- 3.5 Summary

### **3.1 Introduction**

In the search for breast imaging techniques that do not use ionising radiation, breast ultrasound (US) has emerged and is the main competing imaging modality to breast MRI. Largely due to progress in computer technology and improvements in performance, it has rapidly developed into a widespread, accessible, rapid, easy to perform, and relatively cheap breast imaging modality. Initially recognised as a useful tool to distinguish solid from cystic lesions, it is being developed into a diagnostic tool which can assist in characterisation of lesions (including differentiation of benign from malignant lesions) rather than just simple detection. The application of two main US modalities, namely B-mode (or grey scale) and Doppler US, to the diagnosis of breast cancer are described in this chapter. Recent interest has focused on the emergence of novel intravascular US contrast-enhancing agents. As opposed to contrast agents for MRI, these agents are purely intravascular and do not extravasate into the extravascular space. Their possible application to breast cancer diagnosis is discussed.

### **3.2 B-mode (Grey Scale) Ultrasound**

The basic principle of grey scale or B-mode breast US is creating real-time cross-sectional images based on differences in the reflection of high frequency sound waves by different tissue structures and at tissue interfaces. A hand held transducer (preferably a linear array transducer) produces high frequency sound waves (at least 7 MHz; usually 10-13 MHz) by electronically inducing crystal vibration. Sound waves reflected off tissue structures and interfaces, are detected by the same transducer and converted into electrical signals, which can then be used to create a cross-sectional image. The higher the frequency emitted by the transducer, the better the spatial resolution but the lower the depth of penetration of the ultrasound beam. State of the art US equipment is a compromise between the use of low frequency sound to optimise Doppler analysis of vascular flow, and high frequency sound which improves spatial resolution of B-mode ultrasound.

The breast is very well suited to US scanning since it is a fluctuant, superficial organ easily accessible to examination with a transducer. The US appearance of breast tissue depends on several factors including the interface between intra-mammary structures, varying densities and varying tissue elasticities (Rubin et al. 1979). Features of benign and malignant masses have been described (Kobayashi, 1977; Cole-Beuglet et al. 1983; Fornage et al. 1989). Fibroadenomas tend to be ellipsoid, have homogeneous echogenicity (iso- or hypoechoic), lobulated margins with a thin echogenic pseudocapsule (Stavros et al. 1995) whereas malignant lesions are irregular with no

visible pseudocapsule, hypoechoic with posterior acoustic shadowing. Since overlap exists between characteristics of benign and malignant lesions these features are by no means diagnostic (Pamilo et al. 1991; Adler et al. 1991). The best results are obtained by experienced radiologists using state of the art ultrasonography. Stavros et al. (1995), studied 750 breast nodules prospectively and could detect breast cancer with a sensitivity of 98.4% (123 / 125) and a negative predictive value of 99.5% (625 / 750 benign lesions).

Indications for B-mode US include differentiation of cystic from solid masses, evaluation of masses in radiologically dense breasts, guidance for interventional procedures, and as an adjunctive investigation to triple assessment in the diagnosis of breast cancer. It has also been used in the assessment of residual disease following primary medical therapy (PMT) and in the detection of breast recurrence.

### ***3.2.1 Differentiation of cystic from solid masses***

Features of a cystic lesion include smooth walls, sharp anterior and posterior borders, no internal echoes, and compressibility with posterior enhancement. When these criteria are strictly adhered to, the accuracy of US for the differentiation of cystic from solid lesions is 96% - 100% (Jellins et al. 1977; Hilton et al. 1986). Occasionally real echoes may be seen in cysts containing blood, cellular debris or even in malignant cysts with mixed solid and cystic components. In a retrospective study of 56 mixed cystic and solid lesions on US (Omori et al. 1993), 43% were found to be malignant. It is therefore important to aspirate all cysts with solid components and send the contents for cytological examination.

### ***3.2.2 Evaluation of masses in radiologically dense breasts***

Breast tissue in young women or in women taking hormone replacement therapy (HRT) is radiologically dense which may obscure small breast masses. Ultrasonography may be useful in this instance but, since not all palpable masses are visible on US (Pamilo et al. 1991), any palpable mass should undergo FNA irrespective of its US appearance.

### ***3.2.3 Guidance for interventional procedures***

US has been used successfully in guidance for several interventional procedures including FNA of palpable (Rubin et al. 1997) and non-palpable breast lesions (Boerner et al. 1999; Okamoto et al. 1998; Sneige et al. 1994; Jokich et al. 1992), FNA of lesions in women with breast prostheses (Fornage et al. 1994), cyst aspiration, core-needle biopsy (Parker et al. 1993) and pre-operative wire localisation. In a recent retrospective study of US guided FNA of 1885 non-palpable breast lesions (Boerner et al. 1999), a sensitivity of 97.1% and specificity of 99.1% for the detection histologically confirmed breast cancer was achieved. Patel et al. (1988) randomised 116 patients with breast



masses into blind FNA or US guided FNA and showed that inadequate FNA cytology (C1) was more frequently seen with blind FNA particularly in lesions measuring less than 3cm. In another study of 705 solid breast lesions (Gordon et al. 1993), US guided FNA had an accuracy of 93%, which was superior to that achieved by mammography or US alone.

The value of US-guided FNA has recently been a subject of debate, since the rate of insufficient aspirate may be as high as 65% in lesions under 10mm (Ogawa et al. 1998) and it is not useful in the diagnosis of small areas of calcification (Klijanienko et al. 1998). In these instances novel stereotactic procedures may be more helpful, as described in chapter 1 of this thesis. Advantages of US guided over stereotactic interventional procedures include patient comfort, good patient access, no need for breast fixation or compression and no ionising radiation.

#### ***3.2.4 Adjunctive investigation to triple assessment of breast cancer***

US is useful as an adjunct to triple assessment in the detection of breast cancer, particularly in young women with dense breasts. In a prospective study of 223 patients, Warwick et al. (1988) showed that in triple assessment of solid breast masses by using sonography in lieu of mammography, a 25% increase in accurate diagnoses would allow definitive surgery avoiding the need for frozen sections. In a prospective study of 400 palpable breast tumours, Perre et al. (1994) showed that US had a sensitivity of 96.6% and specificity of 94.2% for breast cancer diagnosis. The combined use of US and mammography, can achieve a sensitivity of 92% and specificity of 97.7% in breast cancer detection (Duijm et al. 1997).

#### ***3.2.5 Assessment of residual disease following primary medical therapy***

US has been used to assess response to PMT (Gawne-Cain et al. 1995; Seymour et al. 1997). In the largest study by Seymour et al. (1997), 52 patients with large (>4cm) breast cancers were scanned before, during and on completion of chemotherapy. Although tumours were measured using B-mode US, the authors have compared response rates using UICC criteria rather than using actual reduction in tumour size. Forouhi et al. (1994) compared pre-operative clinical, US and mammographic measurements of tumour volume with pathological volume in 35 consecutive patients. Tumour volume was estimated from the average of 2 (mammography) or 4 (clinical and US) diameters using the formula for volume of a sphere (for clinical and mammographic measurements) or ellipsoid (for US measurements). Correlation coefficients for tumour volume were highest for US ( $r^2=0.89$ ) and mammography ( $r^2=0.84$ ), compared with clinical examination ( $r^2=0.68$ ). Clearly, B-mode US is more accurate than clinical examination in the measurement of palpable residual lesions. However, B-mode US may

miss impalpable microscopic residual disease and may lead to misinterpretation of residual scarring as representing residual disease. Another drawback is that baseline measurements of very large tumours cannot be made if the lesion is larger than the field of view of the transducer. In one study (Kedar et al. 1994), 68% (21/31) of tumours could not be measured. New improvements in 3D acquisition and composite 2D imaging, may resolve this problem (Carson et al. 1997).

### ***3.2.6 Detection of breast cancer local recurrence***

Post-operative scarring and post-radiation change pose a challenge in their differentiation from malignancy. As a non-invasive modality, ultrasound is often used in this context especially in young women who have undergone breast conservation surgery. However, to date it has not been shown sufficiently sensitive for the detection of breast recurrence. In a large retrospective study (Balu-Maestro et al. 1991), data on clinical, mammographic and ultrasound for 171 breast cancer follow-up patients were compared. In the early post-operative period, ultrasound was found to be useful in the diagnosis and treatment of haematomas, lymphocoeles and abscesses post breast conservation surgery. With respect to detection of recurrence, ultrasound was found to be less sensitive (90%) when compared with mammography (95.5%).

Recent technological improvements include the detection of tissue harmonics, which could not be detected by previous US scan heads. Tissue harmonics are integer multiples of the fundamental frequency, reflected by tissues in response to high frequency sound waves. The use of Tissue Harmonic Imaging was pioneered by ATL US Inc. (Washington, USA) in 1991, but was first commercially available in 1996. Most commonly, the second harmonic is detected by the transducer. The detection of tissue harmonics dramatically improves spatial resolution, particularly by reducing noise (Shapiro et al. 1998). These and other developments, such as 3-dimensional reconstruction of images, may improve resolution of breast US images to such an extent that images may be more readily interpreted by less experienced observers.

## **3.3 Colour Doppler Ultrasound**

When a moving object reflects high frequency sound waves, there is a frequency difference as the object approaches or moves away from the detector or transducer, called the Doppler effect. The increased Doppler signal of malignant breast tumours first demonstrated by Wells et al. (1977), generated interest in characterisation of breast tumours. Since then, advances in sonographic equipment and computer software resulted in the detection of vascular flow in increasingly smaller vessels. Although the rapidly evolving technology has improved Doppler sensitivity to detecting small vessels, this also

resulted in the generation of differing results and much uncertainty. Results in different studies also vary due to a combination of other factors including: differences in patient populations, the subjectivity of the procedure, experience of ultrasonographers, variations in sonographic equipment and variations in sonographic settings.

Two basic types of Doppler ultrasound imaging are used in practice. Colour Doppler US, provides velocity and directional information by detecting Doppler frequency shifts, which are displayed as a colour overlay (coded red or blue) on the B-mode image. Power Doppler (amplitude Doppler) US, is a newer, more sensitive technology for detection of slow blood flow. Signal intensity on power Doppler, depicted as a two-dimensional map of the vascular space in a section of a tumour, is proportional to vessel flow volume and provides information on the quantity of moving particles as opposed to their direction of flow. Other terms are used to describe power Doppler commercially including Power Mode (ATL, Bothell, WA, USA), Energy Mode (Acuson, Mountain View, CA), amplitude Doppler and 2 dimensional B-mode ultrasound.

The possible additional benefit of Doppler US in comparison to B-mode US alone, has been investigated in relation to breast tumour characterisation, assessing response to PMT and detecting lymph node involvement.

### ***3.3.1 Breast tumour characterisation***

The most clinically important aspect of breast tumour characterisation, is the differentiation of malignant lesions from benign ones. The vasculature of malignant tumours is believed to penetrate a tumour mass from its periphery and to consist of irregular blood vessels with chaotic anastomoses and shunts (Lee et al. 1995). Both colour and power Doppler signals have been studied qualitatively (e.g.: grading of vascularity as none, slight, moderate or high), semi-quantitatively (e.g.: number of vessels / cm<sup>2</sup> or average colour pixel intensity on selected images) or quantitatively. Quantitative assessment has been carried out using vessel flow parameters such as vessel flow rates, resistance index (RI) and systolic diastolic frequency ratios. In some cases computer assisted image analysis was used to obtain and analyse semi-quantitative and quantitative parameters.

In the largest study, by Madjar et al. (1997), 471 patients (133 with breast cancer) underwent colour Doppler US using state of the art US equipment and a 10MHz transducer. Presence of vascularity, number of vessels and vessel flow parameters (RI index, AB ratio, peak systolic flow velocity and sum of all peak systolic flow velocities in a tumour) were assessed for every tumour and a comparison was made between benign

and malignant lesions. A significant difference was found in systolic flow velocity and number of tumour vessels between the 2 groups. However with respect to the other parameters, overlap between benign and malignant lesions produced a trend that did not reach statistical significance. These data confirmed findings in a previous study by the same author (Madjar et al. 1995). Cosgrove et al. (1993), studied 210 patients (58 with breast cancer) and demonstrated, using a semi-quantitative analysis, that vessels were detected in 98% (57/58) of cancers compared with 14% (5/36) of fibroadenomas and 5% (5/104) of women found to have benign breast change. Again, these findings were similar to those in a smaller previous study by the same author (Cosgrove et al. 1990).

Most other studies on the use of colour Doppler are small and demonstrated variable results. Adler et al. (1990), subjectively classified tumour vascularity in 55 patients, as minimal moderate and marked. Although 82% (45/55) of tumours were found to have either moderate or marked vascularity, 69% (20/29) of normal breast tissue areas, were also found to have moderate or marked vascularity. Other studies (Kuijpers et al. 1994; Holcombe et al. 1995) also confirmed that although vessels are more frequently seen with malignancy, this finding is by no means specific. Dixon et al. (1992) studied 53 patients (32 cancers) and demonstrated that when the lowest velocity scale (0.06 m/s) and lowest colour Doppler filter setting are used to maximise detection of small vessels, the specificity for breast cancer detection is 100% (PPV 100%) but with a lower sensitivity (78%). However, a single cut-off point was used and the authors did not establish detection rates at different colour Doppler settings. Clearly, colour Doppler US may provide useful information for tumour characterisation if performed by an experienced ultrasonographer and using up-to-date US equipment, but as yet, histology is still essential in all cases.

Studies using power Doppler US are also small and demonstrate overlap between appearances of benign and malignant lesions. Raza et al. (1997) used power Doppler to study the vascularity of 86 solid breast lesions (25 cancer). Patterns of vascular distribution (no vessels, peripheral vessels, penetrating vessels) were compared with tissue diagnosis. A penetrating pattern of vascularity gave a low sensitivity of 68% but a high specificity of 95% for the detection of malignancy. On the other hand, Birdwell et al. (1997) studied the degree of flow in 69 breast masses lesions (35 cancers) using power Doppler US categorised as none, less than 25%, 25% to 50%, and more than 50%. Due to significant overlap between the vascularity of benign and malignant lesions, they concluded that assessing the extent of vascularity with power Doppler US, has limited value in diagnostic evaluation of breast masses. However, the addition of power Doppler information on blood vessel distribution, to colour Doppler information, may improve overall diagnostic accuracy and work in this vein is currently underway.

### **3.3.2 Assessing response to primary medical therapy**

The principle of assessing tumour vascularity has also spiked an interest in its application to assess response to primary PMT. Since a tumour is only available for pathological examination on completion of treatment, and even then tumour vessels collapse following tissue fixation (Shubik, 1982), colour Doppler may be better than histology at demonstrating and assessing tumour vascularity. Imaging of tumour vascularity using colour Doppler US, may provide functional evidence of response that is independent of tumour size. Kedar et al. (1994) scanned 34 patients with large breast carcinomas prior to a total of 126 chemotherapy treatments. In 77% (97/126) of treatment cycles, qualitative assessment of colour Doppler vascularity, correctly predicted response, compared with 56% of cycles for clinical tumour size and 58% of cycles for B-mode US. Interestingly, in 40% (50/126) of cycles, the reduction in tumour colour Doppler vascularity preceded that in tumour size by 4 weeks whereas in 19% (24/126) of cycles, changes in clinical tumour size preceded changes in colour Doppler vascularity.

On completion of PMT, US has been used to assess final response to treatment. Seymour et al. (1997) scanned 52 women after PMT and showed that although post treatment diffuse parenchymal distortion or mass without Doppler signal were associated with more favourable histology, these changes were by no means specific for absence of residual disease. In addition, in those patients without a complete clinical response, 71% had residual disease irrespective of US findings. Lagalla et al. (1998) used both power and colour Doppler to study patients before and after chemotherapy. Of 18 patients with initial intra-tumoural colour Doppler signals, 16 had no colour signals following treatment. In 2 with detectable colour Doppler signals following treatment, residual disease was demonstrated histologically. These results are promising, but further work is needed to assess the precise impact of visualising tumour vascularity on diagnostic accuracy in this context.

### **3.3.3 Detection of lymph node involvement**

Histopathological correlation of axillary node excision specimens with pre-operative US scans is inherently difficult since it is difficult to prove that a particular lymph node corresponds to one that has been scanned. Results from studies so far, vary greatly.

Some studies demonstrated a correlation between colour Doppler vascularity in the primary tumour and lymph node involvement (Dixon et al. 1992; Lee et al. 1995; Sterns et al. 1996; Lee et al. 1996; Kubek et al. 1996), but not in all studies (Cosgrove et al. 1993). Stern et al. (1996) scanned 207 patients with breast cancer pre-operatively demonstrating that high vascularity (25% of patients) was associated with an increased risk of lymph node involvement. Lee et al. in 2 separate studies (Lee et al. 1995; Lee et al. 1996), found a significant correlation between tumour vessel flow velocity and lymph

node involvement but only in T1 (<2cm) tumours. In another study (Kubek et al. 1996), the finding of a characteristic curvilinear or branching signal pattern in the tumour periphery was seen in 50% (10/20; n=33) of patients with lymph node involvement compared with 10% of patients without lymph node involvement (NPV = 90%).

Yang et al. (1998) studied pre-operative colour Doppler flows in lymph nodes of 186 axillas (81 patients with cancer; 106 without breast pathology) and found that vessels could be identified in over 80% of patients irrespective of lymph node involvement (87.5% in women with involved nodes vs. 83.6%; specificity 9.5%) or absence of breast pathology (86.7%). In the same study, B-mode US could detect lymph node involvement (sensitivity 79.5%; specificity 94%; accuracy 87.6%) based on the finding of loss of central hilar fat or eccentric cortical hypertrophy.

Doppler US provides information on intra-lesional blood flow, which is superimposed on a B-mode scan. This additional information may improve breast tumour characterisation but the main problem remains overlap between findings in benign and malignant lesions.

### **3.4 Doppler Ultrasound Contrast Agents**

Improvements in sensitivity of colour Doppler, have led to detection of increasingly smaller intra-lesional vessels even in benign tumours (Kedar et al. 1996; Kedar et al. 1995), so that the simple criterion of vessel detection, may not be enough to diagnose malignancy. Since blood flow within tumours is slow and of low volume, un-enhanced Doppler US does not detect some vessels, even when the beam to vessel angle is optimised. The use of contrast agents for Doppler US not only enables detection of even smaller vessels, but provides an opportunity to study blood flow characteristics of these vessels. It has been suggested that these additional features may increase overall diagnostic confidence for differentiation of benign from malignant breast lesions (Kedar et al. 1996) or even expand the range of applications and patients that can be successfully studied. The essential requirement for this technique is an US system sensitive to small differences in contrast agent concentration.

Ultrasound contrast agents have acoustic impedance that is very different to that of tissue. Usually, contrast agents increase the echo intensity facilitating vessel detection. Contrast agents fall into two main categories. Fluid contrast agents increase the echo

intensity of blood, extracellular fluid or the reticuloendothelial system depending on their uptake and distribution. An example is *Fluosol* (Mattry et al. 1987), a mixture of 2 perfluorochemicals (organic compounds in which the hydrogen atoms are replaced by fluorine), used to image liver metastases since it is taken up selectively by the reticuloendothelial system. Disadvantages include a delay between intravenous injection and enhancement (24-48 hours), and allergic reactions. Such agents have not been used for the detection of breast lesions and, in view of the delayed enhancement, are not suitable for imaging vascular flow.

Bubble contrast agents, were initially introduced for studying hepatic and gastrointestinal tumours (Matsua and Yabuuchi, 1986) and were not suitable to study organs in the systemic circulation since the bubbles produced, were too large to cross the lung capillaries. Over the last decade, microbubble contrast agents were developed. Since surface tension increases with the fourth power of the radius, small microbubbles dissolve very quickly and, in order to produce clinically useful enhancement, require the addition of stabilising agents. When insonated, microbubbles reflect acoustic information at higher frequency than originally transmitted. As opposed to contrast agents for MRI, microbubble contrast agents are purely intravascular and therefore do not enter the extravascular space through leaky capillaries. By increasing the signal-to-noise ratio (enhancing the echo signal while reducing background signal), microbubble contrast agents enable the detection of Doppler signals from smaller vessels.

Drawbacks of microbubble contrast agents include artefacts such as 'blooming', an intense enhancement disproportionate to the actual vascular flow rate. The use of power Doppler and harmonic ultrasound to detect signal produced by insonating microbubbles, may reduce blooming since some sound frequencies are almost exclusively reflected by microbubbles and not surrounding tissue. This improves signal intensity and suppresses background noise, significantly.

*Levovist* (SH U 508; Schering, Germany), is a microbubble US contrast-agent which has been used to study the characterisation of breast tumours (Spreafico et al. 1994; Kedar et al. 1996). The powder preparation is mixed with sterile water, 2 minutes prior to intravenous injection, to produce a suspension of galactose microparticles, microbubbles and palmitic acid (stabilising agent). Since the microbubbles are small (<4 µm), they

cross the pulmonary circulation to be distributed in the systemic circulation. The multiple interfaces between microbubbles and surrounding fluid, produce a dose-dependant acoustic scatter which increase the echogenicity of blood by 10-20dB. Kedar et al. (1996), scanned 34 patients with breast complaints (18 cancers) before and after contrast enhancement with *Levovist* noting the number of vessels, tortuosity and number of interconnections between vessels. After intravenous injection, the degree of enhancement and the dynamic bolus transit time was noted. The degree of contrast enhancement, number of new vessels, tortuosity of vessels, time to peak enhancement and duration of enhancement, were statistically significantly raised in carcinomas compared with benign lesions. No difference was seen in surrounding breast tissue vessel enhancement, in the two groups. The sensitivity for breast cancer detection improved from 88.9% to 100%, specificity from 87.5% to 100% and accuracy from 88.2% to 100%. Although very encouraging, these results require confirmation by larger studies.

### 3.5 Summary

B-mode US has a recognised place in the imaging *armamentarium* for differentiation of cystic from solid lesions, evaluation of masses in radiologically dense breasts, in young women as part of triple assessment and in the guidance of interventional procedures especially core biopsy of small lesions and wire localisation. With respect to colour Doppler US, an overall impression on clinically useful applications is still lacking since the literature is currently rich in contrariety. However, new developments are still underway and results on its application to tumour characterisation and assessment of residual disease following PMT, are very promising. As such, it may have novel applications in the prognostic assessment of breast cancer, some of which have been examined in this thesis.

With respect to non-invasive axillary staging, sentinel node localisation by nuclear medicine has recently over-shadowed interest in the use of US to stage the axilla. Although B-mode US can detect architectural distortion in some metastatic lymph nodes, lack of these features does not exclude lymph node involvement.



# **Chapter 4: Prognostic Assessment of Breast Cancer**

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- 4.1 Introduction
- 4.2 Prognostic and Predictive Indicators for Breast Cancer
- 4.3 Staging and Assessing Response to Treatment
- 4.4 Tumour Angiogenesis as a Prognostic Indicator
- 4.5 Imaging Modalities in the Prognostic Assessment of Breast Cancer
  - 4.5.1 Assessment of breast cancer angiogenesis
  - 4.5.2 Correlation with other prognostic indicators
- 4.6 Summary

#### **4.1 Introduction**

Current understanding of the natural history of breast cancer is largely based on clinical and pathological observations of the disease. Since clinical assessment of important prognostic indicators including both tumour size (Yorkshire Breast Cancer Group, 1980; Pain et al. 1992) and lymph node status (Barr and Baum, 1992; de Freitas, Jr. et al. 1991) is often inaccurate, pathological evaluation is regarded as the gold standard for both tumour staging and prognostic assessment (Elston et al. 1998). However, prior to surgery and some forms of modern breast cancer treatments (table 4.1), a pathological specimen is not available and pre-treatment histological evaluation is solely based on assessment of representative core-biopsies. In these circumstances, management decisions are taken without knowledge of definite disease stage. In the context of surgery, management decisions are based on clinical assessment, FNA and core biopsy. Some prognostic information, such as ER and PR status, can be obtained reliably from core-biopsy specimens. Although a clinical need exists to obtain additional useful information from core-biopsy, tumours are heterogeneous and core biopsies may not be representative of the entire tumour.

An exciting development is the possible application of minimally invasive imaging modalities to derive prognostic, as well as diagnostic, information on breast cancer. What remains to be demonstrated, is whether this will provide information which is as accurate (or perhaps more accurate) as that provided by pathological tumour assessment. Enthusiasm surrounding this area of research is fuelled by the theoretical possibility of obtaining functional information on the tumour, which cannot be obtained by pathological evaluation of specimens.

#### **4.2 Prognostic and Predictive Factors for Breast Cancer**

Most biological information on breast cancer, is derived from tissue factors although the utility of some blood markers has been investigated, including CEA and CA15.3 (Coveney et al. 1995). Whereas tissue factors provide static information, blood markers provide dynamic information, which can be repeated. However, in the context of breast

cancer, blood markers are at the experimental stage and none are currently in routine clinical use.

**Table 4.1: Availability of resection specimens for full pathological evaluation prior to management decision making.**

<b>Pathological Specimen Evaluation</b>	<b>Treatment Modality</b>
<i>Available before treatment</i>	<b>Adjuvant chemotherapy</b> <b>Adjuvant radiotherapy</b> <b>Adjuvant endocrine therapy</b>
<i>Available after treatment</i>	<b>Surgery</b> <b>Primary medical therapy</b> (neoadjuvant chemotherapy and primary endocrine therapy) - only in patients with no response or partial response to treatment
<i>Not Available</i>	<b>Primary medical therapy</b> – in complete responders <b>Novel experimental ablative therapies</b> (e.g. Interstitial laser photocoagulation,)

Tissue markers are qualitative or quantitative alterations or deviations from normal of a molecule, substance or process that can be detected by some type of assay (Hayes et al. 1996). Tissue factors include tissue markers, as well as other routine histopathologic or laboratory evaluations. It is important from the outset, to make a distinction between *prognostic* and *predictive* factors. Prognostic factors are clinical, pathologic or biological features of cancer patients that predict clinical outcome (Allred et al. 1998), in terms of outcome variables such as disease free or overall survival. Predictive factors, are clinical, pathologic or biological features that are used to estimate the likelihood of a response to a particular type of adjuvant therapy (Allred et al. 1998). The recognition of specific prognostic groups is of current interest since it helps identify subgroups of patients who may be targeted by a specific therapeutic approach and thus avoid the need to treat a large group of patients for the benefit of a minority. However, although patients with more advanced disease are most in need of adjuvant therapy, they may not necessarily be the best responders to treatment. Predictive factors help identify patients who are more likely to respond to adjuvant medical treatment.

Since there are numerous studies on different prognostic and predictive factors, official guidelines have been published to assist in the evaluation of putative factors (McGuire,

1991; Goldhirsch et al. 1995; Henson et al. 1994; Hayes et al. 1996; American Society of Clinical Oncology, 1996). Initially, all tests for prognostic or predictive factors must be technically validated in order to determine their sensitivity, specificity and reproducibility. Subsequent clinical validation ensures that the technique used identifies a subgroup of patients with significantly different relative risks for recurrence, survival or response to treatment. Lastly, and most importantly, it is essential to demonstrate that the prognostic or predictive factor is both an *independent* prognostic indicator and is *clinically useful*. For example, the absolute risk reduction in overall survival in postmenopausal women achieved by combined chemoendocrine therapy over tamoxifen alone, is in the order of only 1% in 5 years (Ravdin, 1998). Clearly, putative prognostic and predictive factors must enable the identification of women with poorer prognosis that are most likely to respond to therapy. Only those putative prognostic and predictive factors that have been demonstrated to have an *independent* significance in multivariate analyses, have a place in clinical management. Furthermore, since the risk reduction in disease free survival is low, a predictive factor will be clinically useful only if it is able to identify a subgroup of women which a significantly greater risk reduction.

The most clinically important prognostic factors to date, are listed in table 4.2. Amongst the clinical, pathological and biological factors identified to date, pathological factors appear to be the most important (Elston et al. 1998; Allred et al. 1998). Of these, lymph node status and tumour size, are the most powerful and form the basis of the TNM classification for breast cancer staging.

Although many prognostic factors have been shown to be associated with a biological process or with biological end points, that does not imply that they will ultimately be considered as clinically useful factors. Several authors have attempted to grade the evidence available on individual prognostic factors in an attempt to define their clinical utility. Hayes et al. (1996) proposed a Tumour Marker Utility Grading System (TMUGS) to evaluate the clinical utility of individual markers in terms of the end points of overall survival, disease free survival, quality of life and cost of care. Levels of evidence for grading

**Table 4.2: Prognostic factors for breast cancer:**

Axillary nodal Involvement  
Tumour size  
Age  
Menopausal status  
Hormone Receptor Status  
Histological Grade  
Other: tumour type, vascular invasion

clinical utility were considered and classified between level I (evidence from prospective controlled clinical trial) and level V (evidence from pilot study). Although the authors did not test their proposed grading system on individual factors, Ravli et al. (1998) did. They concluded that only ER and PR status were backed by level I/II evidence and only as predictive markers, not prognostic indicators.

At the College of American Pathologists Conference XXVI (Henson et al. 1994), working groups subdivided breast cancer prognostic factors into 3 categories (table 4.3). Category I factors are well supported by outcome studies and are used in therapeutic decision making. Category II factors have been tested in clinical trials (category IIA) or have undergone biological and correlational studies with few clinical outcome studies (category IIB). Category III factors, have not been studied or have not been found to be clinically useful. Again, the only biological prognostic markers to have been included in category I were ER and PR status assessed by dextran coated charcoal (biochemical ligand-binding assay). Although many centres use immunohistochemical techniques to assess ER and PR status, techniques vary, currently fall short of requirements for a good prognostic or predictive marker, and both technical and clinical validation by individual centres is advised (Allred et al. 1998). Immunohistochemistry for angiogenic markers (FVIIIRAg, CD31, CD34) has been classified in category IIB since few comparative studies exist showing the association of these with other prognostic markers.

**Table 4.3: College of American Pathologists' Classification of prognostic markers for breast cancer:**

- I *Well supported factors*
  - Pathological TNM
  - Histological tumour type
  - Histological tumour grade
  - ER /PR status by dextran coated charcoal
- II. *Extensively studied factors*
  - A. *Factors tested in clinical trials*
    - Proliferation markers: mitotic count; S-phase; Ki-67 (MIB1)
    - ER / PR by immunoassay (tissue or cytosol)
  - B. *Factors backed by biological and correlative studies*
    - c-erb/B2*
    - p53
    - Angiogenesis by immunohistochemistry
    - Vascular invasion by histology
- III. Factors that do not meet criteria for category I or II
  - All other markers

The American Society of Clinical Oncology (ASCO) produced clinical guidelines for the use of tumour markers for breast and colorectal cancer (Toi et al. 1996; American Society of Clinical Oncology, 1998). A multidisciplinary panel considered 7 breast cancer markers (serum CA 15.3, serum CEA, ER and PR status, DNA flow cytometry DNA index and proliferation index, p53, *c-erb B2*). Again, only ER and PR status were recommended to be measured routinely on all primary breast cancers, although they were considered predominately predictive markers and only weak prognostic markers. Present data was considered insufficient for recommending the other markers and recommendations remained unchanged when the committee reconvened a year later (American Society of Clinical Oncology, 1996).

Inevitably, some factors will eventually be shown to be clinically useful independent factors. Large numbers of prognostic indices may require the development of a multivariate prognostic index. A more simple index, the Nottingham Prognostic Index (NPI), already exists and combines tumour size, lymph node stage (stage 1 - no lymph node involvement; stage 2 - 1-3 nodes involved; stage 3 -  $\geq 4$  nodes involved) and grade

to subdivide patients into good, moderate or poor prognostic groups. It has been shown to provide clinically useful management information (Galea et al. 1992).

Computer software is also available which can provide individual patients with prognostic information and information on probable benefit of adjuvant chemotherapy based on data from large clinical studies (Ravdin, 1998). In the future, it may be possible to derive complex information on prognostic variables and compute it using both linear as well as non-linear mathematics.

### **4.3 Staging and Assessing Response to Treatment**

Staging is used to assess local disease extent and categorise patients into different groups. The ultimate aim of staging is to aid in planning treatment, assist in and standardise the evaluation of response to treatment, provide an indication of prognosis and facilitate exchange of information between centres.

The TNM classification (table 4.4) of the *Union International Contre le Cancer* (UICC), is the most widely used classification for staging breast cancer (UICC, 1997). It uses primary tumour size (T) lymph node involvement (N) and metastatic spread (M) to classify disease stage. However, it is well recognised that clinical staging is quite often inaccurate. The error rate of clinical evaluation of axillary nodal status can be as high as 30%, and clinical assessment of tumour size may over or underestimate histological tumour size (Schottenfield et al. 1976). Furthermore, concerns about the clinical usefulness of the TNM classification, have been raised (Barr and Baum, 1992). Pathological evaluation of TNM (pTNM) is accurate but is not available until after surgery and thus cannot be of assistance in planning the extent of surgical resection or PMT.

In 1975, the UICC published guidelines on clinical assessment of response to treatment (Hayward et al. 1977). The classification suffers from a pronounced loss of precision since it is composed of only 3 categories. Despite the additional limitations of clinical assessment, the UICC criteria have resulted in international standardisation of published results of clinical studies over the last 2 decades, and are still in use today (table 4.5).

**Table 4.4: Staging of breast cancer:**

**(1) The UICC TNM Classification: primary tumour (T); regional lymph nodes (N); and distant metastasis (M).**

- TN: Primary tumour cannot be assessed**
- T0: No evidence of primary tumour**
- Tis: Carcinoma *in situ***
- T1: Tumour < 2cm**
- T2: Tumour  $\geq 2$  cm  $\leq 5$ cm**
- T3: Tumour > 5cm**
- T4: Tumour fixation to chest wall or skin**
  
- NN: Regional lymph nodes cannot be assessed**
- N0: No axillary involvement**
- N1: Mobile involved axillary nodes**
- N2: Fixed axillary lymph nodes**
- N3: Supraclavicular lymph node involvement or arm swelling**
  
- MN: Presence of distance metastasis cannot be assessed**
- M0: No metastatic spread**
- M1: Distant metastases present**

**(2) Stage grouping**

<b>Stage 0</b>	<b>Tis N0 M0</b>
<b>Stage 1</b>	<b>T1 N0 M0</b>
<b>Stage IIA</b>	<b>T1, or T0, N1 M0 / T2 N0 M0</b>
<b>Stage IIB</b>	<b>T2 N1 M0 / T3 N0 M0</b>
<b>Stage IIIA</b>	<b>T0, or T1, or T2, or T3, N2 M0 / T3 N1 M0</b>
<b>Stage IIIB</b>	<b>T4 any N M0 / any T N3 M0</b>
<b>Stage IV</b>	<b>Any T, any N, M1</b>

It is evident that there is a need for novel ways to classify breast tumours in a more accurate fashion. Perhaps more pragmatic future schemes, may incorporate prognostic factors more closely related to prognosis, and this may replace anatomical staging. But it is likely that anatomical staging will still be required in the pre-operative assessment of breast cancer. Whether MRI or US can fulfil all the requirements of a clinically useful staging modality awaits to be proven.

**Table 4.5: UICC criteria for the assessment of response to treatment:**

- CR** – Complete Response
- PR** – Partial Response
- NC** – No change
- PR** – Progression



#### **4.4 Tumour Angiogenesis as a Prognostic Indicator**

Angiogenesis or neoangiogenesis, is the process which permits a tumour to stimulate the formation of new blood vessels from a pre-existing vascular bed. It is now well recognised that the growth of a tumour is dependant on its ability to develop a blood supply. Angiogenesis is an essential component of many physiological and pathological processes but recent developments in angiogenesis research have had important clinical implications on both the determination of prognosis and on the implementation of anti-angiogenic therapeutic strategies for cancer treatment (Gasparini, 1996b).

Studies have shown that tumours cannot grow beyond  $10^6$  cells or about 1-2 mm in diameter, in the absence of a blood supply (Folkman et al. 1989; Folkman, 1990). The initial growth phase or prevascular phase (Folkman et al. 1989; Weidner et al. 1991), is followed by a vascular phase in which tumour induced angiogenesis is the rate limiting step for further tumour growth but also provides tumour cells with direct access to the circulation. The poor prognostic implication of extensive angiogenesis quantified by intratumoural microvessel density (MVD) in histological sections is well recognised in a wide variety of cancers (Engel et al. 1996; Bikflavi, 1995; Carrau et al. 1995; Frank et al. 1995; Jaeger et al. 1995; Ogawa et al. 1995; Wiggins et al. 1995) including breast cancer. Although not all studies on breast cancer have demonstrated an association between MVD and prognosis (Morphopoulos et al. 1996; Axelsson et al. 1995), several studies of between 155 and 191 patients with multivariate analysis, have demonstrated a significant association between MVD and both relapse free as well as overall survival (Weidner et al. 1992; Bosari et al. 1992; Ogawa et al. 1995; Bevilacqua et al. 1995; Gasparini et al. 1995; Gasparini et al. 1996). An overview of the literature by Gasparini et al. (1996a) showed that in 69% and 80% of breast cancer studies with multivariate analysis, MVD was an independent prognostic indicator for relapse free survival and overall survival, respectively.

It is thus likely that MVD is associated with tumour aggressiveness. This association may be explained in several ways:

- (1) *propensity to metastasis*: angiogenic tumours may have an increased propensity for haematogenous tumour cell spread or MVD may be proportional to the size of the vascular bed through which tumour cells can metastasise.
- (2) *increased tumour perfusion*: MVD may be a surrogate marker of the ability of a tumour to induce stromal cell growth, leading to improved tumour perfusion.
- (3) *tumour – stromal paracrine effects*: MVD may be proportional to the density of endothelial cells producing overall promoting factors that stimulate tumour growth.
- (4) *tumour – stromal endocrine effects*: Systemic factors may play a role in both stimulation of tumour as well as stromal growth, and increased MVD may result. A recent animal study by Haran et al.(1994) suggested that tamoxifen's antioestrogenic effects, also lead to reduced vascularity in MCF7 breast cancer cells. This particular mechanism may also be responsible for the association between timing of surgery in the menstrual cycle, and patient outcome.

The standard technique used to stain microvessels, is immunohistochemistry using antibodies specific to endothelial markers the most frequently used being FVIIIIRAg, CD31 and CD34. Studies in which MVD has been demonstrated to be an independent and significant prognostic factor have used CD31 (Gasparini et al. 1995; Gasparini et al. 1996; Bevilacqua et al. 1995) and FVIIIIRAg (Weidner et al. 1992; Bosari et al. 1992; Ogawa et al. 1995). In a retrospective study of 167 node negative breast cancers, only MVD (using CD34) was found to be an independent prognostic indicator for disease free and overall survival, in a multivariate analysis (Heimann et al. 1996). In a recent comparative study of MVD quantification using CD31, CD34 and FVIIIIRAg, CD34 was found to be the most reproducible and reliable (Martin et al. 1997). CD31 and CD34 stain both large and small vessels with equal intensity in normal and tumour tissue, whereas FVIIIIRAg stains predominantly large vessels and capillaries with variable intensity. With CD31 occasional plasma cell staining is seen whereas with CD34 and FVIIIIRAg, some lymphatic endothelial staining is seen.

Following immunohistochemical staining, several techniques are available to quantify MVD:

(1) *Weidner's method of counting vascular hot spots*: In the first report of the correlation of breast cancer angiogenesis with metastasis, Professor Noel Weidner et al. (1991) described scanning slides at low power to select areas of vascular 'hot spots', and subsequently at high power, counting individual capillaries and venules in 3 regions. The training and experience of the investigator is important in the selection of vascular 'hot spots'. Once these have been selected, any separate endothelial cell or cell cluster is counted as a separate vessel. It is important to validate the results by using 2 experienced observers (Vermeulen et al. 1996). A major drawback of this technique is that it can be very time consuming.

(2) *Grading of MVD*: This involves semi-quantitative grading of microvessel density in vascular 'hot spots'. It is of course subjective and relies on the experience of the investigator. An advantage is the reduced analysis time compared with Weidner's method. Weidner et al. (1992) demonstrated with both the grading and Weidner's counting method that MVD is an independent prognostic indicator in breast cancer.

(3) *Chalkley counting*: In an attempt to speed up microvessel counting, Fox et al. (1995) applied Chalkley point counting to MVD estimation and demonstrated that it gave independent prognostic information. Initially, slides are scanned to identify vascular 'hot spots', and subsequently at high power, a Chalkley eyepiece graticule containing 25 randomly positioned dots, is used for counting. The graticule is rotated so that the maximum number of points, are overlying microvessels. Rather than counting each microvessel, the dots that overlay vessels are counted to obtain a score, out of a total of 25. Despite the obvious advantage of increased speed of counting, it appears that the counting step in this technique is less subjective than that in Weidner's counting method, since a decision on the independence of individual vessels is not necessary (Vermeulen et al. 1996).

(4) *Computerised image analysis methods*: Computer analysis enables analysis of several additional morphometric parameters such as vessel lumen area, vessel lumen perimeter and the percentage of immunostained area per microscopic field (Vermeulen et al. 1996). An obvious advantage is the reduced subjectivity of analysis but this method is quite time consuming since individual 'hot spots' still require selection by experienced

observers. In a series of 91 node-negative breast cancers stained using CD31 (Barbareschi et al. 1995), microvessel area determined by computer image analysis, was shown to be an independent prognostic indicator for disease free survival but not for overall survival. In another study of 178 breast cancers (Simpson et al. 1996), total immunostained area was estimated using CD34 and shown to be an independent prognostic indicator for overall survival only in node negative patients and for disease free survival only in node positive patients. Although the authors found that tumour grade was independently associated with overall survival, Weidner's counting method was not, raising doubts on the reproducibility of these data. Additional information on microvessels can be obtained by computerised image analysis, but this technique requires further refinement as well as standardisation of the counting method itself, to enable inter-centre comparison.

Of the novel prognostic indicators, angiogenesis appears to be the most powerful. On balance, it is evident that the most reliable technique appears to be Chalkley counting, using CD31 for immunostaining. Prospective studies to assess clinical usefulness, are still lacking.

#### **4.5 Imaging Modalities in the Prognostic Assessment of Breast Cancer**

For the reasons stated at the beginning of this chapter, there is a growing interest in the potential application of modern imaging modalities to derive prognostic information on breast tumours. Research in this field is currently at the initial stage and several imaging modalities have been studied including MRI, spiral CT, colour and power Doppler US. However, MRI and colour Doppler ultrasound appear to be the most promising and have been used in this thesis.

##### ***4.5.1 Assessment of breast cancer angiogenesis***

Most research into angiogenesis has been conducted from a basic scientific perspective. Novel clinical imaging techniques are currently being evaluated as tools to study angiogenesis. Contrast enhancement of both MRI and US has been instrumental in improving the differentiation of normal from abnormal breast tissue, and the mechanism for this, is thought to be differences in tissue vascularity. Furthermore, the spatial

resolution of MRI is such that lesions greater than 2mm, which are angiogenesis dependant (Folkman et al. 1989; Folkman, 1990), may be detected.

Dynamic contrast enhanced MRI, allows the acquisition of kinetic information on tumour enhancement. As was discussed in chapter 2, breast tumours enhance more rapidly than benign lesions, although a certain amount of overlap exists. In animal studies, using extremely high magnetic field strength (e.g.: > 4.7T), some studies have demonstrated that angiogenesis may be an important mechanism for certain clinical observations. Haran et al. (1994) demonstrated that tamoxifen reduces the MVD of implanted MCF7 breast tumours as well as their contrast-enhancement. They postulated that the anticancer effects of tamoxifen are at least partly mediated by an antiangiogenic mechanism of action. Abrahamovitch et al. (1998), used high magnetic field strength MRI to demonstrate that in nude mice, if cancer cell spheroids are implanted within 0.5cm of a fresh wound, wound healing is delayed and tumour growth is promoted. They postulated that the tumour angiogenesis triggered by a surgical wound, may stimulate tumour growth. Vandijke et al. (1996) used albumin-bound Gd-DTPA, a prototype intravascular contrast agent, to estimate plasma volume and microvascular permeability of implanted R3230 mammary carcinoma in nude mice, using dynamic contrast-enhanced MRI. They demonstrated a correlation between kinetic measurements of contrast enhancement and histologic capillary density measurements. It was postulated that MRI may provide physiologic information, in addition to anatomical information on entire breast tumours, non-invasively. Unfortunately, this albumin-bound contrast agent cannot be used clinically as a result of the risk of anaphylactic reactions. Hoffmann et al. (1995), also described a very elegant technique in which signal intensity on dynamic MRI was used to derive pharmacokinetic parameters which relate to tissue perfusion and capillary permeability. Unfortunately, the mathematical equations used are complex and image analysis time consuming.

Recent clinical studies have correlated contrast enhancement with tumour angiogenesis. In a qualitative comparative study of 9 patients with palpable breast lumps, Frouge et al. (1994) suggested that early contrast enhancement on dynamic MRI, may be used to characterise different small invasive carcinomas since it appeared to correlate with a qualitative grading of MVD, as assessed by immunohistochemistry with FVIIIIRAg. Later studies demonstrated a correlation between percentage contrast enhancement on high

resolution MRI (Bone et al. 1998) as well as the initial rate of contrast-enhancement on dynamic MRI (Buadu et al. 1997b; Buadu et al. 1996; Frouge et al. 1994) and MVD. These studies suggest a potential role for MRI in monitoring intratumoural angiogenesis from kinetic information on contrast-enhancement. Furthermore, these findings suggest that the mechanism for breast tumour characterisation is due to differences in angiogenesis, between benign and malignant lesions. Although this is an important observation, it is not as yet clear whether it would result in a substantial improvement in specificity for breast cancer detection. Furthermore, the association between rate of enhancement and MVD has not been demonstrated by all studies. Buckley et al. (1997), studied 40 patients with dynamic contrast-enhanced MRI and correlated parameters from dynamic data with MVD. None of the overall parameters correlated with MVD. Subgroup analysis, demonstrated a correlation between MVD and enhancement index at 1 minute, in node-positive patients. However, this study was retrospective and paraffin blocks could not be correlated with MRI images. More research work is necessary to define any correlation between enhancement intensity and MVD.

Colour Doppler ultrasound has been used in patients with breast cancer to study microvascular blood flow patterns and characteristics within tumour areas, but studies of histopathologic correlation have failed to demonstrate an association between MVD and colour Doppler signal (Lee et al. 1995; Peters-Engl et al. 1998; Buadu et al. 1997a). With respect to prediction of lymph node status, some studies have shown a higher risk of lymph node involvement in tumours that are vascular on ultrasound (Sterns et al. 1996; Kubek et al. 1996; Lee et al. 1995; Lee et al. 1996; Peters-Engl et al. 1998) and some demonstrated no correlation (Cosgrove et al. 1993; Yang and Metreweli, 1998). Although Lee et al. (1995; 1996) found an association between high tumour flow velocity and lymph node involvement in tumours under 2 cm in size, no correlation was found in larger tumours. Dixon et al. (1992) used colour Doppler ultrasound to scan the axillas of 29 patients prior to axillary clearance and demonstrated, based on a subjective assessment of vascularity, that it was possible to predict axillary nodal involvement with a sensitivity of 75% and specificity of 100%. However, this data has not been confirmed by other studies. None of the studies to date demonstrated a correlation between quantitative assessment of lymph node vascularity on ultrasound and lymph node status.

Refinements in three dimensional spatial and real-time flow replication using colour Doppler ultrasound are underway. In combination with the clinical introduction of novel ultrasound contrast agents, such as microbubble contrast agents (Goldberg et al. 1993), visualisation of smaller vessels and their non-invasive quantification may be achievable and may result in closer correlation with tumour angiogenesis.

Research in the field of angiogenesis is extending to include other imaging modalities such as breast scintimammography using  $^{99m}\text{Tc}$ -sestamibi. Scopinaro et al. (1994), found a correlation between MVD and  $^{99m}\text{Tc}$ -sestamibi uptake in a study of 19 breast cancer patients. Although encouraging, confirmatory studies as well as studies of clinical utility, are still required.

#### ***4.5.2 Correlation with other prognostic indicators***

Amongst the novel imaging modalities, the striking histopathologic correlation seen with MRI, makes it the most promising imaging modality with respect to obtaining prognostic information from breast tumours. Measurement of tumour size by MRI correlates closely with histological tumour size (Mumtaz et al. 1997b; Boetes et al. 1995; Kerslake et al. 1995). In a study of 51 women, Mussurakis et al. (1997b) suggested that maximum enhancement intensity on dynamic MRI is a stronger predictor of axillary node status than patient age or maximum tumour cross-sectional area.

As yet pharmacokinetic parameters related to tissue perfusion and capillary permeability (Hoffmann et al. 1995), have not been shown to correlate with known prognostic indicators. In a semidynamic study (1 pre- and 2 post-contrast scans) Bone et al. (1998), demonstrated a univariate correlation between early contrast enhancement on MRI and MVD, as well as proliferating cell activity, but no correlation was demonstrated with tumour size, lymph node status, histological type or malignancy grade. Buadu et al. (1996), demonstrated a good correlation between dynamic parameters of signal intensity and MVD, but no correlation with 2 prognostic indicators, namely age and tumour size.

The association of other prognostic factors with signal intensity on MRI has been studied but results to date have been disappointing. Mussurakis et al. (1997a) in a study of 53 patients, used signal intensity ratios derived from ROI's on dynamic contrast-enhanced

MRI, and correlated them with lymph node status, tumour size, tumour type, multifocality, tumour grade, presence of extensive *in situ* component and lymphovascular invasion. Multiple regression analysis, showed that only lymph node status and histological grade, were independent statistically significant predictors of automated ROI's enhancement ratio. Unfortunately, despite the fact that this was a retrospective study, no attempt was made to correlate prognostic indicators and enhancement ratio, to either disease free or over all survival, using multiple regression analysis. Bone et al. (1998) demonstrated a correlation between MVD and contrast intensity but univariate analyses between contrast intensity and other important prognostic indicators (lymph node status, tumour size, histological type and grade) showed no correlation. Enhancement ratio as an independent prognostic indicator, is thus as yet unproven.

Ultrasound has also been used in an attempt to derive prognostic information on breast cancer. Grey-scale ultrasound can be used to assess tumour size although it often underestimates it (Pain et al. 1992). Several studies have attempted to correlate colour Doppler ultrasound with other prognostic indicators, but results have so far been disappointing (Lee et al. 1996; Sterns et al. 1996).

#### **4.6 Summary**

Clearly, no single test, prognostic factor or consensus statement by expert panels can replace good clinical judgement combined with an informed patient involvement in decision making. However, additional clinically useful information, will assist the realistic assessment of benefits of therapy for a given patient. The optimal management of patients with breast cancer should then be carried out on a multidisciplinary basis. Prospective decisions should be taken in the presence of surgeons, oncologists, histopathologists and radiologists.

So far, the most powerful prognostic indicators remain the histopathological ones. Of the biological prognostic indicators, MVD appears to be the most promising and has been demonstrated to be an independent prognostic indicator for breast cancer. Assessment of MVD by immunohistochemistry appears to be optimal using CD31 or CD34. Of the histological techniques used to quantify MVD, the Chalkley point counting method



appears to be the quickest and least subjective. Prospective studies are now necessary to assess the clinical usefulness of MVD.

Of the novel imaging modalities, some studies suggest that contrast enhancement on dynamic MRI, correlates with tumour angiogenesis. The strength of this correlation varies in different studies and some even found no correlation. Studies using colour Doppler US, have failed to demonstrate a correlation with MVD. Further studies are required to define or refute the correlation between these imaging modalities and angiogenesis. It is also necessary to ~~confirm~~ confirm the clinical utility of a correlation with tumour angiogenesis in order to decide whether prognostic information may be obtained in this way and if imaging modalities may be of assistance in assessing response to novel antiangiogenic therapies.

With respect to the use of MRI and US to obtain prognostic information on breast cancer, the jury is still out. Assessment of tumour size and extent appear to be the only reliable prognostic information at present. Further research is necessary before MRI or US may be shown to be useful in assessing prognosis in breast cancer.

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## **Section B: MRI In The Management Of Breast Cancer**

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**Chapter 5: Current Clinical Indications for Breast MRI**

**Chapter 6: Additional Enhancing Foci Detected by MRI**

**Chapter 7: Current Influence of Pre-operative MRI on Extent of  
Surgical Resection**

**Chapter 8: Comparison of Surgical and MRI Assessments of Tumour  
Resectability**

**Chapter 9: MRI in the Assessment of Response to Primary Medical  
Therapy**

# **Chapter 5: Current Clinical Indications for Breast MRI**

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5.1 Introduction

5.2 Patients and Methods

5.3 Results

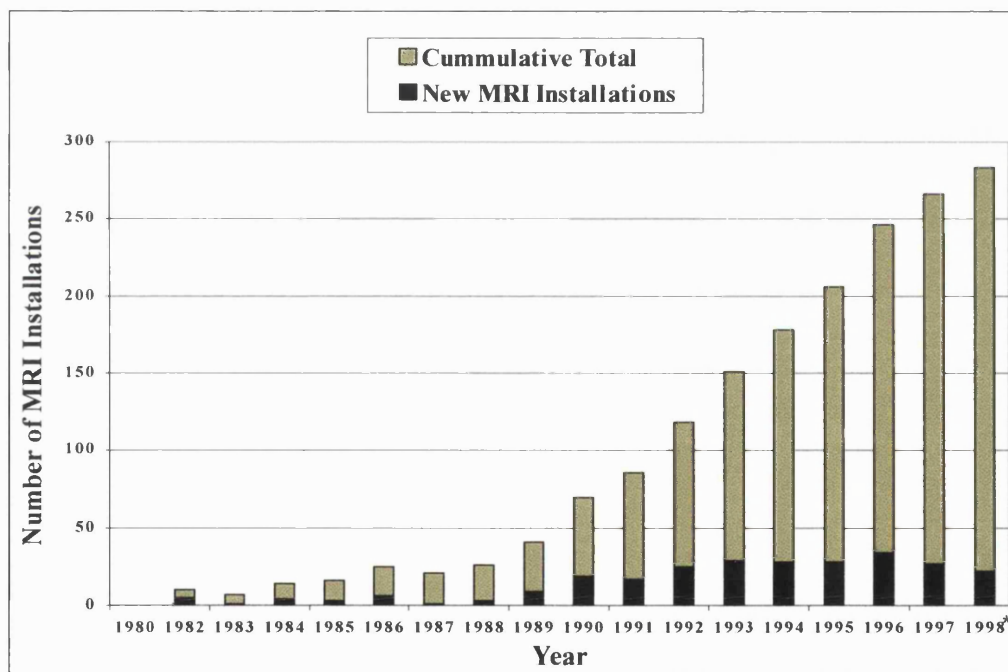
5.4 Discussion

## 5.1 Introduction

The availability of MRI (figure 5.1), and its application to novel anatomical sites, has increased dramatically in the UK over the last decade. As a result, an increasing number of centres are now imaging the breast. Breast MRI has been introduced to the Middlesex Hospital in 1994 and since then, the number of breast MRIs has increased from under 40 in 1994 to 148 in 1997. It is thus important from the outset to determine the current indications for breast MRI at this unit.

**Figure 5.1: Cumulative total and yearly MRI installations in the UK:**

Figures were obtained from Dr J. De Wilde, MRI Evaluation Centre for the Medical Devices Agency (MagNET) Database, Electrical Engineering Department, Imperial College of Science Technology and Medicine, London.



\* Figures for 1998 are until April 1998 only.

## 5.2 Patients and Methods:

All patients scanned in 1997 were reviewed and indications for breast MRI determined. Definitive histology, when available, was obtained from patient records. Breast MRI was performed within 3 research slots per week although occasionally patients underwent

scans privately outwith these slots. Clinical patients were scanned within these research slots although patients scanned for research were given priority.

### 5.3 Results

A total of 124 patients underwent 148 breast MRIs. These are listed in table 5.1 with respective indications.

**Table 5.1: Breast MRI performed at The Middlesex Hospital in 1997:**

<i>Indication for MRI</i>	<i>Total Number of Patients (%)</i>	<i>Total Number of MRI scans</i>
<b>Detecting local recurrence (clinical)</b>	<b>41 (33%)</b>	<b>41</b>
<b>Pre-operative staging and correlation with prognostic (research)</b>	<b>37 (30%)</b>	<b>37</b>
<b>Laser therapy (research)</b>	<b>18 (15%)</b>	<b>31</b>
<b>Primary medical therapy (research)</b>	<b>10 ( 8%)</b>	<b>21</b>
<b>Other (clinical)</b>	<b>18 (15%)</b>	<b>18</b>

Of 124 patients, 65 patients (52%) underwent breast MRI within research protocols and 59 patients (48%) for clinical reasons. Out of the research protocols, most patients were entered into the pre-operative MRI protocol for staging and correlation with prognostic variables (37 patients; 30%). Pre- and post-treatment high resolution MRI was performed on 18 patients who had undergone interstitial laser photocoagulation (ILP). Of these, 19 scans were performed on 12 patients with fibroadenomas and 12 scans on 6 patients with breast cancer. Twenty one MRI scans were performed on 10 patients undergoing PMT.

Of those patients undergoing MRI for clinical reasons, detection of local recurrence was the commonest indication (figure 5.2). Of 41 patients, 16 were diagnosed with recurrence (3 axillary) and 25 patients had negative scans. Of those diagnosed with recurrence, one patient was a false positive since extensive lesion enhancement was seen. Cytology in this case was reported as C4 and a mastectomy was performed. Extensive

histological sampling showed scarring and inflammation but no malignant lesions were detected. An additional 2 patients, with breast implants following mastectomy for breast cancer, underwent MRI but no enhancing lesions were identified.

Eighteen patients underwent breast MRI for a range of other indications (table 5.2).

**Table 5.2: Other clinical indications for breast MRI:**

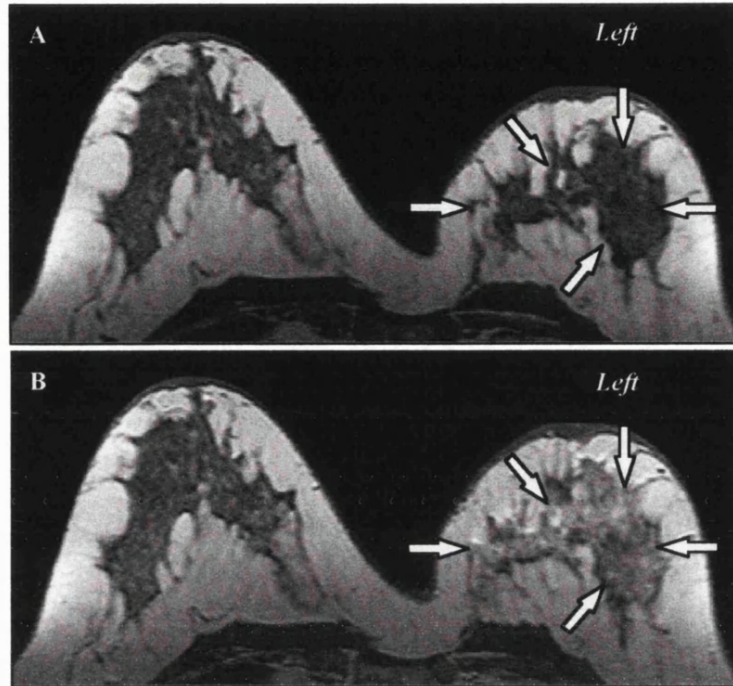
<i>Indication for MRI</i>	<i>Total Number of Patients</i>	<i>Number of patients with positive scans</i>
<b>DCIS ?small focus of invasive cancer</b>	<b>5</b>	<b>0</b>
<b>Detection of silicon implant leak</b>	<b>4</b>	<b>0</b>
<b>Bilateral fibromatosis ? tumour</b>	<b>3</b>	<b>0</b>
<b>Axillary node metastasis ? breast primary</b>	<b>2</b>	<b>0</b>
<b>Axillary staging – patient refused surgery</b>	<b>1</b>	<b>0</b>
<b>Paget’s disease ? breast primary</b>	<b>1</b>	<b>1</b>
<b>Pregnant women with breast mass</b>	<b>1</b>	<b>0</b>
<b>Christmas disease and previous breast cancer - ? recurrence</b>	<b>1</b>	<b>0</b>

A patient with nipple asymmetry and an eczematous induration of the left nipple underwent cytology and was found to be suspicious (C3). A clinical diagnosis of Paget’s disease of the nipple, was made and mammogram requested. The mammogram did not show any abnormality apart from asymmetry of the nipples, and was reported as within normal limits when compared to a previous mammogram. MRI was undertaken in order to determine whether a primary was present. It showed an intensely enhancing mass involving and distorting the left nipple in an otherwise normal breast (figure 5.3). These findings were highly suggestive of a malignancy and at subsequent wide local excision, a diagnosis of mixed tubular carcinoma of the nipple was made.

All other patients had negative scans. A pregnant women underwent MRI prior to wide local excision of a Phylloides tumour, but since contrast could not be administered, MRI did not assist in clinical management of this case. In a women with Christmas disease,

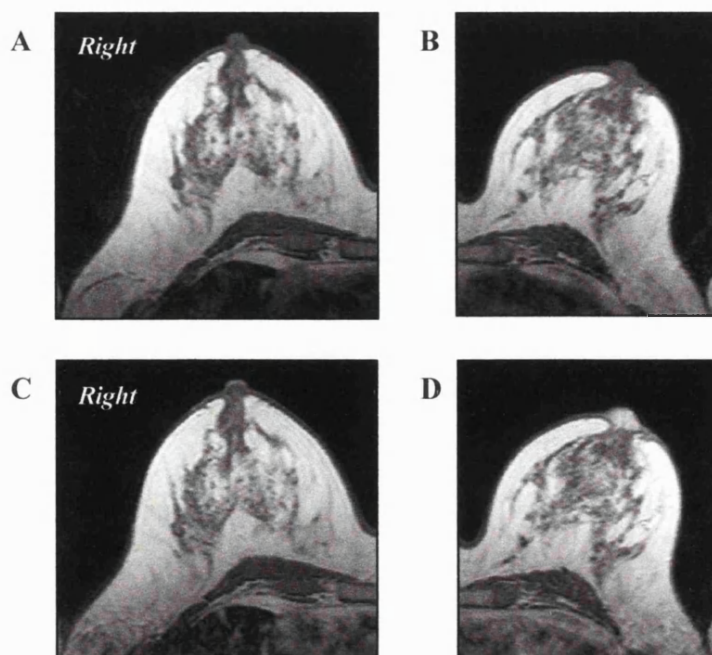
cytology of a clinically suspicious scar was reported as C3 (suspicious) and biopsy advised. This patient refused a biopsy and an MRI scan was negative for recurrence.

**Figure 5.2:** Pre- (A) and post- (B) contrast-enhanced MRI scan showing extensive enhancement of local recurrence in the left breast (arrows).



**Figure 5.3:** Pre- and post contrast enhanced MRI of a tubular carcinoma of the nipple:

MRI of the left and right nipples before contrast enhancement (A & B) and after contrast enhancement (C & D). The 'enhancing cap' appearance of the normal right nipple (C) is distinctly different from the distortion and intense enhancement seen in the left nipple (D).



## 5.4 Discussion

In 1997, 52% (65/124) of patients underwent breast MRI for research and 48% (59/124) for clinical indications. Of the clinical indications, detection of recurrence (41/124) was the commonest indication, and this is in agreement with data produced by this centre for 1996 (Mumtaz et al. 1997a). In these cases, MRI demonstrated both the extent of recurrence as well as its relation to underlying structures. However, diagnosis was based on triple assessment and histology. Since tumours that are clinically mobile are regarded as resectable, it is not clear whether the additional information obtained from MRI has had an impact on management. Interestingly, in the patient with a false positive MRI, a C4 cytology in combination with strong and extensive enhancement on MRI 18 months after initial surgery, was regarded as strong enough evidence to recommend a mastectomy. In this case, MRI was positively unhelpful.

In unusual situations (table 5.2), MRI has been requested in order to assist in decision making but since there is little data to support its use, it is not possible to come to any conclusions. With respect to detecting silicone implant leaks, although the literature suggests that MRI is useful, very few MRI scans were requested at this centre. In the patient with tubular carcinoma of the nipple, MRI was the only investigation to detect a tumour of the nipple and also suggested that it was amenable to wide local excision since no other breast lesion was detected. Interestingly, in 2 patients with axillary metastasis, no primary tumour was found. One of these patients had been on tamoxifen for 1 year (since diagnosis) and a second MRI did not detect any primary. It is thus possible that tamoxifen has suppressed the growth of a small subclinical breast tumour.

Breast MRI may prove useful in many clinical situations but potentially could also be unhelpful. Prior to assessing ways in which MRI *could* assist in surgical management, it is important to determine the significance of additional enhancing lesions on MRI and whether MRI *does* influence surgical management at present. This was evaluated in the subsequent chapters.



# **Chapter 6: Additional Enhancing Foci Detected by MRI**

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- 6.1 Introduction
- 6.2 Patients and Methods
  - 6.2.1 Patients
  - 6.2.2 Ethics
  - 6.2.3 Breast MRI
  - 6.2.4 Surgery
  - 6.2.5 Histopathology and specimen radiography
- 6.3 Results
- 6.4 Discussion and Conclusion

## **6.1 Introduction**

When mastectomy specimens are examined by detailed radiological-histological correlational methods, small additional invasive or in-situ cancer foci are found in over 60% of patients; 80% of these lie remote from the previously operated quadrant (Vaidya et al. 1996). Since 90% of local recurrences occur in the operated quadrant, the clinical relevance of small cancer foci has been questioned (Baum et al. 1997). However, so far small cancer foci have been diagnosed solely by histology since no imaging modality has been able to detect such small lesions, pre-operatively.

The aim of this study was to determine whether small enhancing foci seen separately from the main tumour on contrast-enhanced MRI, are cancer foci and whether MRI could detect all cancer foci identified by radiological-histological correlation.

## **6.2 Patients and Methods**

### ***6.2.1 Patients***

Ten consecutive breast cancer patients were recruited prospectively following diagnosis of breast cancer by triple assessment (clinical examination, mammography and fine needle aspiration cytology). Patients undergoing excisional biopsy were excluded. All 10 patients were suitable for MRI and consented for the procedure.

### ***6.2.2 Ethics***

Ethical approval for patient recruitment and histopathological correlation was obtained from University College Hospital Ethics Committee.

### ***6.2.3 Breast MRI***

High resolution transverse T1-weighted 3D FLASH images (TR=18 ms, TE=7 ms, FA=40°, TA=4 m 56 s, FOV=410 mm) before and after an intravenous bolus hand injection of dimeglumine gadopentetate (Magnevist, 0.2 mL/kg) were acquired. A 1.0 T Siemens Magnetom Scanner (42 SP) with dedicated breast coil, was used. The 3D volume was 64 mm thick with 32 partitions giving an effective slice thickness of 2 mm

and this was sufficient to cover the entire breast in all cases. All MRI images were reviewed by the investigator and a breast radiologist.

#### **6.2.4 Surgery**

After surgical excision (four mastectomies, six wide local excisions), the specimens were orientated and marked with silk sutures (long single suture=Lateral; double suture=Deep; single short suture=superior). All specimens were taken fresh to the histopathology department.

#### **6.2.5 Histopathology and specimen radiography**

Wide local excision specimens were immediately fixed in 10% formal saline. A transverse cut was made into mastectomy specimens, prior to fixation, in order to ensure fixation of deep tissues. Specimens were sliced transversely after 24 hours, at 5 mm intervals, in the same plane as the MRI. Routine histopathological examination was performed by an experienced breast pathologist.

The remaining specimen slices were pinned on cork sheets for orientation and then transferred onto acrylic sheets. Specimen slices were radiographed at equal exposure (27kV, 3mAs) using mammographic equipment (Siemens Mammomat 3000). Two experienced observers identified and noted on the x-ray films any radiological abnormalities (calcifications, densities, or spiculations). All lesions that were deemed suspicious by either observer were sampled, mounted in paraffin and examined histologically by a breast pathologist. The paraffin blocks which were found to have no discernible histological abnormality on sectioning, were radiographed and blocks deemed abnormal by either observer, sent for sectioning at multiple levels.

MRI finding were compared with histopathology results.

### **6.3 Results**

On MRI, 19 enhancing foci separate from the main tumour were identified in seven out of ten patients (figure 6.1). On radiography of specimen slices, 71 suspicious areas were sampled and histological examination revealed 15 areas of in-situ cancer (nine) or

*Chapter 6: Additional Enhancing Foci Detected by MRI*

invasive cancer (six) in five patients (table 6.1). All five patients with cancer foci were amongst the seven patients who had enhancing foci on MRI. In two of these five patients, the tumour was surrounded by widespread enhancement on MRI and all 11 (four+seven) areas sampled showed cancer foci. In all wide local excision specimens, the enhancing foci on MRI were within 11 mm of the tumour edge and therefore within the resected specimen.

**Table 6.1 Summary of (a) MRI and (b) histological findings:**

**(a) MRI: 19 foci in 7 / 10 patients**

<b>Enhancement</b>	<b>Number of Patients</b>	<b>Number of foci</b>
No enhancement	3	0
Focal enhancement	5	8
<u>Multiple enhancing foci</u>	<u>2</u>	<u>11*</u>
<b>Total</b>	<b>10</b>	<b>19*</b>

\* In the 2 patients with multiple enhancing foci, all 11 histological samples were found to harbour invasive or in-situ cancer and a count of 11 was awarded to MRI for comparison with histology.

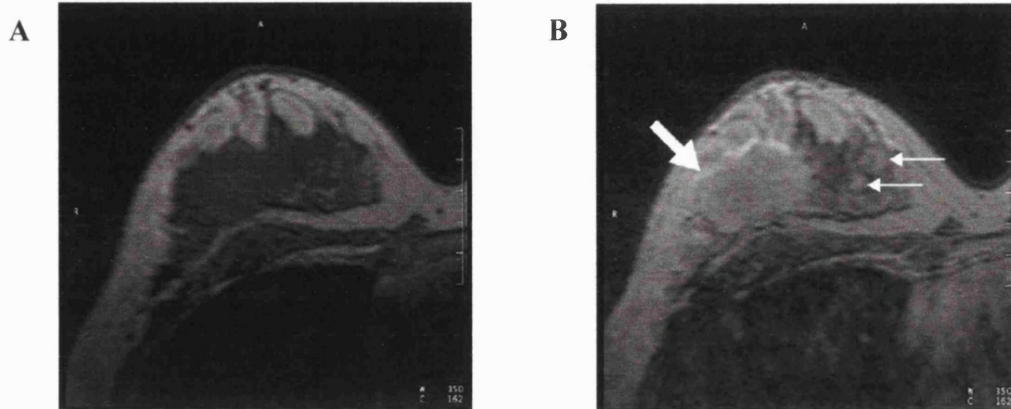
**(b) HISTOLOGY: 15 foci in 5 / 10 patients**

<b>Histology</b>	<b>Number of Patients</b>	<b>Number of foci</b>
No foci	5	0
DCIS only	2	5
IDC only	1	1
<u>IDC + DCIS</u>	<u>2</u>	<u>5 IDC + 4 DCIS</u>
<b>Total</b>	<b>10</b>	<b>15</b>

\* IDC – invasive ductal carcinoma

**Figure 6.1: T1-weighted breast MRI before (A) and after (B) contrast-enhanced MRI:**

Two separate enhancing foci (small arrows) are visible away from the primary tumour (large arrow) after contrast enhancement.



#### 6.4 Discussion and Conclusion

The data suggest that enhancing foci on MRI represent cancer foci and that MRI detected 14 out of 15 cancer foci (sensitivity 93%). Of course, this is based on the assumption that the radiological-histological correlational method is indeed the gold standard. If that is so, the specificity of MRI for tumour detection would be 79% (15/19). However, bearing in mind that the spatial resolution of MRI is of the order of 1-2 mm, it may yet transpire that MRI could have even greater sensitivity and specificity, which may become apparent with an even more obsessional sampling of the specimen.

These findings provide strong circumstantial evidence that small enhancing foci on MRI represent cancer foci and that MRI is highly sensitive for the detection of invasive or in-situ cancer foci. Since MRI-biopsy techniques are currently limited to the spatial resolution of low field strength scanners (0.5 T), as yet very small breast lesions cannot be biopsied to confirm these findings.

However, small cancer foci may never become clinically apparent in a woman's lifetime (Vaidya et al. 1996; Baum et al. 1997) and therefore resecting them may not be necessary. Furthermore, resecting these foci may be unethical since the clinical decision to resect would be based on an unproven assumption that sub-clinical MRI-detected

cancer foci, if left surgically untreated, would result in a worse outcome in terms of local control.

Leaving ethical considerations aside for a moment, evidence on the clinical significance of these additional sub-clinical cancer foci should be appreciated. Large studies of breast conservation have shown that more than 90% of local recurrences occur in the operated quadrant, irrespective of breast radiotherapy (Fisher et al. 1992), whereas cancer foci occur throughout the breast. It is as a result of these findings that the clinical significance of cancer foci (in-situ or invasive), away from the operated quadrant, has been questioned (Baum et al. 1997). The high sensitivity of MRI for breast cancer detection should therefore not lead to overzealous resections. If it does, then lessons learned from large randomised studies showing the equivalence in survival of breast conservation surgery over mastectomy, would be ignored. Whether MRI influences surgical management at present, was evaluated in the next chapter.

The results suggest that MRI could be used to investigate prospectively the clinical significance of unresected cancer foci in order to convincingly determine their natural history in the context of breast conserving surgery and radiotherapy. Such a study would be deemed ethical since breast MRI is still considered experimental for preoperative planning of surgery. To ascertain the value of MRI in clinical management, patients with enhancing foci on MRI should be randomised to either surgical excision of these foci along with the primary tumour, or excision of the primary alone with MRI follow-up. In this way, as well as establishing the natural history of enhancing foci, it would be determined whether removing these foci is feasible by conservative surgery and whether this practice would ultimately influence local recurrence rates. However, since the specificity of MRI is reduced as a result of post-operative inflammation and scarring (Heywang-Kobrunner et al. 1993), MRI scans may only be repeated 6-9 months post-operatively, although this may improve with the introduction of new contrast-agents and scanning techniques.

# **Chapter 7: Current Influence of Pre-operative MRI on Extent of Surgical Resection**

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- 7.1 Introduction
- 7.2 Patients and Methods
  - 7.2.1 Patients
  - 7.2.2 Breast MRI
  - 7.2.3 Surgical management
  - 7.2.4 Histopathological assessment
  - 7.2.5 Statistical analysis
- 7.3 Results
- 7.4 Discussion and Conclusion

## **7.1 Introduction**

In breast cancer detection, the sensitivity of MRI approaches 100 per cent (Davis and McCarty, Jr., 1997) but its specificity is relatively low (Heywang-Kobrunner et al. 1997). Additional enhancing foci on MRI have frequently been noted adjacent to the main tumour. Although these may represent foci of in-situ or invasive cancer (chapter 6), their clinical relevance has been questioned (Baum et al. 1997) and it remains uncertain whether excision margins need to be extended in cases where such additional foci are detected. Breast MRI provides accurate information on tumour size (Davis et al. 1996) which correlates closely with histological measurements (Yang et al. 1997; Mumtaz et al. 1997b). However, since clinical assessment often overestimates tumour size (Pain et al. 1992) and incorrectly stages breast cancer patients (Dixon et al. 1984), the wider availability of MRI may influence surgeons to alter the amount of tissue removed during breast conservation. The potential impact of information derived from breast MRI on the extent of surgical resection, was assessed prospectively.

## **7.2 Patients and Methods**

### ***7.2.1 Patients***

Over a period of 18 months (September 1996 to March 1998), 190 breast cancer patients with 193 breast cancers (3 patients with bilateral tumours) were diagnosed by triple assessment. Of these, 131 cancers (67.9%) were treated by breast conservation surgery (wide local excision or quadrantectomy) and comprised the study group. Patients undergoing excisional biopsies were excluded. Breast MRI was offered to any eligible pre-operative patient according to availability of the MRI scanner. Patients were unselected but those with known claustrophobia (n=1), obesity(n=1), poor mobility(n=1), metal implants(n=2) or those who refused (n=2) were included in the no-MRI group for analysis.

### ***7.2.2 Breast MRI***

Breast MRI was performed using a high resolution transverse T1-weighted three dimensional (3D) FLASH sequence (TR=18ms, TE=7ms, FA=40°; TA=4m 56s, FOV=410mm) with a 1.0 T (Siemens Magnetom Scanner 42 SP with dedicated breast



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coil) during the initial 8 months. The scanner was then up graded and a dynamic transverse T1-weighted three dimensional (3D) FLASH sequence (TR=8.1ms, TE=4ms, FA=20°; TA=1m 18s, FOV=410mm) with a 1.5T (Siemens Magnetom Vision) scanner was used thereafter. Scans were performed before and after an intravenous bolus hand injection of dimeglumine gadopentetate (*Magnevist*, 0.2 mL/kg).

### **7.2.3 Surgical management**

MRI scans were not used for decision-making on type of surgery (conservative surgery vs mastectomy) but hard copies of matched pre- and post-contrast images were shown immediately before surgery to the operating surgeon to indicate the location of lesions, size and local extent. The written report was supplemented by comments from the investigator.

It is standard practice for the deep excision to include the pectoral fascia in patients undergoing wide local excision or quadrantectomy. The extent of conservative breast resection was decided upon by the surgeon (TD, MB, IT). Intraoperatively, all specimens were orientated using sutures and sent fresh for histological assessment.

### **7.2.4 Histopathological assessment**

Histopathological assessment was performed by a single breast pathologist. Specimens were weighed, inked and sectioned in a transverse plane. The largest tumour cross-section was processed whole as a large block. Additional small blocks were taken for estimation of closest margins. Excision margins were classified as positive (invasive carcinoma present at an inked margin); close (invasive carcinoma  $\leq 1$ mm from an inked margin); negative (no invasive carcinoma within  $\leq 1$ mm from an inked margin) or indeterminate (not measured). Circumferential margin status was determined by excluding deep margin involvement.

Breast specimen weights and excision margin status were used to estimate the actual volume of tissue removed and compared between those patients who underwent pre-operative MRI and those who did not. A ratio of tumour volume over specimen weight ( $\frac{4}{3} \times \Pi \times r^3 / \text{specimen weight}$ ;  $r$  = tumour radius) was used as a measure of extent of surgical excision, using specimen weight as an estimate of actual specimen volume. This

*Chapter 7: Current Influence of Pre-operative MRI on Extent of Surgical Resection* takes account of the relative volume of specimen required to remove a tumour of a particular size.

### **7.2.5 Statistical analysis**

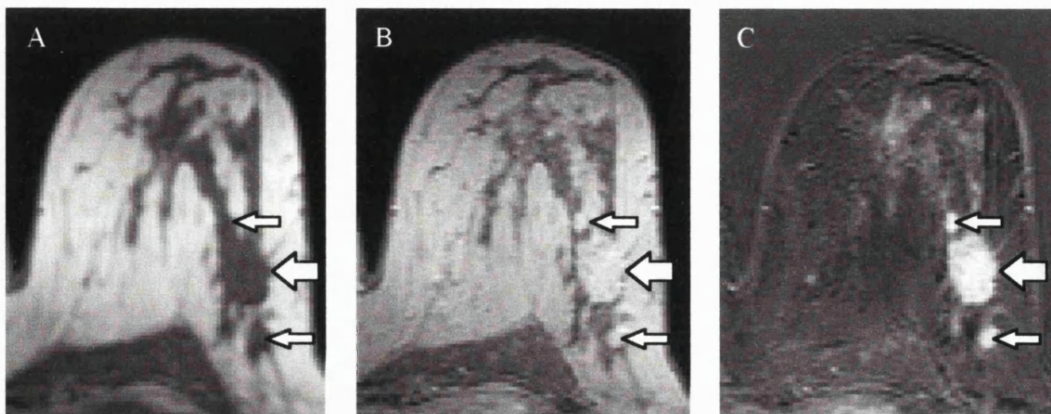
Statistical analysis was performed using the Independent Sample T-Test for comparing mean specimen weights between the MRI and no-MRI groups. Multiple regression analysis was used to identify variables which correlate with the ratio of tumour volume to specimen weight. Logistic regression analysis was used to identify factors which correlate with circumferential margin status.

## **7.3 Results**

Clinical and pathological characteristics of the 131 patients studied are summarised on table 7.1. Of those patients who underwent MRI, 4 had a primary tumour represented by only a few small enhancing foci and 2 had multifocal primary tumours (figure 7.1). Additional enhancing foci away from the primary tumour (figure 7.2) were identified and reported in 8 patients. None of the patients who underwent MRI was subsequently converted to a mastectomy as a result of MRI findings.

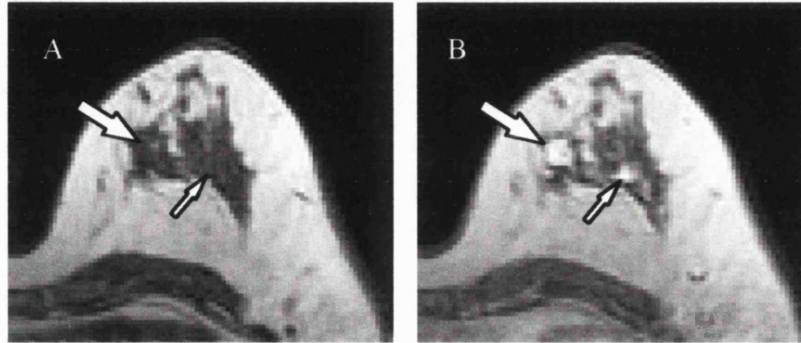
**Figure 7.1: Pre- (A), post- (B) and subtracted (C) contrast-enhanced MRI of a multifocal breast tumour:**

Two distinct enhancing foci (small arrows) were detected away from the primary tumour (large arrow).



**Figure 7.2: Pre- (A) and post-(B) contrast-enhanced breast MRI of a multifocal breast tumour:**

The large arrow indicates a clinically palpable primary tumour. The smaller enhancing focus (small arrow) away from the index quadrant was an incidental finding. The surgeon decided not to attempt to remove this lesion and a follow-up MRI, was recommended.



No significant difference between the MRI group (n=41) and no-MRI group (n=90) was seen in average specimen weight (table 7.2a) or resection margin status (table 7.2b). Multiple regression analysis was performed using the ratio of tumour size to specimen weight as the grouping variable; and age, MRI (performed / not performed) and resection margin status, as explanatory variables. There was no statistically significant correlation with the explanatory variables (table 7.3). When close margins were regarded as positive, a logistic regression model demonstrated a positive correlation between resection margin status and patient age, and a negative correlation between resection margin status and MRI status (table 7.4a). These correlations were not present when close margins were regarded as negative (table 7.4b).

Re-excision for histologically confirmed residual disease was performed in 11 patients. Of these, 3 patients underwent pre-operative MRI and 8 did not.

**Table 7.1: Patient Characteristics:**

A comparison between patients who underwent pre-operative MRI and those who did not.

		MRI (n=41)	No-MRI (n=90)
Age (years)	mean ± sd	50.2 ± 14.2	56.3 ± 14.5
	range	28-84	28-87
Axillary node surgery	clearance	27 (65.9)	52 (57.8)
	sampling	2 (4.9)	4 (4.4)
	None	12 (29.3)	34 (37.8)
Tumour grade	I	5/31 (16.1)	11/80 (13.8)
	II	13/31 (41.9)	39/80 (48.8)
	III	13/31 (41.9)	30/80 (37.5)
Tumour size (cm)	<2	14/32 (43.8)	37/83 (44.6)
	2-5	17/32 (53.1)	43/83 (51.8)
	>5	1/32 (3.1)	3/83 (3.6)
Vascular Invasion		13/41 (31.7)	31/90 (34.4)

Values in parenthesis are percentages

**Table 7.2: Comparison of specimen weight(a) and resection margin status(b) between patients who underwent pre-operative MRI and those who did not:**

(a):

	MRI (n=41)	No-MRI (n=90)	Mean Difference	SE of Difference	95% CI for SE of Difference	2-Tailed p*
<b>Specimen mean weight (g)</b>	<b>98.8</b>	<b>93.4</b>	<b>-5.40</b>	<b>15.16</b>	<b>-35.4, 24.6</b>	<b>0.72</b>
<b>sd</b>	<b>84.1</b>	<b>78</b>				

\*T-test for equality of the means

CI - confidence interval; SE - standard error

(b):

	MRI (n=41)	No-MRI (n=90)	P*
<b>Margin status</b> (distance of invasive carcinoma from nearest resection margin)			<b>0.41</b>
	<b>positive (0mm)</b>	<b>8 (19.5%)</b>	<b>15 (16.7%)</b>
	<b>close (= 1mm)</b>	<b>8 (19.5%)</b>	<b>28 (31.1%)</b>
	<b>negative (&gt;1mm)</b>	<b>23 (56.1%)</b>	<b>44 (48.9%)</b>
	<b>indeterminate</b>	<b>2 (4.9%)</b>	<b>3 (3.3%)</b>
<b>Circumferential margin status -</b> (distance of invasive margin from nearest resection margin other than the deep margin)			<b>0.17</b>
	<b>positive (0mm)</b>	<b>4 (9.8%)</b>	<b>10 (11.1%)</b>
	<b>close (= 1mm)</b>	<b>4 (9.8%)</b>	<b>21 (23.3%)</b>
	<b>negative (&gt;1mm)</b>	<b>31 (75.6%)</b>	<b>56 (62.2%)</b>
	<b>indeterminate</b>	<b>2 (4.9%)</b>	<b>3 (3.3%)</b>

\* Chi square test with 2 DF (indeterminate cases were excluded).

**Table 7.3: Multiple regression analysis to determine which factors influence the ratio of tumour volume to specimen weight ( $4/3\pi r^3$  / specimen weight):**

	<i>Coefficient</i>	<i>SE</i>	<i>P</i>
Age	2.108	1.367	0.126
Margin status	- 51.981	28.878	0.075
MRI / No MRI	31.580	43.020	0.465

*SE - standard error*

**Table 7.4: Results of fitting a logistic regression model to determine which variables independantly correlate with resection margin status:**

Close margins were regarded as positive (a) or negative (b).

(a):

	<i>Coefficient</i>	<i>SE</i>	<i>P</i>
Age	0.032	0.016	0.036
Tumour diameter	-0.049	0.030	0.098
Tumour volume / specimen weight	-0.001	0.002	0.653
MRI / No MRI	-1.261	0.550	0.022

(b):

	<i>Coefficient</i>	<i>SE</i>	<i>P</i>
Age	0.038	0.022	0.091
Tumour diameter	-0.083	0.040	0.038
Tumour volume / specimen weight	0.003	0.003	0.231
MRI / No MRI	-0.263	0.681	0.699

#### 7.4 Discussion and Conclusion

In an era of breast conservation, the detection of additional small enhancing foci away from the primary tumour by MRI represents a clinical problem since it may lead to more extensive wide local excisions or even unnecessary mastectomies. It is essential therefore to determine the impact of MRI on patient management as well as the clinical significance of additional small enhancing foci by prospective randomised studies. However, as a result of the rapid expansion of MRI in the UK (figure 5.1) breast MRI may become widely used before the results of such trials are available. In this prospective

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study we evaluated whether MRI currently influences the extent of surgical resection in conservative breast surgery. We used resection margin status and specimen weight as surrogate markers of the actual volume of tissue removed during surgery.

The deleterious effects of large resections on cosmesis are well recognised (Veronesi et al. 1994; Liauw et al. 1987). Smaller resections are thought to lead to higher positive margin and local recurrence rates (Vicini et al. 1991). Resection margin closeness or involvement by tumour is a recognised risk factor for the development of local recurrence (Noguchi et al. 1997; Kurtz et al. 1990) , especially in the absence of radiotherapy (Veronesi et al. 1994; Renton et al. 1996) , and it is therefore important to allow for an adequate resection margin during breast conservation.

Positive and close margin rates vary between different studies depending on the pathological technique used for assessment (Guidi et al. 1997); the pathological criteria used (Fisher et al. 1996), and whether or not the deep margin (pectoral fascia) is included in the assessment. However, deep margin involvement is less clinically significant since an adequate conservative resection includes the pectoral fascia and when the pectoral fascia is involved, it does not appear to be a risk factor for recurrence even following mastectomy (Mentzer et al. 1986). In this study we compared resection margin status between the groups using the deep as well as circumferential margins. Our margin positive rates compared favourably with those of other surgical studies (Umpleby et al. 1988; MacMillan et al. 1994; Chinyama et al. 1997; Spivack et al. 1994; Macmillan et al. 1997; Fisher et al. 1992).

When mastectomy specimens are sampled extensively, cancer foci are seen throughout the breast in up to 60 per cent of cases (Vaidya et al. 1996). However, large clinical studies have shown that 90 per cent of local recurrences following breast conservation occur in the operated quadrant and consequently the significance of these cancer foci has been questioned (Baum et al. 1997). Since there is no evidence to suggest that sub-clinical enhancing foci on MRI if left surgically untreated, lead to poor local control, resecting them may not be necessary. If they are resected this may result in many women undergoing larger resections or suffering unnecessary mastectomies.

### *Chapter 7: Current Influence of Pre-operative MRI on Extent of Surgical Resection*

In this prospective study we have demonstrated that knowledge of breast MRI findings pre-operatively does not significantly influence the extent of surgical resection. The negative correlation between margin status and MRI suggests that pre-operative MRI may increase tumour clearance margins if close margins are regarded as unacceptable (table 7.4a). This may ultimately result in larger resections.

Advances in real time three dimensional (3D) breast MRI (Daniel et al. 1998a) and interventional biopsy techniques (Gould et al. 1998) may influence surgical management in the future but these techniques are still at the experimental stage. At present, surgeons are still primarily guided by their clinical assessment of local tumour extent in deciding how much tissue to excise at the time of breast conservation.

# **Chapter 8: Comparison of Surgical and MRI Assessments of Tumour Resectability**

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- 8.1 Introduction
- 8.2 Patients and Methods
  - 8.2.1 Patients
  - 8.2.2 Primary medical therapy
  - 8.2.3 Breast MRI and evaluation
  - 8.2.4 Phantom volume measurements
  - 8.2.5 Statistical analysis
- 8.3 Results
- 8.4 Discussion and Conclusion



## 8.1 Introduction

Breast-conserving surgery is now accepted by most surgeons as the standard treatment for small and localised breast tumours. What is still controversial, is the optimal size of a primary tumour selected for breast conservation and how much breast tissue must be removed to provide an "adequate" margin in order to achieve local control. Although it is widely accepted that a 1cm margin should be obtained in all cases, it is clear that in reality this is not achieved (chapter 7). It is evident from chapter 7, that in deciding on how much tissue to excise surgeons are primarily guided by their clinical assessment of local disease extent.

When deciding upon the optimal surgical approach (mastectomy *versus* wide local excision) of an operable breast tumour a surgical assessment and informed patient preference are considered. The surgical assessment is influenced by factors such as breast size, tumour size, position of the tumour within the breast and the distance of the tumour from the nipple. A subjective summation of all these factors leads to a surgical opinion. A small tumour in the upper outer quadrant of a large breast, clearly required a wide local excision. However, it can be more challenging to decide on the optimal surgical approach in individual cases. Unfortunately, a more objective assessment is not available at present.

Breast MRI can accurately assess tumour size and since the entire breast is scanned, an assessment of breast volume can be made. The aim of this study, was to develop an objective assessment of tumour resectivity, using MRI. The study was performed on patients undergoing primary medical therapy (PMT), since these patients undergo surgical assessment twice: pre-recruitment and after completion of chemotherapy. All these patients are assessed at presentation and found to have locally advanced disease which is unsuitable for breast conserving surgery. Primary medical therapy offers patients the hope of breast conserving surgery if the disease is successfully down-staged following treatment. Surgical assessment of resectability, pre- and post-chemotherapy, was compared with MRI assessment.

## 8.2 Patients and Methods

### 8.2.1 Patients

Between 1996 and 1998, 11 consecutive patients with 12 newly diagnosed breast cancers were recruited prospectively prior to PMT. All patients were assessed jointly by a consultant surgeon and medical oncologist at a combined breast clinic and were deemed unsuitable for breast conservation. Patients were offered a pre-treatment MRI scan and another one on completion of the full course of chemotherapy. Subsequently, patients were re-assessed at the same joint breast clinic by the same consultant surgeon and medical oncologist, prior to surgery. The surgical opinion (mastectomy versus breast conserving surgery) was compared with objective MRI measurements.

Patients did not undergo ultrasound or mammography during chemotherapy and the MRI scan was not used in the decision making process. The study was approved by the ethics committee of the Royal Free University College Hospitals Trust.

### 8.2.2 Primary medical therapy

Primary medical therapy was undertaken within the TOPIC trial (Trial of Primary Infusional Chemotherapy), a randomised comparative trial of infusional ECF (epirubicin, cisplatin and 5FU) vs conventional AC (adriamycin and cyclophosphamide) as PMT for patients with potentially operable but large ( $\geq 3$  cm diameter) breast tumours. ECF were administered as 5FU by a continuous intravenous (iv) infusion for 18 weeks (via a Hickman line), epirubicin and cisplatin by iv bolus 3 weekly for 6 cycles (18 weeks). Conventional AC were administered by iv bolus 3 weekly for 6 cycles (18 weeks).

### 8.2.3 Breast MRI and evaluation

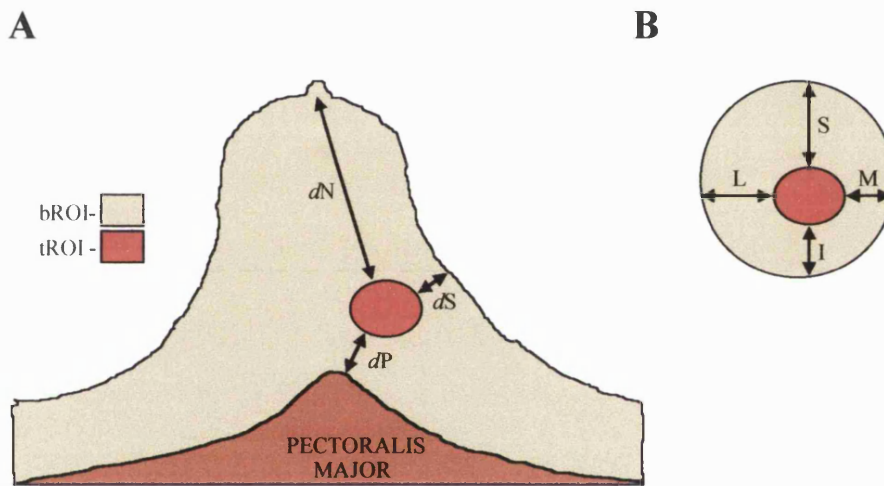
High resolution MRI was performed using the same protocol as for patients studied in chapter 7 (section 7.2.2). From the image with largest tumour cross-section, the shortest distance from the tumour edge to the nipple ( $d_N$ ), skin ( $d_S$ ) and pectoral muscle ( $d_P$ ) were measured (figure 8.1A). Furthermore, distances from the tumour edge to the skin in the saggital plane (figure 8.1B) were measured to assess the 'centricity' of the tumour in relation to the breast.

Breast cross-sectional area was measured by tracing around the skin and breast contour in each MRI slice (figure 8.2). Since the breast is not well demarcated towards the axilla and midline, the anterior axillary breast fold (anterior axillary line) was used as the lateral

margin and mid-line as the medial margin. Breast volume (bVol), was measured by multiplying each breast cross-sectional area by slice thickness (2mm) and summing them up. Registration software was then used to register and then subtract pre- from post-contrast images. Tumour volume (tVol) was measured by summation of individual tumour ROIs from subtracted images, and multiplying by slice thickness (2 mm).

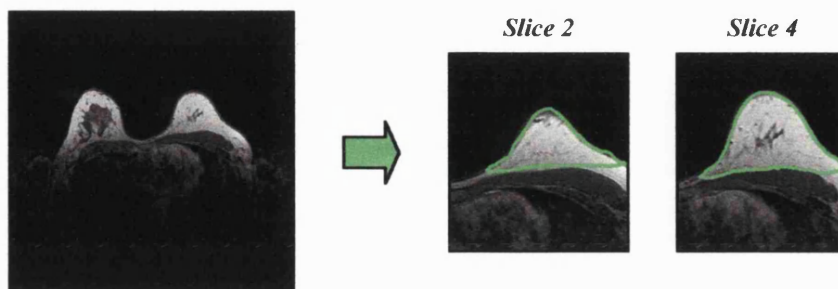
**Figure 8.1: Evaluation of tumour distance from surrounding structures and tumour area, from image with largest tumour cross-section.**

The breast was evaluated in both the axial (transverse) plane (A) and saggital plane (B). (bROI- breast region of interest; tROI- tumour region of interest; shortest distance from tumour edge to base of nipple ( $dN$ ), skin ( $dS$ ) and pectoral muscle ( $dP$ ); distance from tumour edge to skin superiorly (S), inferiorly (I), medial (M) and lateral (L))



**Figure 8.2: Measurement of left breast volume:**

Area was measured within breast regions of interest (bROI), by tracing around the breast contour. This was repeated for all 64 slices. Areas were multiplied by slice thickness to obtain the slice volume and summated to give the total breast volume (bVol).



#### **8.2.4 Phantom volume measurements**

The accuracy of volumetric assessment using in-house software, was evaluated on phantoms. Breast prostheses (200-300ml) and volumetric flasks (containing 5-15ml water) were scanned using the same MRI protocol as that used on patients. The area of each phantom slice was determined by region of interest (ROI) measurements multiplied by slice thickness. Volume was derived from the summation of all ROI slice volumes for a given phantom. The results are shown in appendix 1.

#### **8.2.5 Statistical analysis**

Since the data was not normally distributed, the difference between the 2 groups was assessed using the Mann-Whitney U-test.

### **8.3 Results**

Eleven patients aged  $42 \pm 6$  years (mean  $\pm$  sd) with 12 breast tumours, were studied. A total of 24 MRI scans were evaluated. Complete response on MRI was achieved in one patient and this scan could therefore not be quantitatively assessed. All 12 tumours were treated by wide local excision. In total, 23 scans could be quantitatively evaluated: 12 scans on patients deemed suitable for mastectomy and 11 scans on patients suitable for breast conserving surgery. A comparison of descriptive statistics for the various parameters measured, is shown in table 8.1. There was a statistically significant difference between the 2 groups only in tumour volume (figure 8.3).

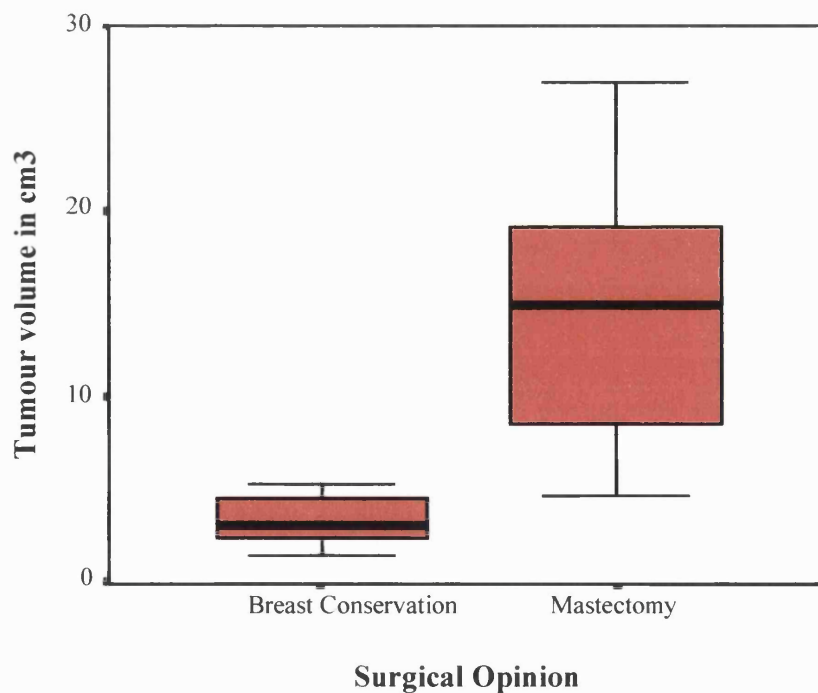
**Table 8.1:** A comparison of MRI parameters and surgical opinion for 23 MRI scans:

	<i>Mastectomy</i> <i>n=12</i> <i>(mean ± sd)</i>	<i>Breast conservation</i> <i>n=11</i> <i>(mean ± sd)</i>	<i>P*</i>
Breast volume (bVol – cm <sup>3</sup> )	799 ± 327	768 ± 275	0.673
Tumour volume (tVol - cm <sup>3</sup> )	15.0 ± 7.1	3.8 ± 2.1	0.001
dN (cm)	3.9 ± 2.5	4.8 ± 2.7	0.955
dS (cm)	1.1 ± 0.7	1.6 ± 0.8	0.232
dP (cm)	2.7 ± 2.0	2.5 ± 1.7	0.955
Medial (M – cm)	4.6 ± 2.7	4.6 ± 2.3	0.955
Lateral (L – cm)	2.4 ± 1.9	2.8 ± 1.5	0.397
Superior (S – cm)	3.7 ± 1.5	3.8 ± 1.6	1.000
Inferior (I – cm)	4.1 ± 1.8	4.9 ± 1.2	0.397

\*p value using Mann-Whitney U-Test

**Figure 8.3:** Boxplot of tumour volume against surgical opinion (breast conservation or mastectomy):

For each group, the median (bold line), quartiles (box) and extreme values (T-bar) are shown.



#### **8.4 Discussion and Conclusion**

MRI can be used to measure breast volume, tumour volume and distances from the tumour edge to the skin and underlying structure. Phantom measurements and repeated breast volume measurements have shown that MRI is highly accurate in determining volume. However, actual breast volume measurements appear to be high when compared to expected volumes of mastectomy specimens as discussed in chapter 7 ( $554\text{g} \pm 374.8$ ; mean  $\pm$  sd). This is likely to be due to the fact that an extensive amount of subcutaneous tissue between breast and axilla is included in volume assessment but is not routinely excised during a mastectomy.

From table 8.1 it is evident that only tumour volume was significantly different between MRI scans of tumours assessed as suitable for mastectomy and those suitable for wide local excision. All other parameters were not significantly different following PMT. This suggests that clinical assessment of tumour extent, is accurate in selecting patients suitable for breast conservation even though clinical assessment of tumour size does not correlate with histological assessment (Chapter 9). At present, more accurate measurements of tumour position within the breast, do not appear to correlate with surgical opinion.

It is likely that a more objective assessment of tumour extent may prove useful if interventional techniques improve to such an extent, that accurate surgical planning could influence surgical resection. Clearly, this would require meticulous histopathological correlation as well as patient follow-up in randomised studies, to determine its benefits. However at present, clinical assessment of tumour extent appears to be sufficient in terms of selecting patients suitable for breast conserving surgery.

# **Chapter 9: MRI in the Assessment of Response to Primary Medical Therapy**

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## 9.1 Introduction

## 9.2 Patients and Methods

### 9.2.1 Patients

### 9.2.2 Patient clinical assessment and follow-up

### 9.2.3 Primary medical therapy and radiotherapy regimens

### 9.2.4 MRI scanning protocol

### 9.2.5 Biopsy and specimen histology

## 9.3 Results

### 9.3.1 Patient assessment at presentation

### 9.3.2 Assessment of response to PMT

### 9.3.3 Patient outcome and correlation of MRI findings with specimen histology

## 9.4 Discussion and Conclusion

## 9.1 Introduction

The systemic treatment of choice in <sup>large operable</sup> ~~advanced~~ breast cancer is neoadjuvant chemotherapy or primary medical therapy (PMT). Although no study demonstrated a survival advantage of pre-operative chemotherapy over post-operative chemotherapy, a good response to PMT may down stage the disease to such an extent that local control can be achieved by wide local excision rather than a mastectomy. This is an important benefit particularly in this group of patients in whom, quite often, prognosis is poor.

Preliminary observations at The Middlesex Hospital suggest that there is a potential role for breast MRI in defining the extent of residual disease prior to surgical treatment (Mumtaz et al. 1996b). Moreover, Abraham et al. (1996), have shown that breast MRI may accurately predict pathologically proven residual disease in 97% of patients on completion of PMT. However, it is not known how MRI assessment of tumour size compares with clinical assessment. Furthermore, MRI may play a role in determining response to chemotherapy at the initial stages of treatment, thus allowing either an alteration to the chemotherapeutic regimen or earlier surgical intervention. In this study we evaluated the role of MRI in defining the extent of residual disease both during the initial stages of PMT as well as on completion of treatment, and compared it to clinical and histological assessments.

## 9.2 Patients and Methods

### 9.2.1 Patients

Over 18 months, newly diagnosed breast cancer patients were recruited prospectively from UCL, prior to PMT. Patients were also recruited from the Royal Surrey County Hospital in Guilford. The diagnosis of breast cancer was established by clinical examination, mammography and fine needle aspiration cytology at a one-stop breast clinic. All patients had <sup>large operable</sup> ~~locally advanced~~ breast cancer (T2-4, N0-2), and consented to undergo MRI. The study was approved both by the UCL and Royal Surrey County Hospital Ethics Committees.



### **9.2.2 Patient clinical assessment and follow-up**

Patients were initially assessed by a senior surgeon and deemed to be unsuitable for breast conservation surgery. Prior to PMT, patients were assessed at a combined breast clinic where a senior clinician measured tumour size in the superoinferior and mediolateral planes, using callipers. The product of these, represented the bidimensional product for each tumour. Assessment was repeated before each cycle of chemotherapy and on completion of treatment.

Response to treatment was assessed using UICC (Union International Contre le Cancer) criteria (Hayward et al. 1977): CR (complete response: no detectable tumour); PR (partial response:  $\geq 50\%$  reduction in bidimensional product); NC (no change:  $< 50\%$  reduction or  $< 25\%$  increase in bidimensional product) and PD (progressive disease:  $\geq 25\%$  increase in bidimensional product).

### **9.2.3 Primary medical therapy and radiotherapy regimes**

As described in chapter 8, anthracycline based PMT was undertaken within the TOPIC trial (Trial of Primary Infusional Chemotherapy), a randomised comparative trial of infusional ECF (epirubicin, cisplatin and 5FU) vs conventional AC (adriamycin and cyclophosphamide) as primary (neoadjuvant) chemotherapy for patients with potentially operable but large ( $\geq 3$  cm diameter) breast tumours. ECF were administered as 5FU by a continuous intravenous (iv) infusion for 18 weeks (via a Hickman line), and epirubicin and cisplatin by iv bolus 3 weekly for 6 cycles (18 weeks). Conventional AC were administered by iv bolus 3 weekly for 6 cycles (18 weeks). Patients who declined the TOPIC trial were prescribed other regimens (see table 9.1).

At the Royal Surrey County Hospital, as part of an on-going study, tumour grade is determined by either FNA or core- biopsy. Patients with grade III tumours, are given 2 cycles of anthracycline based chemotherapy (Methotrexate and 5-FU on days 1 and 8; cyclophosphamide for 2 weeks) prior to resection whereas those with grade I/II are given tamoxifen for 21 to 43 days, in an attempt to reduce tumour invasiveness pre-operatively. This protocol provided an opportunity to directly assess the effect of chemotherapy on tumour size following 2 cycles of chemotherapy rather than a total of 6 cycles.

## *Chapter 9: MRI in the Assessment of Response to Primary Medical Therapy*

Post-operative radiotherapy was administered to all patients via a linear accelerator using a simulator for pre-planning. A total dose of 50Gy given in 25 fractions over 5 weeks, delivered as 5mV photons using opposing tangential fields (medial and lateral). An additional boost of 15 Gy over 5 fractions was delivered to the tumour bed, using a short distance Cobalt 60 machine.

### **9.2.4 MRI scanning protocol**

All patients underwent MRI prior to treatment, between the 2nd and 3rd courses of chemotherapy (4-6 weeks) and on completion of the full course of chemotherapy (at least 6 cycles or 18 weeks). Pre- and post-contrast enhanced breast MR imaging were performed using a standard technique (transverse T1-weighted three dimensional FLASH imaging at 2 mm effective section thickness) with a Magnetom 42SP (Siemens, Germany). All films were reported by a Consultant Radiologist, without prior knowledge of biopsy findings. Pre- and post-treatment MRI scans were compared and both tumour size and enhancement distribution noted. Breast lesions were measured in 2 dimensions (superoinferior and mediolateral) from the slice with the largest tumour diameter to obtain MRI tumour bidimensional products. These measurements as well as enhancement distribution, were then correlated with both clinical and histological findings.

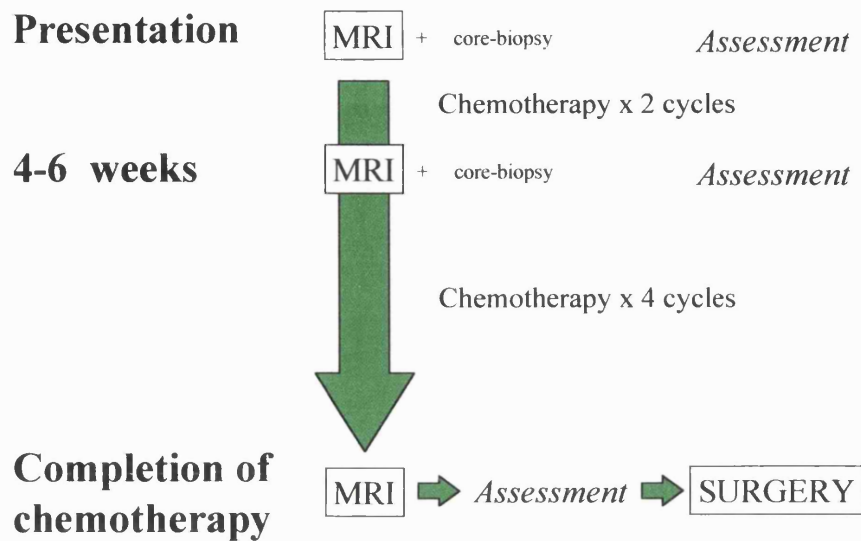
### **9.2.5 Biopsy and specimen histology**

Following breast MRI, needle-core biopsies of both the centre and rim of the tumour were taken using a 14 gauge biopsy needle, to assess tissue viability. Biopsy specimens were fixed in formalin, embedded in paraffin, sectioned at 5 µm section-thickness and stained with H&E. All slides were reviewed and reported by a Consultant Pathologist.

Following tumour resection, the fixed specimens were sliced in the transverse plane, the same plane as the MRI. Large blocks were prepared from the specimen cross-section with the largest tumour diameter and 5 µm sections were stained with H&E. Tumour bidimensional products were determined from the superoinferior and mediolateral tumour measurements.

A summary of the methodology is shown in figure 9.1.

**Figure 9.1: Study Methodology:**



### 9.3 Results

#### 9.3.1 Patient assessment at presentation

A total of 16 patients with 17 tumours were enrolled into the study. One patient was subsequently excluded because a cochlear implant precluded MRI studies. Another patient received only 2 cycles of CMF. The remaining 14 patients aged 34–80 (median 42) years with 15 breast tumours, underwent a full course of at least 6 cycles of primary medical therapy. During the study, a total of 42 breast MRI scans were performed.

Patient characteristics for the 16 cases of breast cancer in 15 patients, who received primary medical therapy, are shown in table 9.1. All patients had locally advanced breast cancer (T2-T4), not suitable for breast conservation. In the patient with bilateral breast cancers, both tumours measured over 5cm in maximal diameter. Initial breast MRI delineated an enhancing mass in all 16 tumours. One tumour demonstrated extensive enhancement of the entire breast suggestive of multicentric disease. Additional enhancing foci away from the main tumour mass were seen in 5/16 (31%). Of these, 1 patient had a single additional enhancing focus (figure 9.2), 1 had several additional foci away from the main mass, 1 had an enhancing focus in the contralateral breast, 1 had linear enhancement extending from the main tumour suggestive of in-situ disease and 1 had

enhancement of the contralateral right upper quadrant suspicious of in-situ disease. The patient with apparent multicentric disease at presentation, did not attend MRI on completion of chemotherapy. Histology showed a ductal carcinoma with extensive lymphovascular permeation within 1mm of the anterosuperior resection margin but the tumour was 7mm from the nearest resection margin. The remaining 4 of 5 patients with additional enhancing foci, did attend MRI on completion of treatment and the enhancing foci were no longer visible. Following breast conservation surgery, histology showed a close resection margin in 1 of these but clear resection margins in the rest.

**Table 9.1: Pre-treatment(a) and post-treatment(b) patient and tumour Characteristics for 16 tumours (15 patients):**

(a)

<i>Patient Characteristics</i>	<i>Number of Cases</i>
Menopausal Status	
Premenopausal:	9
Postmenopausal:	7
Surgical Assessment	
Operable by mastectomy:	16
Operable by breast conservation:	0
TNM Clinical Classification	
T2 (> 2cm ≤ 5cm):	6
T3 (> 5cm):	9
T4 (extension to chest wall or skin):	1
N0:	14
N1:	3
M0:	15
M1 (supraclavicular lymph node):	1
Histological Grade Pretreatment (Core biopsy)	
Grade I:	0
Grade II:	10
Grade III:	0
No grading:	3
No biopsy	3

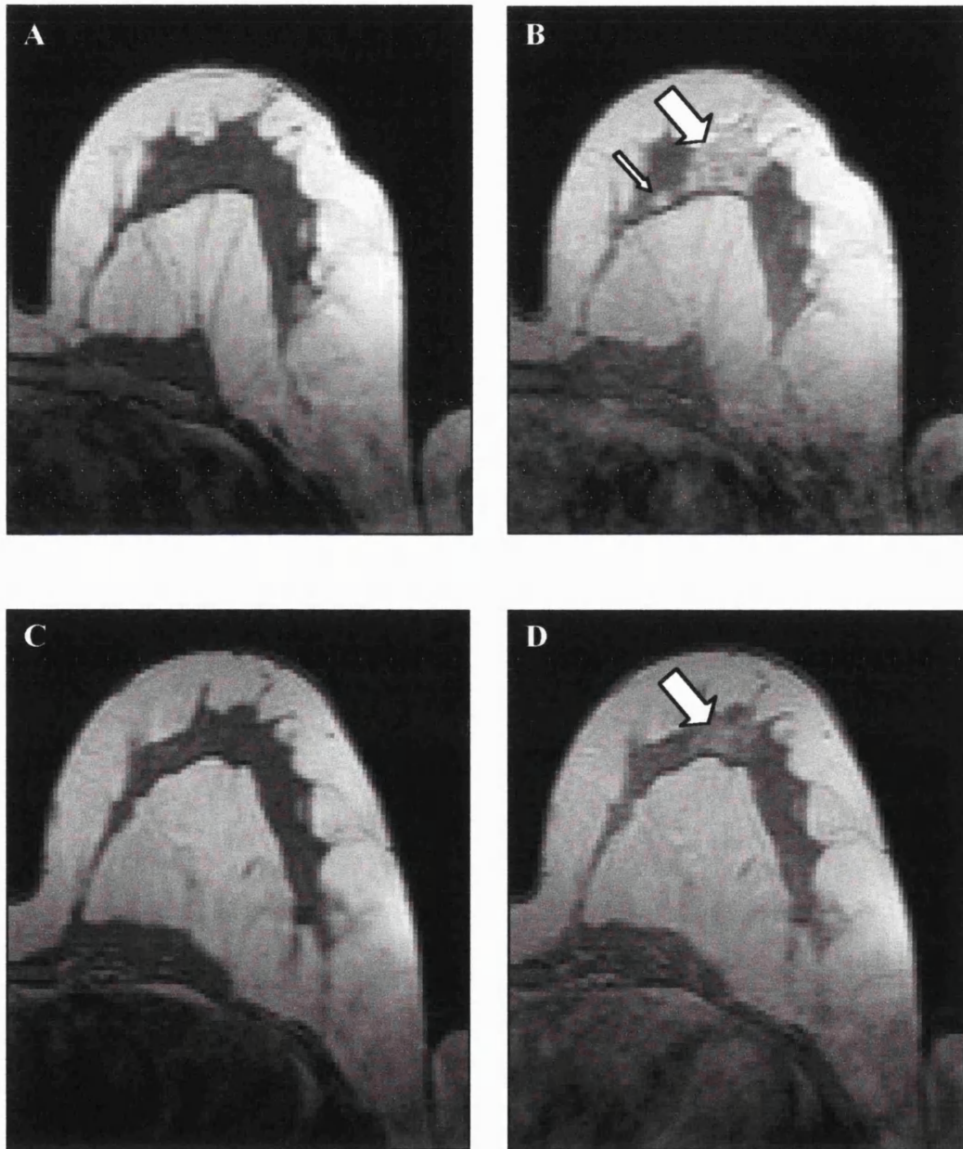
*TOPIC - Trial of Primary Infusional Chemotherapy; AC - adriamycin, cyclophosphamide; ECF - epirubicin, cisplatin, 5FU; CMF - cyclophosphamide, methotrexate, 5FU; MMM - methotrexate, mitoxantrone, mitomycin ; EC-epirubicin, cyclophosphamide ; FEC - - 5FU, epirubicin, cyclophosphamide; DC - doxorubicin, cyclophosphamide*

(b)

<i>Patient Characteristics</i>	<i>Number of Cases</i>
<b>Breast Surgery</b>	
Mastectomy:	0
Breast conservation:	16
<b>Axillary Surgery</b>	
Axillary node clearance (ANC):	13
ANC + internal mammary nodes:	1
Axillary node sampling:	1
No axillary surgery:	1
<b>Primary Medical Therapy</b>	
TOPIC AC:	2
TOPIC ECF:	4
CMF:	4
MMM:	1
EC	2
EC changed to FEC:	1
FEC:	1
DC:	1
<b>pTNM Clinical Classification</b>	
Tis	1
T1 ( $\leq 2$ cm)	5
T2 ( $> 2$ cm $\leq 5$ cm):	10
T3 ( $> 5$ cm):	0
T4 (extension to chest wall or skin):	0
N0:	6
N1:	9
NX:	1
<b>Tumour Type</b>	
Ductal:	11
Lobular:	2
Tubular:	1
Mixed:	1
DCIS:	1
<b>Histological Grade</b>	
Grade I:	2
Grade II:	2
Grade III:	7
Not graded:	5
<b>Resection margins</b>	
Positive (DCIS only):	1
Positive or close (invasive):	2
Negative ( $>1$ mm):	13

**Figure 9.2: Additional enhancing foci, away from main tumour mass.**

Pre- (A) and post-contrast (B) MRI of a breast tumour (large arrow) at presentation. An additional enhancing focus was seen away from the main mass (small arrow). On completion of chemotherapy, pre- (C) and post-contrast (D) images demonstrate a reduction in tumour size as well as enhancement intensity. The additional enhancing focus was no longer seen.



### 9.3.2 Assessment of response to primary medical therapy

A comparative assessment of tumour size by bidimensional product, during PMT, is shown in table 9.2. On presentation, 1 tumour was not measurable by either MRI or

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mammography since it appeared to involve the whole breast on both imaging modalities, but a distinct mass was apparent on clinical examination. Two palpable tumours were not detected by mammography but were detected by MRI and one of them, by ultrasound as well. Ultrasound was performed selectively on a small number of patients, by the same radiologist who performed the mammograms. Measurements by using this modality were therefore not assessed.

**Table 9.2: Assessment of tumour bidimensional product during PMT.**  
All measurements are in cm<sup>2</sup>.

No.	Presentation				4-6 weeks		Completion		
	Clinical	MRI	US	Mammo	Clinical	MRI	Clinical	MRI	Histology
1	41.3	12.4	ND	25.0	ND	ND	12.3	6.5	4.5
2	48.8	11.5	8.2	16.0	6.0	8.4	6.0	1.4	1.7
3	20.0	4.8	ND	22.5	20.0	6.0	12.0	0.5	2.2
4	21.6	10.0	ND	16.0	15.0	7.6	9.0	3.5	1.5
5	24.8	5.6	ND	6.25	20.3	6.8	12.3	5.3	7.0
6	25.0	6.7	0.0	0.0	22.5	0.9	0.0	0.0	64.0
7	72.0	Diffuse	1.8	Diffuse	12.3	ND	20.9	5.0	4.4
8	20.0	5.0	6.2	14.0	10.0	2.9	7.5	1.7	2.0
9a	27.5	8.0	ND	Missing	6.0	2.6	6.0	1.2	1.1
9b	22.5	4.8	ND	Missing	0.0	2.3	0.0	0.8	0.8
10	37.5	3.9	20.0	0.0	27.0	5.3	20.0	0.2	26.1
11	68.0	17.2	12.0	24.0	27.5	8.8	25.0	6.8	10.6
12	50.0	26.4	ND	25.0	ND	ND	18.5	10.5	18.9
13	12.3	6.3	1.2	4.0	9.6	ND	3.0	0.6	4.4
14	25	9.0	3.3	9.0	20	ND	9.6	3.3	4.4
15	20.0	13.0	6.3	15.0	16.0	11.6	ND	ND	ND

*ND – not done; Diffuse – extensive lesion, not well demarcated; Missing – films could not be found; Mammo – mammography*

At presentation, estimation of bidimensional product by clinical assessment was on average 3 times greater than by MRI and 2 times greater than by mammography (table 9.3). Although the sample means were significantly different (table 9.3b), there was a statistically significant correlation between the 3 modalities. (table 9.3c).

**Table 9.3: Assessment of tumour size by bidimensional product (cm<sup>2</sup>) at Presentation:**

Mean and standard deviation (a), paired samples t-test for mean difference (b) and Pearson's correlation coefficient (c) were used to compare clinical examination, MRI and mammography in the assessment of bidimensional product. Tumours that were not measurable by one modality in the paired comparisons, were excluded.

(a) Mean and standard deviation of bidimensional products at presentation (n=11):

	Clinical	MRI	Mammography
Mean ± sd (cm <sup>2</sup> )	32.0 ± 17.4	11.0 ± 6.4	16.1 ± 7.5
Std. Error of mean	5.2	1.9	2.3

(b) Significance of difference between sample means using Paired Sample t-test (n=11):

	Mean Difference	SE of Difference	95% CI for SE of Difference	2-tailed p
Clinical examination vs MRI	21.0	4.1	11.9, 30.1	*0.000
Clinical examination vs mammography	15.9	4.1	6.7, 25.1	*0.003
MRI vs mammography	-5.05	1.9	-9.2, -0.9	*0.023

CI - confidence interval; SE - standard error

(c) Assessment of correlation using Pearson's correlation coefficient:

	n	Correlation coefficient	2-tailed p
Clinical examination vs MRI	15	0.67	*0.007
Clinical examination vs mammography	11	0.65	*0.030
MRI vs mammography	11	0.61	*0.046

\* Significant at the  $p < 0.05$  level

Of 15 patients (16 tumours), 10 (11 tumours) underwent MRI at 4-6 weeks and 14 underwent MRI after at least 6 cycles of chemotherapy. On completion of chemotherapy, there was a correlation between clinical and MRI assessments of bidimensional product, but not at 4-6 weeks (table 9.4).



**Table 9.4: Assessment of tumour size by bidimensional product (cm<sup>2</sup>) during PMT:**

Mean and standard deviation (a), Paired Samples t-test for mean difference (b) and Pearson's correlation coefficient (c) were used to compare clinical examination and MRI. Tumours that were not measurable by one modality in the paired comparisons, were excluded.

(a) Mean and standard deviation of bidimensional products at presentation:

	<b>n</b>	<b>Clinical</b>	<b>MRI</b>
<b>Presentation</b>			
Mean ± sd (cm <sup>2</sup> )	15	31.0 ± 15.1	9.6 ± 6.0
Std. error of mean		3.9	1.5
<b>At 4-6 weeks:</b>			
Mean ± sd (cm <sup>2</sup> )	11	15.5 ± 9.1	5.7 ± 3.3
Std. error of mean		2.7	1.0
<b>On completion:</b>			
Mean ± sd (cm <sup>2</sup> )	14	10.8 ± 7.6	3.2 ± 3.1
Std. error of mean		2.0	0.8

(b) Significance of difference between sample means using Paired Sample t-test:

<b>Clinical examination vs MRI</b>	<b>Mean Difference</b>	<b>SE of Difference</b>	<b>95% CI for SE of Difference</b>	<b>2-tailed p</b>
Presentation	21.3	3.1	14.7, 27.9	*0.000
At 4-6 weeks	9.7	2.6	3.8, 15.6	*0.004
On completion	7.7	1.6	4.2, 11.1	*0.000

(c) Assessment of correlation using Pearson's correlation coefficient:

<b>Clinical examination vs MRI</b>	<b>n</b>	<b>Correlation coefficient</b>	<b>2-tailed p</b>
Presentation	15	0.67	*0.007
At 4-6 weeks	11	0.27	0.429
On completion	15	0.63	*0.012

*CI - confidence interval; SE - standard error*

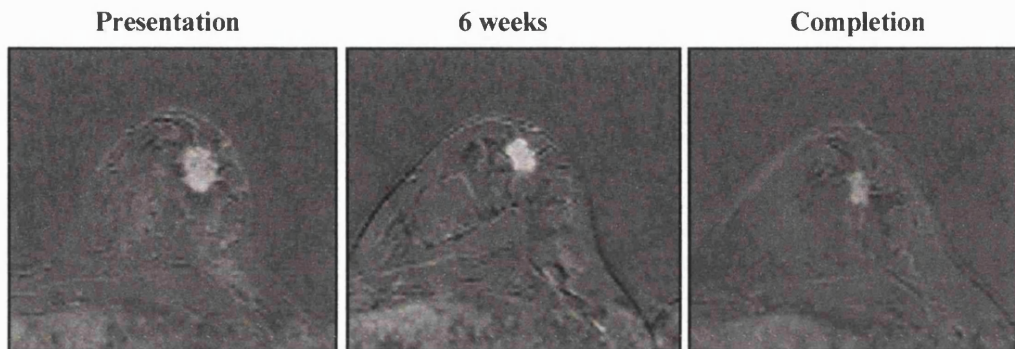
*\*Significant at the  $p < 0.05$  level*

Eleven tumours were assessed by MRI at 4-6 weeks and MRI agreed with clinical assessment in 5/11 (45%). Assessment of response to PMT using UICC criteria, is shown in table 9.5 . A reduction in MRI bidimensional product was observed at 4-6 weeks, in 8 of 11 (73%). In these cases, enhancement of the residual tumour mass was more patchy and less intense. Tumours showed some central contrast enhancement and viable tumour was obtained from all biopsies of the tumour centre. Two patients had

progressive disease on MRI (PD) and in these, enhancement intensity was not reduced following treatment.

**Figure 9.3: Serial registered subtracted MR images of a breast tumour during primary medical therapy:**

This 4.5 x 5 cm tumour in the left upper outer quadrant, was no longer palpable at 6 weeks. Clinically, response to chemotherapy was complete. Pre-contrast images were subtracted from post-contrast images, correcting for patient movement between scans (registration). A reduction in both size and enhancement intensity was observed during chemotherapy. On MRI, the tumour measured 2.2 x 2.2 cm at presentation, 1.9 x 1.2 cm at 6 weeks and 1.2 x 0.9 cm on completion. Histology showed a 1.5 x 1.0 invasive ductal carcinoma.



On completion of chemotherapy, a reduction in bidimensional product on MRI was observed in 13/14 (93%) of tumours and this was associated with a reduction in enhancement intensity. MRI agreed with clinical assessment of bidimensional product in 10/14 (71%) tumours. Clinical examination detected 2 complete responses but in one of these residual tumour was detected on MRI (figure 9.3), whereas in the other no residual enhancement was seen. Histology showed residual disease in both patients. In the patient with a partial response on MRI, a small tumour mass was seen whereas in the other patient with clinical and MRI complete response, only microscopic invasive disease was present. One patient with a partial clinical response but no response on MRI, had a 7cm<sup>2</sup> tumour on histology. Of 2 patients with no clinical response but partial response on MRI, 1 patient had a 2.2cm<sup>2</sup> tumour mass and the other no tumour mass but a 26cm<sup>2</sup> area of fibrosis infiltrated by scattered lobular carcinoma cells.

**Table 9.5: Clinical and MRI response to PMT at 4-6 weeks (a) and on completion (b):**

(a)

	Clinical Response				Total
	CR	PR	NC	PD	
<b>MRI response:</b>					
CR					0(0%)
PR	1	2	1		4(36%)
NC		2	3		5(45%)
PD			2		2(18%)
<b>Total</b>	1(9%)	4(36%)	6(55%)	0(0%)	

(b)

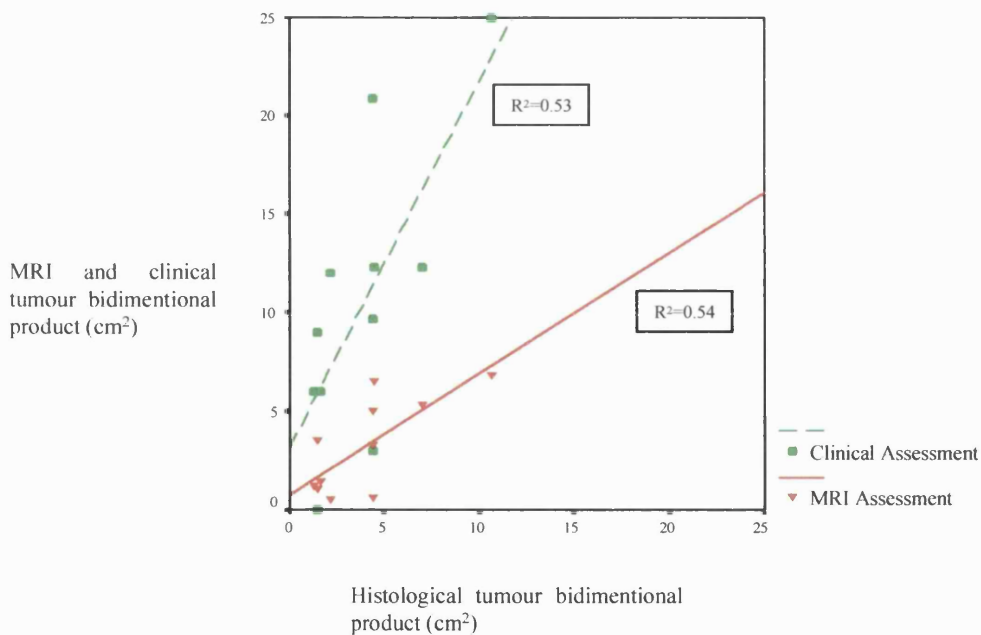
	Clinical Response				Total
	CR	PR	NC	PD	
<b>MRI response:</b>					
CR	1				1 (7%)
PR	1	9	2		12(86%)
NC		1			1 (7%)
PD					0 (0%)
<b>Total</b>	2(14%)	10(71%)	2(14%)	0(0%)	

### **9.3.3 Patient outcome and correlation of MRI findings with specimen histology**

Following PMT, 16/16 (100%) of tumours were operable by nipple preserving breast conservation surgery and 2 patients had close or positive resection margins for invasive disease. Of these, 1 patient with lobular carcinoma had positive resection margins and 1 with ductal carcinoma had a close resection margin, but neither underwent further resection. All 15 patients were followed up for a median of 34 months (range 12-41). Twelve patients (80%) are alive and free of recurrence. Two patients (13%) died of metastatic disease and one (7%) has residual disease in a supraclavicular lymph node and is currently undergoing adjuvant chemotherapy.

On histological examination, a macroscopic tumour mass was found in 7/15 (47%), microscopic tumour focus in 4/15 (27%) whereas in the remainder (26%), there were only scattered cancer cells within a fibrous stroma. In patients with a measurable gross or microscopic tumour mass (n=11), the extent of contrast enhancement correlated with the distribution of residual tumour on histology. Histological tumour bidimensional product correlated with both MRI and clinical assessment (figure 9.6c). However, clinical assessment significantly overestimated tumour size. MRI slightly underestimated tumour size, although this was not statistically significant (table 9.6b). This is illustrated in the overlay scatter diagram (figure 9.4).

**Figure 9.4:** Overlay scatter diagram of MRI and clinical (y-axis) against histological (x-axis) tumour bidimensional products (n=11). The regression coefficient ( $R^2$ ) is shown.



**Table 9.6: A comparison between MRI, clinical and histological assessments of tumour bidimensional product, on completion of chemotherapy:**

Tumours that were measurable by all modalities were compared (n=11). Mean and standard deviation (a), paired samples t-test for mean difference (b) and Pearson's correlation coefficient (c) are tabulated below.

(a) Mean and standard deviation of tumour bidimensional products:

	n	Mean ± sd (cm <sup>2</sup> )	Std. error of mean
Clinical Assessment	11	10.6 ± 7.3	2.2
MRI Assessment	11	3.2 ± 2.4	0.7
Histological Assessment	11	4.0 ± 2.9	0.9

(b) Significance of difference between sample means using Paired Sample t-test:

	Mean Difference	SE of Difference	95% CI for SE of Difference	2-tailed p
Clinical vs histological assessments	6.6	1.7	2.8, 10.4	*0.003
MRI vs histological assessment	-0.8	0.6	-2.1, 0.6	0.228

(c) Assessment of correlation using Pearson's correlation coefficient:

	n	Correlation coefficient	2-tailed p
Clinical vs histological assessments	11	0.73	*0.011
MRI vs histological assessment	11	0.74	*0.010

*CI - confidence interval; SE - standard error*

*\* Significant at the p<0.05 level*

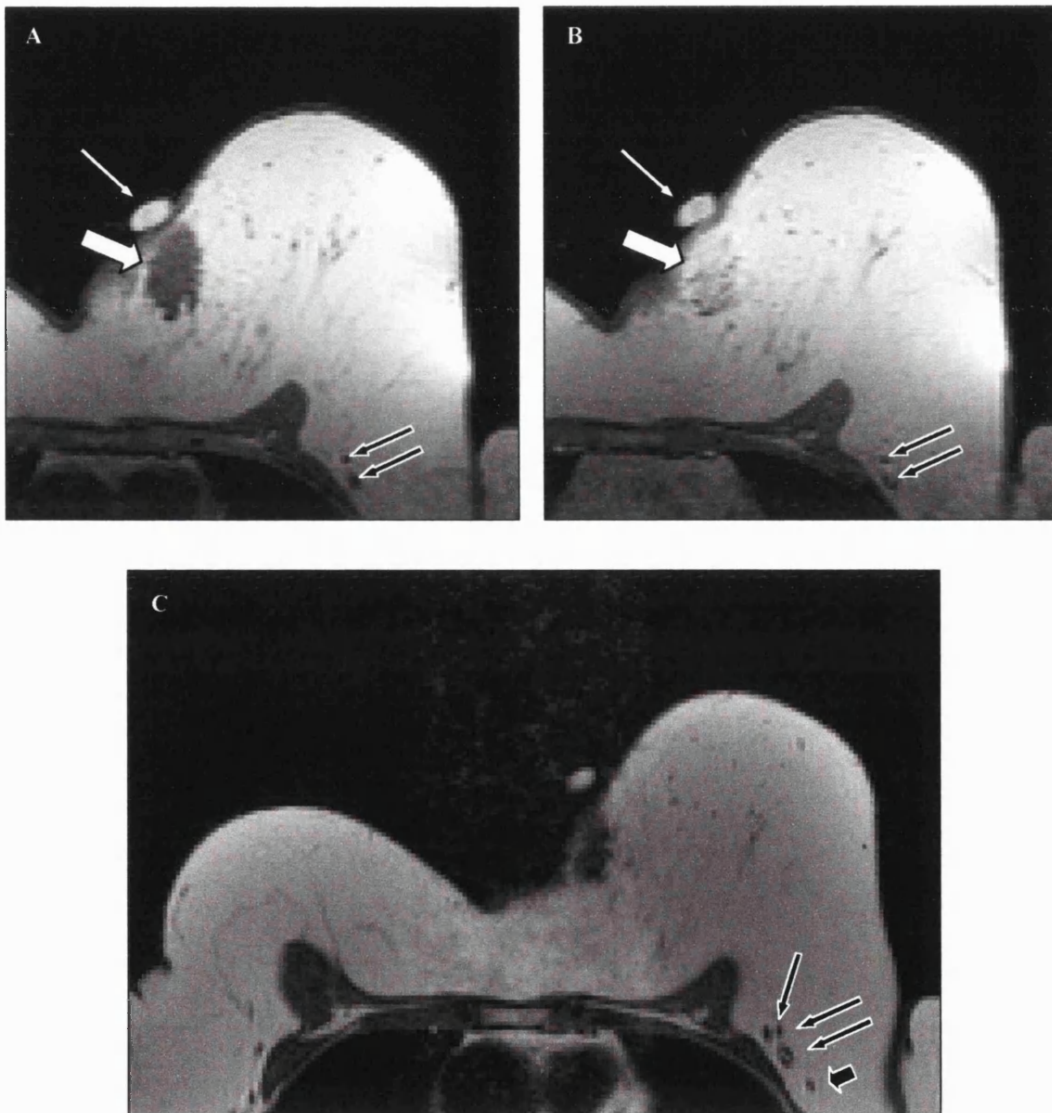
Of 5 patients with no discrete focal mass, 1 patient had pure DCIS, 2 had lobular carcinoma, 1 tubular carcinoma and 1 ductal carcinoma. In these extensive fibrosis was seen interspersed with malignant cells of doubtful viability. Of the residual lobular carcinomas, 1 tumour showed no enhancement on MRI (complete response) and in the other, only a small focus of enhancement was evident (0.4 x 0.4 cm). On histology, scattered cancer cell were seen throughout an area of fibrosis measuring approximately 8 x 8 cm and 4.5 x 5.8 cm respectively.

*Chapter 9: MRI in the Assessment of Response to Primary Medical Therapy*

Adequate axillary visualisation on MRI was not possible since full breast coverage was regarded as clinically essential and was obtained with the same field of view on all patients. Level I nodes were visible in 12/14 patients and level I and II in only 2/14 patients. Neither number of nodes nor size of the largest node, correlate with histological lymph node status. Mediastinal lymph nodes were not seen on MRI in any of the patients but 1 patient with no axillary node involvement was found to have mediastinal lymph node involvement (figure 9.5).

**Figure 9.5: Imaging of axillary lymph nodes**

Pre- (A) and post-contrast (B) MRI of a 4 x 5 cm tumour in the upper inner quadrant of the left breast (large white arrow). A skin marker is visible overlying the tumour (thin white arrow). Two lymph nodes (thin black arrows) are seen in the left axillary tail and demonstrate some peripheral enhancement. From an image with the largest lymph node cross section (C), the size of the largest node was found to be 11 mm and no nodes were seen in the contralateral axilla. An axillary vessel (large black arrow) was seen on several slices. Histology showed that none of the 25 axillary nodes contained metastatic carcinoma but that 2/2 of the left internal mammary nodes contained metastatic carcinoma. The internal mammary nodes were not seen on MRI.



#### 9.4 Discussion and Conclusion

Although there is no demonstrable survival advantage in patients with <sup>large operable</sup> ~~advanced~~ breast cancer who have received PMT (Scholl et al. 1991; Mauriac et al. 1999; Makris et al. 1998; Herrada et al. 1997), a higher breast conservation rate is a clear gain (Mauriac et al. 1999; Powles et al. 1995; Makris et al. 1998; Herrada et al. 1997) without an increase in local or distant relapse (Makris et al. 1998). In our study, patients received a range of chemotherapeutic regimens but all tumours were operable by breast conservation surgery on completion of PMT and none developed local recurrence at  $31.2 \pm 10.7$  months (mean  $\pm$  sd) follow-up. Several randomised (Zurrída et al. 1994; Veronesi et al. 1995) and large non-randomised (Bonadonna et al. 1998) studies reported a breast conservation rate of 85-90% following PMT. Other studies reported a lower breast conservation rate probably deterred by a high incidence of residual disease of over 70% in specimens examined (Merajver et al. 1997).

Clinical examination, mammography and ultrasound are poor at defining residual disease following PMT. Clinical assessment of response is a less accurate predictor of outcome than pathological assessment of response (Feldman et al. 1986) and persistent mammographic densities or calcification correlate poorly with pathology (Moskovic et al. 1993; Vinnicombe et al. 1996). Breast ultrasound, may be useful in the assessment of residual disease in patient with a complete clinical response but not in those with palpable residual tumour on completion (Seymour et al. 1997). Clearly, for modern pre-operative surgical planning, an imaging modality that could accurately delineate the extent of residual disease, is of critical importance in assessing patients after PMT.

Breast MRI can image the breast with a high spatial resolution. MRI assessment of tumour size at presentation is more accurate than mammography and correlates with histology (Mumtaz et al. 1997). In comparison to MRI (table 9.2), we have shown that clinical examination overestimates tumour bidimensional product by a factor of 3 ( $p < 0.000$ ) and mammography by a factor of 2 ( $p < 0.003$ ). Clinical staging pre-PMT is thus based on a gross overestimation of tumour size. A more accurate assessment of tumour size at presentation may improve selection of suitable patients. With respect to surgical resection on completion, it is palpable disease that ultimately guides surgical



resection. Unless intraoperative interventional MRI techniques that guide surgical resection are developed beyond the experimental stage, palpable disease will continue to guide surgical resection.

In addition to imaging the primary tumour, MRI frequently detects additional enhancing foci away from the primary tumour, which could lead to a higher mastectomy rate (Conrad et al. 1999). Although additional enhancing foci on MRI probably represent in-situ or invasive disease, their natural history and prognostic significance is not known (chapter 6). In chapter 7, we demonstrated that these findings do not influence the extent of surgical resection at our centre. The equivalent survival in large clinical trials comparing breast conservation surgery to mastectomy (Fisher et al. 1995), suggests that these foci are probably not significant. In this study, 5 of 16 patients were found to have additional enhancing foci at presentation and 4 of these underwent a further MRI on completion. Interestingly, in all 4 patients the foci were no longer seen suggesting regression. Since regression would also continue with post-operative radiotherapy, excision of additional enhancing foci is probably unnecessary and may only result in overzealous resections.

In a prospective study of 38 patients with locally advanced breast cancer, 22 accepted PMT (George et al. 1999). Clinical response after 2 cycles of treatment was assessed using UICC criteria and subsequent surgical or non-surgical management, was planned after this. Of 22 patients, 15 (68%) were offered breast conservation, based on a clinical reduction in tumour size. However, chemotherapy may induce an initial inflammatory reaction or tumour necrosis, which may lead to overestimation or underestimation of tumour pathological size, respectively. Furthermore, during PMT, complete histological assessment is not available and it is thus unclear whether the assessment of disease extent, accurately reflects pathological disease extent. In our study, biopsies of the tumour cores after 2 cycles of PMT showed viable tumour and none showed necrosis. Despite the gross clinical overestimation of tumour size at presentation and on completion of PMT, there was a statistically significant correlation between the MRI and clinical bidimensional product. This was not the case at 4-6 weeks (after 2 cycles of PMT) suggesting that tumour softening in response to chemotherapy influences clinical assessment of tumour size. Furthermore, MRI detected progressive disease at 4-6 weeks in 2 patients who showed a response on completion of treatment. This may be due to a

reactive inflammatory reaction, generating an increased vascularity which leads to increased tumour enhancement on MRI.

Unlike clinical assessment, MRI assessment of tumour size at 4-6 weeks is independent of tumour consistency. MRI detected some response to chemotherapy in 13/14 (93%) patients on completion of chemotherapy and only in 4/11 (36%) at 4-6 weeks. Similarly clinical assessment detected a response in 12/14 (86%) on completion and in 5/11 (45%) at 4-6 weeks. It is thus evident that lack of MRI or clinical response during the initial stages of treatment does not predict actual response on completion of PMT.

On completion of chemotherapy, MRI detected residual disease in 13/14 (93%) which is in agreement with 97% determination of pathological residual disease in the study by Abrahams et al. (1996). Although clinical examination detected residual disease in 12/14 (86%), its extent was grossly overestimated (figure 9.4). MRI assessment of tumour size accurately predicted histological size in patients with palpable or macroscopically visible residual tumours but not in patients with microscopic disease. MRI often underestimated the extent of microscopic disease particularly in patients with lobular carcinoma. Axillary lymph nodes were seen in most patients but this did not represent tumour involvement. Others have found that MRI could detect axillary node involvement pre-operatively with a sensitivity of 90% and a specificity of 82% (Mumtaz et al. 1997), and more recently internal mammary node involvement with a sensitivity of 93.3% and specificity of 89.3% (Kinoshita et al. 1999). Neither size of largest node nor number of axillary nodes predicted histological axillary lymph node involvement in our study. Internal mammary nodes were not detected in any of the patients and 1 patient was found to have internal mammary node involvement (figure 9.5). Since the axilla was consistently imaged to visualise level I nodes and nodes above this level were not imaged in all patients, more accurate axillary imaging may improve detection of lymph node involvement but histological confirmation will be required until the technique is perfected.

Our results suggest that MRI is more accurate than clinical examination in the preliminary assessment of patients for PMT and in assessing the extent of residual disease following PMT but not in lymph node staging. In patients with microscopic residual disease and no visible tumour mass, the extent of residual enhancement underestimates the extent of microscopic disease. After 2 cycles of chemotherapy,

neither MRI nor clinical examination were able to predict the extent of response to treatment.

In the future the clinical indications for PMT may change. The strategic downstaging of disease by pre-operative chemotherapy was initially applied to non-operable breast cancer in the 1970's and operable breast cancer in the 1980's and 1990's, in an attempt to render tumours operable and operable by breast conservation, respectively. But key questions remain for the future. Does chemotherapy or endocrine therapy have a place in PMT of all breast tumours? One randomised study demonstrated that even in small primary cancers there is a significant reduction in mastectomy rates with PMT (Powles et al. 1995). Furthermore, in a proportion of women with smaller tumours, could PMT combined with radiotherapy potentially prevent the need for surgery altogether without a deleterious effect on overall survival? In such future studies MRI would certainly be the optimal imaging modality to accurately assess response. Both tumour size and tumour angiogenesis are recognised prognostic indicators for breast cancer (Henson, 1994). Breast MRI can accurately assess tumour size and contrast enhancement has been shown to correlate with tumour angiogenesis (Buadu et al. 1996). However, the future role of MRI in the management of breast cancer should now be further evaluated within large randomised prospective studies.

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## **Section C: Imaging Techniques In The Biological Assessment Of Breast Cancer**

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**Chapter 10: Classification of Breast Tumours Based on  
Contrast-enhanced MRI**

**Chapter 11: Correlation of Contrast-enhanced MRI with Tumour  
Angiogenesis and Pathological Prognostic Variables**

**Chapter 12: Contrast-enhanced Colour Doppler Ultrasound in the  
Detection of Recurrence Based on Tumour Vascularity**

# **Chapter 10: Classification of Breast Tumours**

## **Based on Contrast-enhanced MRI**

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10.1 Introduction

10.2 Patients and Methods

10.2.1 Patients

10.2.2 MRI

10.2.3 Breast cancer classification

10.2.4 Pathology

10.3 Results

10.4 Discussion

## **10.1 Introduction**

Breast MRI is extremely useful in delineating tumour local extent. Furthermore, it is thought that contrast-enhancement relies on tumour vascularity for breast cancer detection (chapter 4). Enhancement distribution may thus be dependent upon tumour physiology.

Registration of pre- and post- contrast-enhanced MRI scans corrects for patient movement between the 2 scans. Subsequent subtraction of pre- from post-contrast data sets produces a clear representation of enhancement distribution. At present, subjective terminology is used to describe enhancement distribution, since an objective classification has not been described.

In this study, a classification of MRI enhancement patterns was proposed. Since enhancement distribution may be related to tumour physiology, the classification was compared with findings on histopathology.

## **10.2 Patients and Methods**

### ***10.2.1 Patients***

Twenty one consecutive breast cancer patients were recruited following triple assessment. Patients gave their consent to undergo pre-operative MRI and the study was approved by the local Ethics Committee.

### ***10.2.2 MRI***

High resolution transverse breast MRI was performed using a T1-weighted 3D FLASH sequence (TR=18 ms, TE=7 ms, FA=40°, TA=4 m 56 s, FOV=410 mm), before and after an intravenous bolus hand injection of dimeglumine gadopentetate (*Magnevist*, 0.2 ml/kg). Images were acquired at 1.0 T (Siemens Magnetom Scanner 42 SP with dedicated breast coil) with a 3D volume of 64 mm thick and with 32 partitions, giving an effective slice thickness of 2 mm, sufficient to cover the entire breast in all cases.

Pre- and post-contrast 3D data sets were matched by a rotational and translational registration algorithm to correct for inter-scan motion and subtracted using in-house computer software. Scans were reviewed and the slice with the largest tumour cross-section, selected. Two observers reviewed the images and classified them according to the classification below. Classification categories were compared with histopathology results.

### 10.2.3 Breast cancer classification

The classification was devised following review of previous MRI scans undertaken in the department. Two distinct enhancement patterns were recognized: well-demarcated and poorly-demarcated enhancement. Well demarcated tumours showed either rim (type I - enhancement was predominantly peripheral) or homogeneous enhancement (type II - enhancement of whole lesion) whereas poorly demarcated tumours showed heterogeneous (type III - uneven enhancement of lesion with associated foci of enhancement) or diffuse patchy enhancement (type IV - main lesion associated with fine punctate peripheral enhancement) (figure 10.1).

**Figure 10.1: Breast cancer classification based on patterns of contrast enhancement on MRI:**

#### *Well demarcated lesion*

**Type I: Rim**



**Type II: Homogeneous**

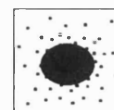


#### *Poorly demarcated lesion*

**Type III: Heterogeneous**



**Type IV: Diffuse patchy**



#### 10.2.4 Pathology

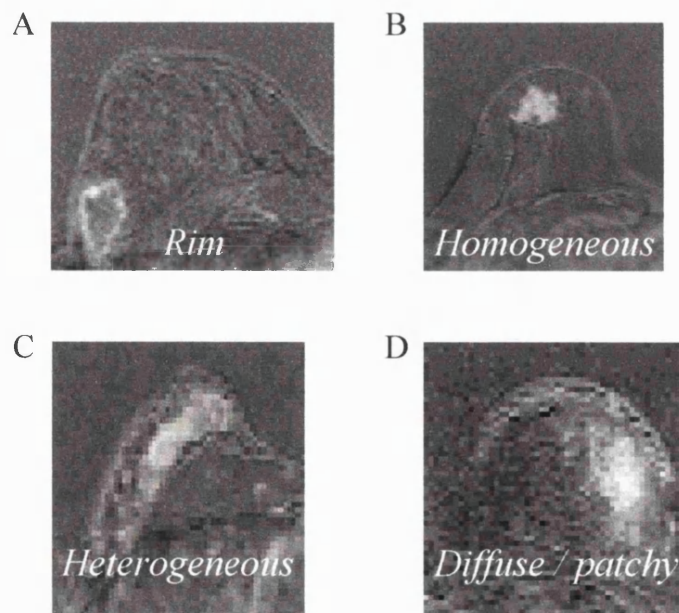
Specimens were examined fresh to note orientation, fixed in 10% formal saline and sliced at 5 mm intervals in the same plane as MRI. Routine histopathological examination was performed by an experienced breast pathologist. The Bloom & Richardson tumour grade and presence of vascular invasion or DCIS were noted.

#### 10.3 Results

Tumours were easily classified into the 4 groups (figure 10.2). Most tumours were invasive ductal carcinomas (n=14) but 4 were mixed tumours, 1 was mucinous carcinoma and 2 were pure DCIS. The frequency of enhancement patterns was: rim (n=3), homogeneous (n=5), heterogeneous (n=9) and diffuse patchy enhancement (n=4).

**Figure 10.2: Breast cancer classification:**

Subtracted contrast-enhanced MRI images of breast tumours classified under each category are shown here (A-D).



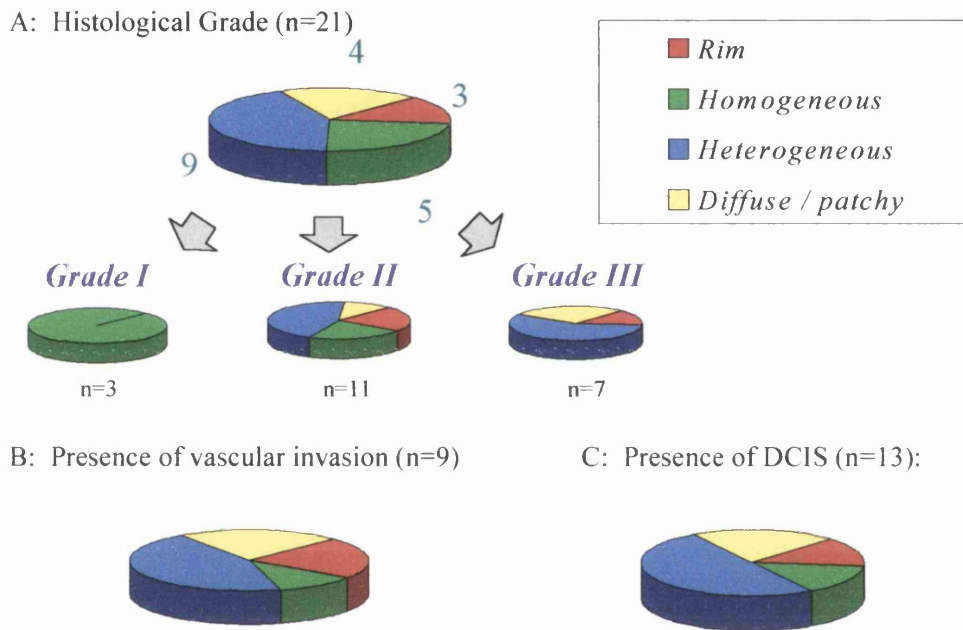
Bloom & Richardson grade I tumours were predominantly homogeneous, grade III tumours were mostly heterogeneous/diffuse patchy and grade II tumours were somewhere in between. Vascular invasion was identified in 9/21 cases and these



Chapter 10: Classification of Breast Tumours Based on Contrast-enhanced MRI tumours were predominantly heterogeneous or diffuse patchy (figure 10.3A&B). High grade DCIS was seen in association with 13 tumours and of these 8 showed heterogeneous or diffuse patchy enhancement (figure 10.3C).

**Figure 10.3: Qualitative correlation between the breast cancer classification and pathological prognostic indicators:**

Histological grade (A), presence of vascular invasion (B) and presence of DCIS (C) for the 4 breast tumour types (I-IV) are shown below (n=21).



#### 10.4 Discussion

The suggested classification of breast cancer, based on subtracted contrast-enhanced MRI, was useful in objectively defining the morphological distribution of contrast-enhancement in a particular tumour imaged by high resolution MRI. Since enhancement distribution changes during dynamic MRI, it may be more difficult to apply one classification category to a particular tumour. However, the classification is simple enough and may at least convey some objective information on the pattern of tumour enhancement at a particular time point. In this study all tumour were assessed with an acquisition time of 5 minutes post-contrast enhancement. Subtraction of pre from post-contrast scans, facilitated the assessment of enhancement distribution.

*Chapter 10: Classification of Breast Tumours Based on Contrast-enhanced MRI*

Correlation with histopathology, suggested that the more aggressive lesions may be more diffuse, without a well demarcated edge. Some studies have suggested that rim enhancement is seen in rapidly growing tumours particularly at 1-2 minutes post-contrast enhancement (Kacl et al. 1998; Gilles et al. 1996; Boetes et al. 1994). However, in this series, rim enhancement was seen in 3 tumour of low histological grade. DCIS and vascular invasion were also associated with poorly demarcated tumours.

The relationship between intensity of contrast-enhancement and histopathological prognostic features, will be assessed quantitatively in chapter 11.

# **Chapter 11: Correlation of Contrast-enhanced MRI with Tumour Angiogenesis and Pathological Prognostic Variables**

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## 11.1 Introduction

## 11.2 Methods

### 11.2.1 Patients

### 11.2.2 MRI

### 11.2.3 Evaluation of pre- and post-contrast MRI images

### 11.2.4 Biopsy and specimen histology

### 11.2.5 Immunohistochemistry

### 11.2.6 Assessment of MVD

### 11.2.7 Statistical analysis

## 11.3 Results

### 11.3.1 Patient and tumour characteristics

### 11.3.2 Detailed histopathological angiogenic mapping and comparison with MRI

### 11.3.3 Correlation of contrast-enhancement intensity with MVD

### 11.3.4 Correlation of contrast-enhancement intensity with other prognostic and histological tumour characteristics

## 11.4 Discussion and Conclusion

## **11.1 Introduction**

Microvessel density (MVD) is a powerful prognostic indicator in breast cancer (section 4.4). A correlation between MVD and contrast-enhancement on MRI would be clinically important since it would demonstrate that prognostic information could be derived from an essentially diagnostic technique.

As was discussed in section 4.5 contrast-enhanced MRI may potentially provide, in addition to information on disease extent, information on the biological and functional behaviour of tumours. Although this would be particularly useful in those situations when a specimen is not available for histopathological assessment until after treatment (table 4.1), it may also be useful as a rapid assessment of the entire tumour to supplement tumour histology. Furthermore, as opposed to histological assessment, MRI can be repeated which may be useful in the assessment of functional response to PMT.

Contrast enhancement in tumours depends on several factors including tumour vascularity. Some clinical studies using dynamic MRI, have demonstrated a correlation between initial rate of contrast enhancement and MVD (Buadu et al. 1997b; Buadu et al. 1996; Frouge et al. 1994) but this was not confirmed by all studies (Buckley et al. 1997). In the clinical setting, spatial resolution is compromised (section 2.3) in order to obtain temporal information on contrast-enhancement. It would thus be an advantage if information on tumour function and biology could be obtained from high resolution MRI.

The aims of this prospective study were to determine:

1. whether enhancement distribution in breast tumours concords with detailed histopathologic angiogenic mapping of resected tumour specimens.
2. whether contrast-enhancement intensity using high-resolution MRI correlates with MVD in resected specimens.
3. whether contrast-enhancement correlates with other prognostic variables and tumour histological characteristics.

## 11.2 Methods

### 11.2.1 Patients

Twenty four patients with 26 breast cancers were recruited prospectively from the Breast Out-Patient Clinics at Middlesex and Elizabeth-Garrett-Anderson (EGA) hospitals. The diagnosis of breast cancer was established by triple assessment. Informed consent for breast MRI was obtained from all patients. An information sheet provided patients with information about the MRI scan.

### 11.2.2 MRI

Pre- and post-contrast enhanced breast MRI was performed pre-operatively, using a transverse T1-weighted three dimensional (3D) FLASH sequence (2mm effective section thickness) with a Magnetom 42SP (Siemens, Germany). All images were then transferred onto a *Sun* workstation (Sun Microsystems, Mountain View, CA) and stored on optical discs.

### 11.2.3 Evaluation of pre- and post-contrast MRI images

Image data sets were retrieved from optical discs onto the *Sun* workstation and in-house computer software was used for measuring enhancement intensity. Firstly, pre- and post-contrast 3D data sets were matched by a rotational and translational registration algorithm to correct for inter-scan motion, using in-house registration software (Davey et al. 1997). Subtraction of these data sets generated subtraction images, which were used to identify the slice with the largest tumour cross-section for further analysis. The distribution of enhancement intensity was then compared with detailed histological angiogenic mapping.

Quantification of enhancement intensity was performed on the selected slice using the original pre- and post-contrast images. This enabled the calculation of percentage enhancement intensity (EI) relative to pre-contrast signal intensity:

$$EI (\%) = \frac{[(\text{post-contrast signal intensity}) - (\text{pre-contrast signal intensity})]}{(\text{pre-contrast signal intensity})} \times 100$$

A region of interest (ROI) was traced around the edge of the tumour on the post-contrast image to generate a mask, which was then applied to the pre-contrast image. The surface area (SA), AP (*dAP*) and medio-lateral (*dML*) tumour diameters were then calculated. Using the formula above, percentage mean tumour enhancement intensity (mean EI) was calculated.

Maximal tumour signal intensity was measured using a small square mask ( $20.5\text{mm}^2$ ) to search the tumour area with highest signal intensity. At the determined location, both pre- and post-contrast values were measured to obtain the maximal enhancement intensity (max EI), using the above formula. Using, irregular ROIs, percentage enhancement intensities of breast fat tissue (fat EI), normal breast tissue (breast EI), pectoralis major muscle (pec EI) and the background outside the breast (background EI) were determined. A small circular ROI ( $87.2\text{mm}^2$ ) was used to measure the enhancement intensity in the aorta (aorta EI). Aortic EI was found to be the highest (appendix 4). In order to obtain a measure of tumour EI in relation to the maximally enhancing tissue in a particular patient, max EI and mean EI were divided by aortic EI to obtain max cEI and mean cEI.

#### ***11.2.4 Biopsy and specimen histology***

Resection specimens (wide local excision or mastectomy) were delivered to the Pathology Department fresh. All specimens were sectioned in half and then fixed in formalin overnight. Specimens were subsequently sliced in the transverse plane at 5 mm intervals. Whole block sections were then prepared to include the whole tumour with adjacent macroscopically normal breast tissue. Sections were stained with H&E.

#### ***11.2.5 Immunohistochemistry (see addendum)***

Immunohistochemical staining was undertaken with monoclonal antibodies for markers of blood vessel endothelium (FVIIIIRAg, CD34, CD31) and for oestrogen receptors. Blocks were cut at 5-10 $\mu$  and fixed onto glass slides (pressure cooker was used for CD31 and CD34; microwave was used for FVIIIIRAg). The standard avidin-biotin technique was used with the following dilution for the 3 primary antibodies:

CD 31	(obtained from Prof David Mason, Oxford)	dilution 1:2
CD 34	(Bionostics, UK)	dilution 1:30
FVIIIIRAg	(DAKO)	dilution 1:50

The secondary antibody used was a mouse anti-rabbit antibody at recommended dilution (Strept ABCComplex/FDRP Duet Kit from, DAKO).

### ***11.2.6 Assessment of MVD***

The distribution and density of microvessels in the whole section was mapped in detail and compared with MRI images from the section with the largest tumour diameter.

In order to validate the counts of the observer against those of an experienced pathologist (appendix 2), MVD was quantified using both Chalkley counting and Weidner's method of counting vascular hot spots (section 4.4), on a series of 20 slides. Chalkley counting was found to be the most reliable method for assessing MVD.

Subsequently, MVD was evaluated by the observer on all the slides by scanning the tumour at low power (x40), and Chalkley point counting of 10 separate non-overlapping x250 power fields. The Chalkley point grid (NG52-21mm, Graticules Ltd, Tonbridge, Kent) had 25 randomly placed spots and covered an area of 0.65 mm<sup>2</sup> at x250 magnification. The highest MVD (max MVD) and average of 3 highest MVD counts (mean MVD), represented the MVD count for each tumour.

### ***11.2.7 Statistical analysis***

On a scatter diagram, the distribution of MVD counts was not normally distributed. Thus, Spearman's correlation coefficient was used in a pairwise assessment for correlation between MVD counts as well as between MVD counts and percentage enhancement intensity. Multiple regression analysis was used to evaluate any linear relationships between prognostic variables and percentage enhancement intensity.

## 11.3 Results

### 11.3.1 Patient and tumour characteristics

A total of 24 patient aged 28-80 (median 41) years with 26 breast tumours, were studied. Patient and tumour characteristics are listed in table 11.1.

### 11.3.2 Detailed histopathological angiogenic mapping and comparison with MRI

Most vascular hot spots were located close to the tumour periphery. In serial tumour sections, microvessel distribution was similar with the 3 endothelial markers (figure 11.1). The distribution of contrast enhancement on MRI, matched the microvessel distribution on histology (figure 11.2; figure 11.3), with higher numbers of capillaries and venules seen in tumour areas with higher signal intensity on MRI.

When MVD counts with the 3 endothelial markers were compared, the best correlation was found between max MVD using FVIIIIRAg and max MVD with the other vascular markers (table 11.2).

**Table 11.1: Patient (a) and tumour (b) characteristics:**

**(a)**

<i>Patient Characteristics</i>	<i>Number of Cases</i>
Menopausal Status	
Premenopausal:	11
Postmenopausal:	15
Breast Surgery	
Mastectomy:	6
Breast conservation:	20
Axillary Surgery	
Axillary node clearance (ANC):	19
No axillary surgery:	7

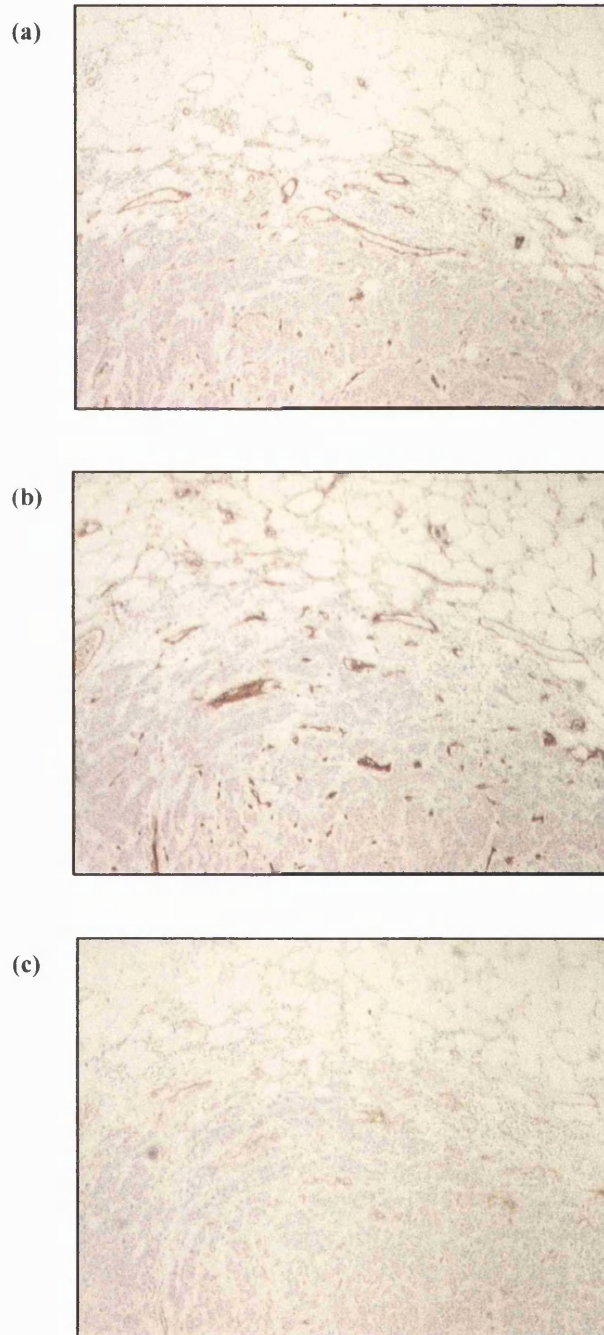


(b)

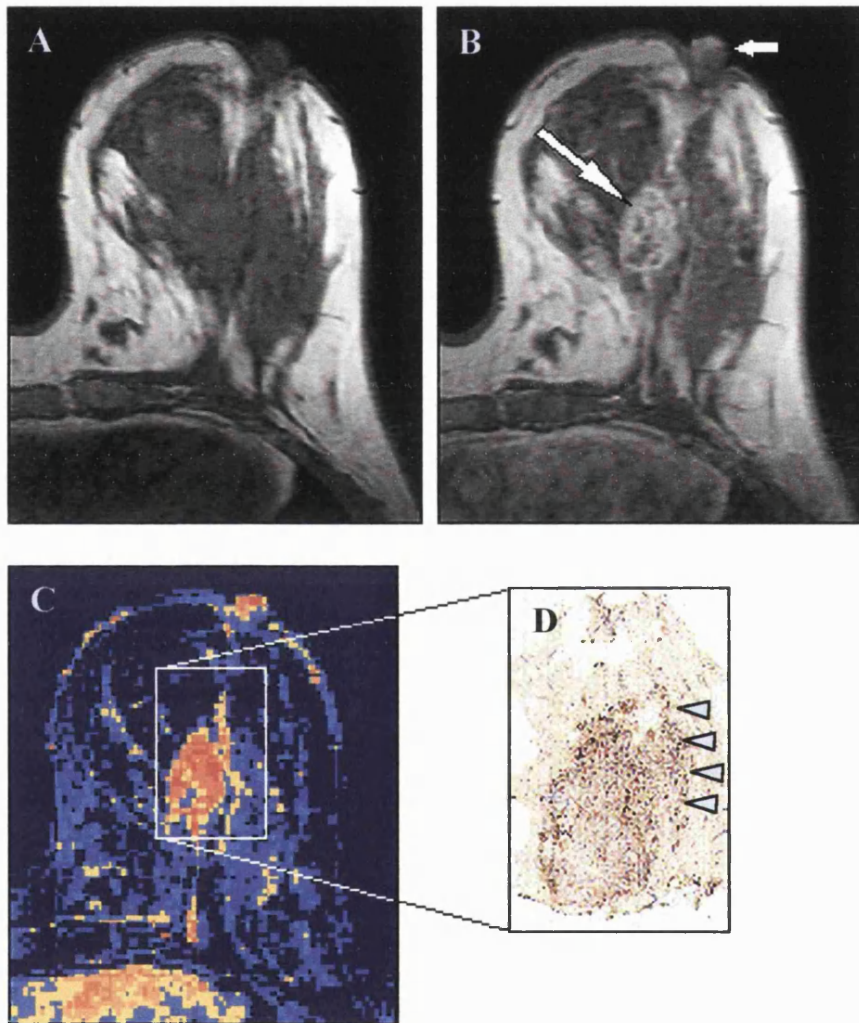
<i>Tumour Characteristics</i>	<i>Number</i>
<b>Tumour Type</b>	
Ductal:	18
Lobular:	1
Mucinous:	1
Mixed:	4
DCIS:	2
<b>Histological Grade</b>	
Grade I:	3
Grade II:	6
Grade III:	12
Not graded:	5
<b>PTNM Clinical Classification</b>	
Tis	2
T1 ( $\leq 2\text{cm}$ )	11
T2 ( $> 2\text{cm} \leq 5\text{cm}$ ):	13
T3 ( $> 5\text{cm}$ ):	0
T4 (extension to chest wall or skin):	0
N0:	5
N1:	14
NX:	7
<b>DCIS</b>	
Absent	6
Low grade	4
High grade	16
<b>Vascular Invasion</b>	
Absent	17
Present	9
<b>Oestrogen receptor status</b>	
Negative (0)	3
Weakly positive ( $>0\% \leq 50\%$ )	3
Positive ( $>50\% \leq 75\%$ )	7
Strongly positive ( $>75\%$ )	6
Not done	7

**Figure 11.1: Immunohistochemical staining for vascular endothelium (x40):**

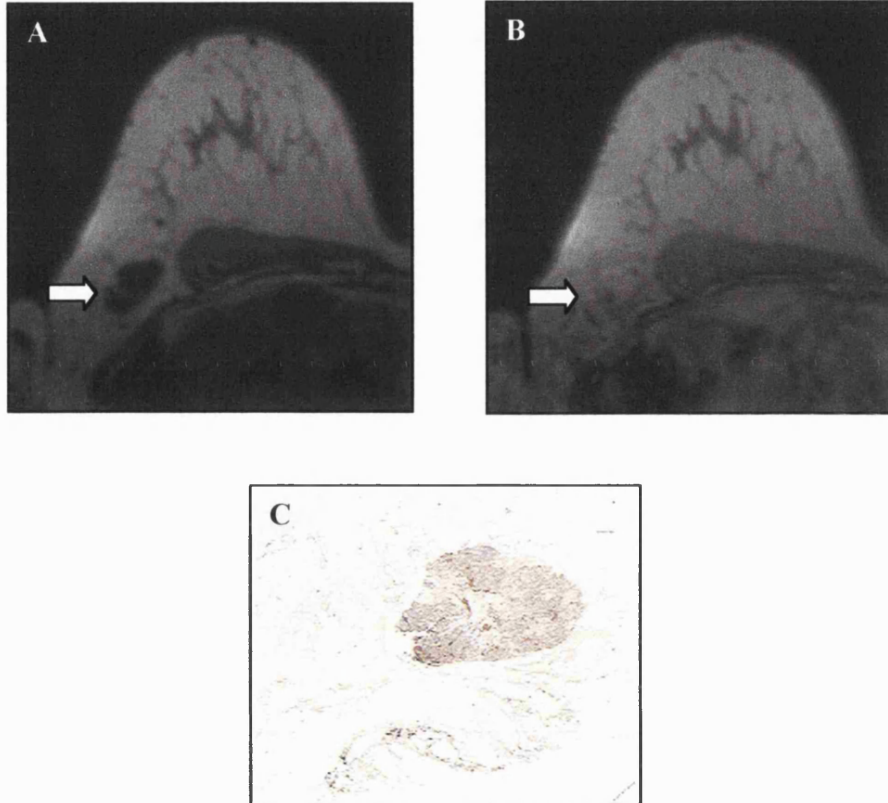
Immunohistochemical staining using anti-CD31(a) anti-CD34 (b) and Anti-FVIIIIRAg (c) monoclonal antibodies. Serial sections of the invasive edge of this breast tumour, demonstrate a similar microvessel distribution (brown staining) with the 3 antibodies.



**Figure 11.2:** T1 weighted pre- (A) and post- (B) contrast-enhanced breast MRI: A breast tumour (large arrow) with nipple involvement (small arrow) and linear enhancement representing DCIS (triangles), were clearly visible after contrast enhancement (B). Digital subtraction of image (A) from image (B) and subsequent colour coding (C), demonstrated a very good correlation between areas of high enhancement intensity (red) and areas rich in blood vessels (brown staining) as seen with anti-FVIIIIRAg immunohistochemistry, performed on sections from the surgical specimen (D).



**Figure 11.3:** T1 weighted pre- (A) and post- (B) contrast-enhanced breast MRI: A breast tumour (arrow) is seen to enhance after contrast (B). Immunohistochemistry with anti-CD31 on a large block section, demonstrated a high microvessel density (brown staining) within the tumour, even at low power (C).



### 11.3.3 Correlation of contrast-enhancement intensity with MVD

In appendix 4, a comparison of percentage enhancement intensity in different tissues showed that maximal enhancement occurs in the aorta. Thus, in addition to comparing mean EI and max EI with MVD counts (table 11.3), mean EI and max EI were corrected for aortic enhancement. This was done by dividing mean EI and max EI by aortic EI and multiplying by 100, to obtain percentage enhancement relative to the individual aortic enhancement. Results of the correlations are tabulated (table 11.3).

A weak correlation was seen between CD34 and percentage enhancement intensity, which reached statistical significance with max EI and mean EI (table 11.3). However, no statistically significant correlation was seen with any of the other markers.

**Table 11.2: Comparison between MVD determined with FVIIIIRAg, CD31 and CD34:**

Descriptive statistics (a) demonstrate similar mean MVD counts with the 3 endothelial markers but that the data has different degrees of skewness. A pairwise comparison of MVD counts using Spearman's correlation coefficient (b) demonstrated a significant correlation only when max MVD using FVIIIIRAg was included in the pair (b).

(a)

	<i>FVIIIIRAg</i>		<i>CD31</i>		<i>CD34</i>	
	<i>Max</i>	<i>Mean</i>	<i>Max</i>	<i>Mean</i>	<i>Max</i>	<i>Mean</i>
<b>No.</b>	19	19	20	20	16	16
<b>Mean</b>	6.79	6.12	6.5	5.88	6.8	6.17
<b>sd</b>	1.69	1.22	1.73	1.56	1.90	1.65
<b>Min</b>	4	3.67	3	2.67	4	3.67
<b>Max</b>	10	8.33	11	9.00	11	9.67
<b>Median</b>	15	24	17	21	15	25
<b>Skew</b>	0.29	-.18	.54	.02	.47	.34

*No.* – number of patients; *sd* – standard deviation; *Max* – max MVD; *Mean* – mean MVD; *Min* – minimum; *Max* – maximum; *Skew* - Skewness

(b)

	<b>n</b>	<b>Correlation coefficient</b>	<b>2-tailed p</b>
FVIIIIRAg vs CD31	19		
Max MVD		0.55	*0.021
Mean MVD		0.43	0.086
FVIIIIRAg vs CD34	16		
Max MVD		0.77	*0.025
Mean MVD		0.61	0.105
CD31 vs CD34	16		
Max MVD		0.22	0.540
Mean MVD		0.25	0.490

\* Significant at the  $p < 0.05$  level

**Table 11.3: Evaluation of degree of correlation between MVD and percentage enhancement intensity:**

		<i>Max EI</i>	<i>Max cEI</i>	<i>Mean EI</i>	<i>Mean cEI</i>
<b>Max MVD FVIIRAg</b>	<b>Correlation coefficient</b>	0.119	0.412	-0.140	0.178
	<b>Sig. (2-tailed)</b>	0.626	0.100	0.954	0.494
	<b>N</b>	19	19	19	19
<b>Mean MVD FVIIRAg</b>	<b>Correlation coefficient</b>	0.145	0.420	-0.063	0.177
	<b>Sig. (2-tailed)</b>	0.555	0.093	0.799	0.496
	<b>N</b>	19	19	19	19
<b>Max MVD CD31</b>	<b>Correlation coefficient</b>	0.036	0.225	-0.147	0.059
	<b>Sig. (2-tailed)</b>	0.881	0.369	0.536	0.817
	<b>N</b>	20	20	20	20
<b>Mean MVD CD31</b>	<b>Correlation coefficient</b>	0.065	0.239	-0.106	0.71
	<b>Sig. (2-tailed)</b>	0.786	0.339	0.655	0.778
	<b>N</b>	20	20	20	20
<b>Max MVD CD34</b>	<b>Correlation coefficient</b>	0.714	0.471	0.652	0.118
	<b>Sig. (2-tailed)</b>	*0.020	0.201	*0.041	0.763
	<b>N</b>	16	16	16	16
<b>Mean MVD CD34</b>	<b>Correlation coefficient</b>	0.695	0.412	0.604	0.050
	<b>Sig. (2-tailed)</b>	*0.026	0.271	0.065	0.897
	<b>N</b>	16	16	16	16

\* Correlation is significant at the 0.05 level (2-tailed)

#### **11.3.4 Correlation of contrast-enhancement intensity with other prognostic and histological tumour characteristics**

A strong linear relationship was found between histological tumour diameter ( $23.0 \pm 2.37$  mm; mean  $\pm$  sd) and tumour diameter on MRI ( $21.4 \pm 2.2$ mm; mean  $\pm$  sd), as illustrated in figure 11.4. There was also a strong linear relationship between histological tumour bidimensional product ( $427 \pm 73$ mm<sup>2</sup>; mean  $\pm$  sd) and cross-sectional area on MRI ( $385 \pm 74$ mm<sup>2</sup>; mean  $\pm$  sd).

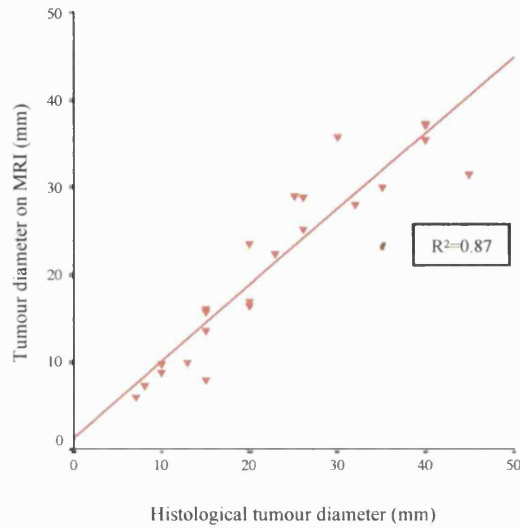
Multiple linear regression analysis was performed sequentially using max EI, max cEI, mean EI and mean cEI as the grouping (dependant) variables and patient age, histological tumour diameter, number of positive lymph nodes, tumour type (ductal,

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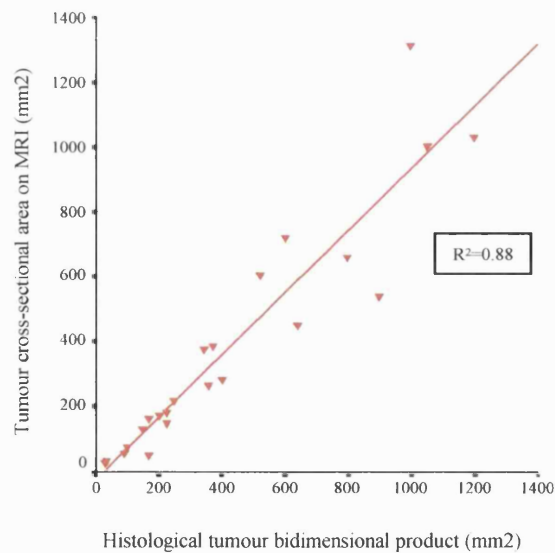
mixed, lobular or mucinous), oestrogen receptor status, presence and grade of DCIS and presence or absence of vascular invasion as the explanatory (independent) variables. There was no statistically significant correlation between percentage enhancement intensity and any of the prognostic variables (table 11.4).

**Figure 11.4: A comparison of MRI and histological assessment of tumour size:**  
Linear regression was used to apply a line of best fit, and the regression coefficients are shown ( $p < 0.000$ ).

(a) Comparison of MRI and histological maximal tumour diameter (n=26):



(b) Comparison of tumour cross-sectional area on MRI and histological tumour bidimensional product (n=26):





**Table 11.4: Multiple regression analysis to determine which factors influence enhancement intensity:**

Multiple regression analysis was used to evaluate any linear relationship between prognostic variables and max EI (a), max cEI (b), mean EI (c) and mean cEI (d).

**(a) Max EI:**

	<i>Coefficient</i>	<i>SE of Coefficient</i>	<i>P</i>
Age	-7.78	4.06	0.306
Histological tumour diameter	3.14	3.58	0.542
Number of positive lymph nodes	18.91	5.98	0.195
Tumour type	24.51	72.42	0.792
Histological grade	-58.79	104.67	0.674
Oestrogen receptor status	49.41	28.25	0.331
Presence and grade of DCIS	-26.69	53.59	0.706
Vascular invasion	-113.87	73.08	0.363

**(b) Max cEI:**

	<i>Coefficient</i>	<i>SE of Coefficient</i>	<i>P</i>
Age	-2.32	0.94	0.246
Histological tumour diameter	0.38	0.83	0.731
Number of positive lymph nodes	5.65	1.39	0.153
Tumour type	2.06	16.83	0.922
Histological grade	-	-	-
Oestrogen receptor status	16.05	6.57	0.247
Presence and grade of DCIS	-19.13	6.82	0.218
Vascular invasion	-36.56	16.99	0.277

**(c) Mean EI:**

	<i>Coefficient</i>	<i>SE of Coefficient</i>	<i>P</i>
Age	-7.70	2.05	0.165
Histological tumour diameter	2.25	1.80	0.431
Number of positive lymph nodes	16.67	3.01	0.114
Tumour type	88.09	34.49	0.250
Histological grade	-179.34	52.74	0.182
Oestrogen receptor status	45.25	14.24	0.194
Presence and grade of DCIS	71.55	27.01	0.230
Vascular invasion	-97.00	36.83	0.231

**(d) Mean cEI:**

	<i>Coefficient</i>	<i>SE of Coefficient</i>	<i>P</i>
Age	-2.30	0.40	0.109
Histological tumour diameter	0.21	0.35	0.659
Number of positive lymph nodes	5.06	0.59	0.074
Tumour type	20.76	7.12	0.210
Histological grade	-	-	-
Oestrogen receptor status	15.10	2.78	0.116
Presence and grade of DCIS	-7.33	2.88	0.239
Vascular invasion	-31.18	7.18	0.144

SE - standard error

#### **11.4 Discussion and Conclusion**

Detailed histopathological angiogenic mapping using large blocks of tumours cut in the same plane as the breast MRI, demonstrated a striking correlation between the distribution of microvessels and enhancement intensity. Although microscopic appearances were similar with the 3 endothelial markers, significant correlations between MVD counts were seen only between FVIIIIRAg and CD31, and FVIIIIRAg and CD34. This may be due to the staining of both large and small vessels with equal intensity by CD31 and CD34, whereas FVIIIIRAg stains predominantly large vessels (section 4.4). When MVD was compared to percentage enhancement intensity, only a weak correlation was seen with CD34, which reached statistical significance. However, the other MVD counts did not correlate with percentage enhancement intensity even when maximal aortic enhancement was accounted for.

To date, data supporting a correlation between MVD and enhancement intensity is weak. In a study using high resolution MRI in 50 breast cancer patients, Bone et al (1998) found a significant correlation between MVD and percentage enhancement intensity using both FVIIIIRAg and CD34. However, the magnitude of the correlation coefficient, number of MVD counts performed on each case and number of excluded cases, have not been disclosed. Several studies using dynamic MRI have found a correlation between early contrast enhancement on MRI and MVD. Buadu et al (Buadu et al. 1996) in a correlational study of 63 lesions, demonstrated that contrast enhancement on dynamic MRI correlates with peak and mean MVD. Although a strong correlation ( $r=0.83$ ,  $p<0.001$ ) was obtained between mean MVD and the steepest slope on the dynamic enhancement intensity curve, several flaws in the study methodology may account for significant study bias. At least 8 of these lesions were benign and their much lower MVD compared to breast cancers, may have accentuated the correlation. Furthermore, dynamic MRI was performed with single 5-inch circular general purpose surface coil (not a breast coil), relatively thick sections (5mm section thickness) and slices were not contiguous (gap of between 1-2.5mm between slices). Quantification of MVD was also sub-optimal since not all specimens were orientated in the same plane as the MRI, and a mean of an unspecified number of vascular hot-spots was obtained before correlating with MRI. Buckley et al (1997) in a study of 40 breast cancers, found a weak correlation

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between early enhancement intensity and MVD( $r=0.47$ ) which reached statistical ( $p=0.002$ ), but the other 7 dynamic parameters measured did not correlate with MVD.

In the assessment of both tumour diameter and cross-sectional area, a strong correlation was found with histological tumour diameter and tumour bidimensional product, respectively. MRI is therefore extremely reliable in the assessment of tumour size pre-operatively. However, no correlation was found between percentage enhancement intensity and patient age, histological tumour size, number of positive lymph nodes, tumour type, histological grade, oestrogen receptor status, presence and grade of DCIS and presence or absence of vascular invasion.

Clearly, any correlation between MVD and enhancement intensity at present remains intuitive with little hard evidence. Since measurement of enhancement intensity is dependant upon the MRI scanning protocol used, and protocols vary between centres, values are not comparable. Thus at present, assessment of enhancement intensity remains an experimental tool. Novel pure intravascular contrast agents, may provide stronger correlations with angiogenesis in the future. Thus, with the exception of assessment of tumour size, the jury must remain out with respect to the role of MRI in providing prognostic information pre-operatively.

# **Chapter 12: Contrast-enhanced Colour Doppler Ultrasound in the Detection of Recurrence Based on Tumour Vascularity**

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## **12.1 Introduction**

## **12.2 Patients and Methods**

### **12.2.1 Patients**

### **12.2.2 Ultrasound**

### **12.2.3 MRI**

### **12.2.4 Breast Biopsy**

### **12.2.5 Assessment of Microvessel density**

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## **12.3 Results**

### **12.3.1 MVD in tumour recurrence compared with benign scar tissue**

### **12.3.2 Correlation between MVD and US vascularity grade**

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### **12.3.4 Decision pathway analysis: possible implications of use of Doppler US on number of benign breast biopsies**

### **12.3.5 Comparison between MRI and Doppler US**

## **12.4 Discussion**

## **12.5 Conclusion**

## 12.1 Introduction

Colour Doppler US is the most important imaging modality that can compete with breast MRI in the evaluation of breast cancer. Since the main clinical application of breast MRI is in the detection of breast recurrence, a possible application of colour Doppler was investigated in this study. Furthermore, Doppler US relies on the detection of increased tumour angiogenesis and this may thus be considered as a direct application of a recognised biological phenomenon to the detection of recurrence.

Conventional grey-scale ultrasound has limited ability to distinguish scar tissue from malignancy (Balu-Maestro et al. 1991). Blood flow characteristics in primary breast tumours have been imaged with colour Doppler US (Madjar et al. 1996), but since intratumoural vessels are small and Doppler signals weak, its use as a diagnostic tool has so far been unsuccessful. However, the development of novel US contrast agents may lead to new applications for colour Doppler US. *Levovist* (Schering, Berlin, Germany), a novel intravenous echo-enhancing bubble contrast agent, lowers the threshold for detection of flow by enhancing the Doppler signal amplitude. It has been used to define vascular characteristics in primary breast tumours with inconsistent results (Madjar et al. 1996; Kedar et al. 1996), but has not been used to look at surgical scars.

In the treatment of small breast cancers, randomised controlled studies of breast conservation versus mastectomy, have demonstrated no difference in disease free or overall survival (Fisher et al. 1989). However with breast conservation surgery followed by post-operative radiotherapy, there is a small increase in local recurrence, 90% of which occur in the operated quadrant (Fisher et al. 1992). Furthermore, scarring and changes after irradiation, often result in clinical and mammographic findings suggestive of local recurrence (Balu-Maestro et al. 1991; Sickles and Herzog, 1984). All patients with suspected recurrence invariably undergo biopsy, and as a result, many unnecessary benign biopsies are undertaken in order to detect only modest numbers of local recurrences. The mere suspicion of recurrence induce unnecessary anxiety in many of these women. Poor post-operative healing due to previous radiotherapy, may increase morbidity in those women who undergo open biopsy. Although the incidence of local recurrence is relatively small (approximately 5-10% in 5 years (Schmolling et al. 1997)),

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in the UK all 27,000 new cases of breast cancer per annum will require some form of post-operative follow-up assessment.

The aims of this prospective study were to determine:

1. whether vascularity is increased in tumour recurrence compared to benign scar tissue;
2. whether vascularity as assessed by colour Doppler US, correlates with histological assessment of lesion vascularity;
3. whether *Levovist* contrast-enhanced colour Doppler US, is superior to conventional colour Doppler US in the detection of breast recurrence.
4. whether the complementary use of colour Doppler US in the investigation of suspected breast cancer recurrence, could reduce the number of patients undergoing benign breast biopsy.
5. to compare MRI with colour Doppler US in those patients who had undergone both investigations.

## **12.2 Patients and Methods**

The clinical aspect of this study was set up in collaboration with Schering Healthcare (Berlin, Germany) to evaluate the clinical utility of *Levovist* in the assessment of breast cancer recurrence. A proposal for angiogenesis assessment of all breast specimens, written by myself and presented to Schering Healthcare, was accepted and sponsored by them. As a result, UCL was selected to be the histology reference hospital for this study.

The study was approved by the Joint UCL/UCH Committee on the Ethics of Human Research at the University College Hospitals and by separate Ethics Committees at each of the other 7 centres. Written informed consent was obtained from all participating subjects.

### **12.2.1 Patients**

Over a 12 month period, consecutive women with a clinical or mammographic suspicion of breast cancer recurrence, were recruited prospectively from 7 centres prior to breast biopsy.

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Patients who had undergone surgery within 3 months, had been given another contrast agent within 24 hours or had been given an investigational drug in the preceding 30 days, were excluded. Pregnancy was excluded in all pre-menopausal women by means of a urinary pregnancy test. The study was carried out under a CTMP (Clinical Trial with a Marketed Product) certificate from the Medicines Control Agency.

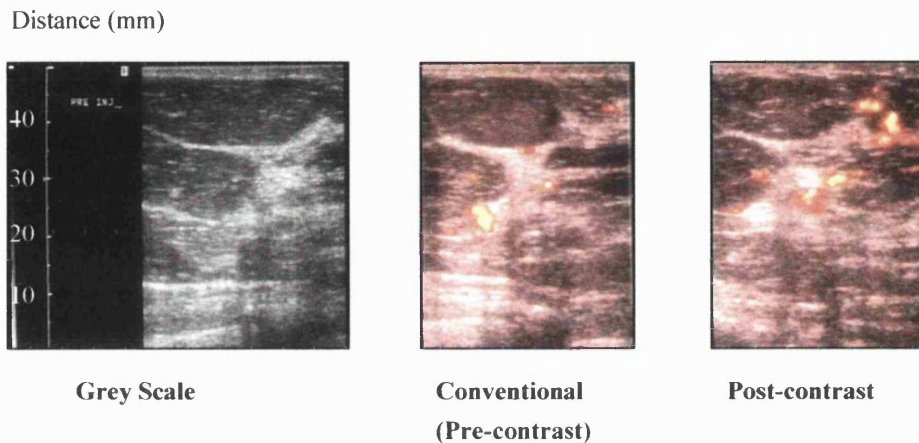
A total of 60 patients were required to obtain a power of 80% to detect an increase in sensitivity from 70% to 95% (with similar specificities), between unenhanced and contrast-enhanced US.

#### **12.2.2 Ultrasound**

Breast ultrasound was conducted using sonographic units capable of power and real-time Doppler and images were recorded on S-VHS video. Standard settings were used for both pre- and post-contrast examinations: pulsed repetition frequency 800-1000 KHz, wall motion filter 50-100 Hz and velocity scale 3-5 cm / sec. A thorough examination of the area of clinical or mammographic concern was made using standard grey-scale and colour Doppler US (figure 12.1). This US probe was then fixed in its optimal position and contrast-enhanced colour Doppler ultrasound performed following an intravenous injection of 8 mls of *Levovist* (400 mgs/ml, Schering, Berlin, Germany) at a rate of 1-2 mls/sec via an indwelling peripheral catheter. In some cases the Doppler gain was adjusted post-injection in order to optimise the signal to noise ratio. Following this examination the videotapes were reviewed and lesion vascularity was graded on a scale of 0-4 (0-avascular; 1-slightly vascular; 2-moderately vascular; 3-highly vascular and 4-very highly vascular) by 2 experienced radiologists who came to a consensus agreement.

**Figure 12.1: Grey Scale (a), conventional (b) and contrast-enhanced (c) colour Doppler sonograms of diffuse breast recurrence:**

This 56 year old women, presented 2 years after WLE of a 1.5 cm invasive ductal carcinoma (Grade III). Note the hypo-echoic posterior shadowing (seen on all illustrations) indicative of malignancy. With colour Doppler Ultrasound, intralesional vessel signal enhancement (red/yellow) is seen (b) and signal enhancement is increased with *Levovist* (c). The scale on left-hand side shows 5mm graduations.



### 12.2.3 MRI

Contrast-enhanced high resolution MRI was performed on patients who had given separate consent for MRI, as part of the other MRI projects in this thesis. When available, the MRI was compared to the US scan. MRI could only be performed after *Levovist* administration since administration of other contrast-agents was a contraindication for *Levovist*.

### 12.2.4 Breast Biopsy

After the US scan, all patients underwent either open biopsy or a minimum of 3 needle-core biopsies (Tru-cut, Travenol Laboratories, Thetford, UK). Experienced breast pathologists reported all biopsies and samples were sent to the investigator for angiogenesis assessment. Pathologists were instructed to send representative paraffin blocks or 10 slides (with 5 $\mu$ m sections) from the largest lesion cross-section. A single reference breast pathologist (at UCL) reviewed and reported all the slides.



### 12.2.5 Assessment of Microvessel Density

Representative paraffin blocks were sectioned at 5 $\mu$ m and stained for endothelial markers CD31, CD34, and FVIIIIRAg (section 11.2.5). Sections were scanned at low power to identify vascular hot spots and vessels were counted at x250 magnification (0.65mm<sup>2</sup>) using a Chalkley grid (section 11.2.6). The mean of the 3 highest readings from a total of 5 counts was used to determine MVD.

### 12.2.6 Statistical analysis

The Wilcoxon Signed Rank Test for paired sample data, was used to assess any differences between MVD with the different endothelial markers, since the distribution of these discrete variables was not normally distributed on a scatter diagram and thus, non-parametric tests were appropriate. Comparisons between MVD in benign and malignant biopsies, was made using the Mann-Whitney U Test. Correlation between MVD and US vascular grade was evaluated using Spearman's rank correlation coefficient, since the ranking variable vascular grade and the discrete variable MVD were not normally distributed.

The number of patient scans with vascularity grades above and below each cut-off point (i.e. <0, 0-1, 1-2, 2-3, 3-4) were counted. Scans with vascularity grades higher than the cut-off point being considered were assumed to be malignant (i.e.: true positives and false positives) and those falling below the cut-off point were regarded as benign (i.e.: true negatives and false negatives). Using the final tissue diagnosis as the gold standard, sensitivity and specificity of the diagnostic test pre- and post-contrast were calculated at each cut-off point. Receiver-operator characteristic (ROC) curves, graphical representations of the relationship between the sensitivity and specificity of a diagnostic test over a range of cut-off values, were constructed for both pre- and post-contrast US. The area underneath each ROC curve represented the overall diagnostic accuracy (a combined measure of sensitivity and specificity) and was calculated using the Wilcoxon statistic (Hanley and McNeil, 1982). *Kendall's tau* (a comparative correction for paired ratings between curves, analogous to that used in the *paired t-test*) (Beck and Shultz, 1986) was used to compare both these areas to determine whether there was a true difference in accuracy between diagnostic tests. This method correctly compared paired ratings, since patients were able to act as their own controls. The overall difference in diagnostic accuracy was assessed for statistical significance.

### **12.2.7 Decision pathway analysis**

Using the percentage of patients found to have recurrence in the study, the impact of the use of US on number of breast biopsied was estimated on a projected population of 100 patients with suspected breast cancer. The clinically optimal cut-off point from the ROC curves was chosen so as to obtain the highest sensitivity for the detection of recurrence. The sensitivity and specificity at this point, was applied to the diagnostic pathway.

## **12.3 Results**

Fifty eight women (41-79 years), were recruited from the 7 centres. Of these, 28% (16/58) had histologically confirmed recurrence (15 invasive carcinomas and 1 DCIS). There was full agreement between the reference pathologist and other reporting pathologists in all cases. Equal numbers of patients (29 vs 29) underwent open and core biopsies.

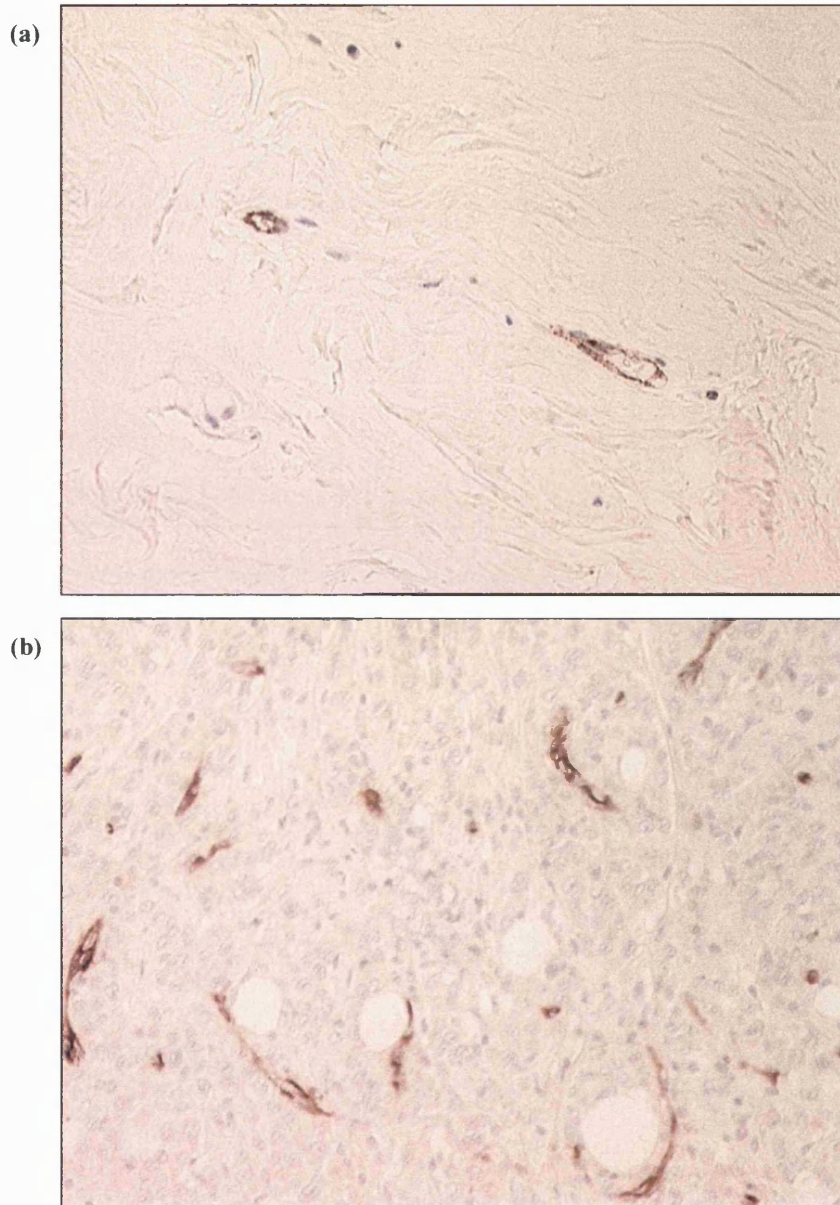
### **12.3.1 MVD in tumour recurrence compared with benign scar tissue.**

Histological material from 44 (76%) patients was received for immunohistochemistry and MVD estimation. Of these, 10 were core biopsies and were excluded since there was insufficient tissue for selection of vascular hot spots. Three incision biopsies taken with the ABBI (Advanced Breast Biopsy Instrumentation) biopsy system (Trinity Health System, Steubenville, USA) were large enough for selection of vascular hot spots and were included amongst the open biopsy specimens. Of 34 open biopsies 4 were excluded from analysis; 2 were excluded due to tissue autolysis, 1 had insufficient tissue and 1 contained only a lymph node and no breast tissue. A total of 27 open biopsies (15 recurrences and 12 benign) were successfully stained by at least 1 endothelial marker.

The distribution of microvessels was found to be higher in breast recurrence as compared to benign scar tissue (figure 12.2).

**Figure 12.2: Immunohistochemical staining for vascular endothelium (x400).**

Immunohistochemical staining using anti-CD31 monoclonal antibodies. The number of vessels seen (brown staining), is lower in scar tissue (a) as compared to breast recurrence (b).



The distribution of microvessels was similar with the 3 endothelial markers. A small number of sections were not successfully stained and were excluded (table 12.1a). Thus, in order to obtain an MVD count for every case, the highest count obtained with any endothelial marker, was evaluated as an additional MVD count. MVD was significantly increased in breast recurrence (table 12.1a&b).

**Table 12.1: MVD with FVIIIIRAg, CD31 and CD34 in benign versus malignant biopsies:**

Descriptive statistics (a) demonstrate skewed distribution of data with no overlap in 95% confidence intervals between benign and malignant biopsies. Mann-Whitney U test demonstrated a statistically significant increase in MVD, in breast recurrence (b):

(a)

		<b>FVIIIIRAg</b>		<b>CD31</b>		<b>CD34</b>		<b>Highest</b>	
		<i>Ben.</i>	<i>Malig.</i>	<i>Ben.</i>	<i>Malig.</i>	<i>Ben.</i>	<i>Malig.</i>	<i>Ben.</i>	<i>Malig.</i>
<b>No.</b>		10	14	10	12	9	11	12	15
<b>Mean</b>		15.60	24.71	15.80	23.50	17.11	24.55	17.17	25.80
<b>sd</b>		3.44	5.85	4.08	6.07	5.28	3.78	4.73	5.67
<b>95% CI</b>	<b>Lower</b>	13.14	21.34	12.88	19.64	13.05	22.01	14.16	22.66
	<b>Upper</b>	18.06	28.09	18.72	27.36	21.17	27.08	20.17	28.94
<b>Min</b>		11	18	10	18	12	20	12	20
<b>Max</b>		23	42	22	40	27	32	27	42
<b>Median</b>		15	24	17	21	15	25	16	25
<b>Skew</b>		1.019	2.087	-0.066	2.082	1.317	0.527	1.146	1.760

*No.* – number of patients; *sd* – standard deviation; *95% CI* – 95% confidence interval for mean; *Ben.* – benign biopsy; *Malig.* – malignant biopsy; *Min* – minimum; *Max* – maximum; *Skew* - Skewness

(b)

	<b>CD31</b>	<b>CD34</b>	<b>FVIIIIRA G</b>	<b>HIGHEST</b>
<b>Number of patients</b>	22	20	24	27
<b>Mann-Whitney U</b>	12.000	14.000	7.000	18.000
<b>Wilcoxon W</b>	67.000	59.000	62.000	96.000
<b>Z</b>	-3.181	-2.707	-3.700	-3.521
<b>Asymp. Sig. (2-tailed)</b>	.001	.007	.000	.000
<b>Exact Sig. [2*(1-tailed Sig.)]</b>	.001	.006	.000	.000

### 12.3.2 Correlation between MVD and US vascularity grade

There was a correlation between MVD and both pre- and post-contrast vascularity grade (VG). However, this reached statistical significance only for CD31 and highest MVD (table 12.2).

**Table 12.2: Evaluation of degree of correlation between MVD and vascularity grade (VG) both pre- and post-contrast:**

Since the data was not normally distributed, a non-parametric test, Spearman's rho correlation coefficient was used.

		<i>Pre-contrast VG</i>	<i>Post-contrast VG</i>
<b>Highest</b>	<b>Correlation coefficient</b>	0.429*	0.410*
	<b>Sig. (2-tailed)</b>	0.037	0.047
	<b>N</b>	27	27
<b>CD31</b>	<b>Correlation coefficient</b>	0.512*	0.473*
	<b>Sig. (2-tailed)</b>	0.021	0.035
	<b>N</b>	22	22
<b>FVIIIIR AG</b>	<b>Correlation coefficient</b>	0.408	0.430
	<b>Sig. (2-tailed)</b>	0.066	0.052
	<b>N</b>	24	24
<b>CD34</b>	<b>Correlation coefficient</b>	0.412	0.366
	<b>Sig. (2-tailed)</b>	0.080	0.123
	<b>N</b>	20	20

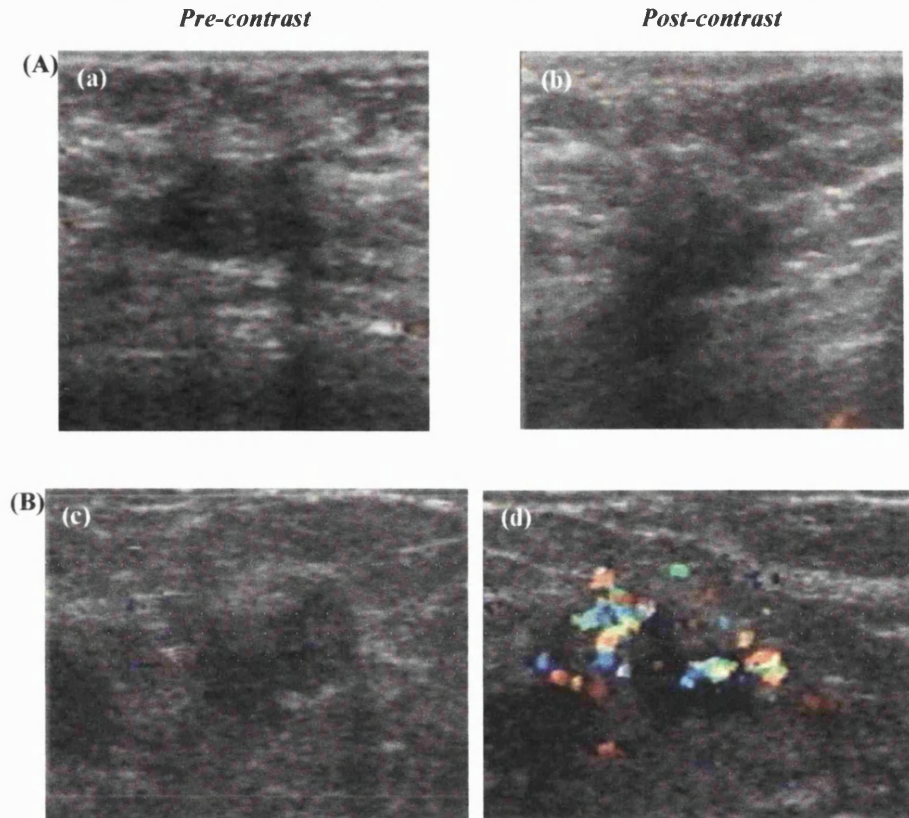
\* Correlation is significant at the 0.05 level (2-tailed)

### 12.3.3 Evaluation of US vascularity grade in malignant and benign biopsies

US vascularity was also increased in breast recurrence compared with benign scar tissue (figure 12.3). The difference in vascular grade between breast recurrence and benign tissues was highly significant, both with conventional and contrast-enhanced colour Doppler US (table 12.3).

**Figure 12.3: Pre- and post-contrast enhanced Doppler US in breast recurrence and scar tissue:**

These images illustrate 2 avascular lesions on conventional Doppler US (A and B). Following contrast-enhancement, it was evident that lesion B was highly vascular (d) compared with lesion A (b). Histology revealed that A was a scar and B was recurrence.



**Table 12.3: Evaluation of difference between US vascularity grade in malignant and benign cases, both pre- and post-contrast:**

	Unenhanced US	Levovist contrast-enhanced US
Number of patients	58	58
Mann-Whitney U	143.5	63.0
Wilcoxon W	1046.5	883.0
Z	-3.931*	-5.065*
Asymp. Sig. (2-tailed)	.000	.000

\* Significant at the 0.001 level (2-tailed)

Sensitivity and specificity for the detection of breast recurrence are illustrated in table 12.4.

**Table 12.4: Sensitivity and specificity at the different vascularity grade cut-off points, before and after contrast-enhanced Doppler US:**

Values for the clinically optimal cut-off grade are shown in bold.

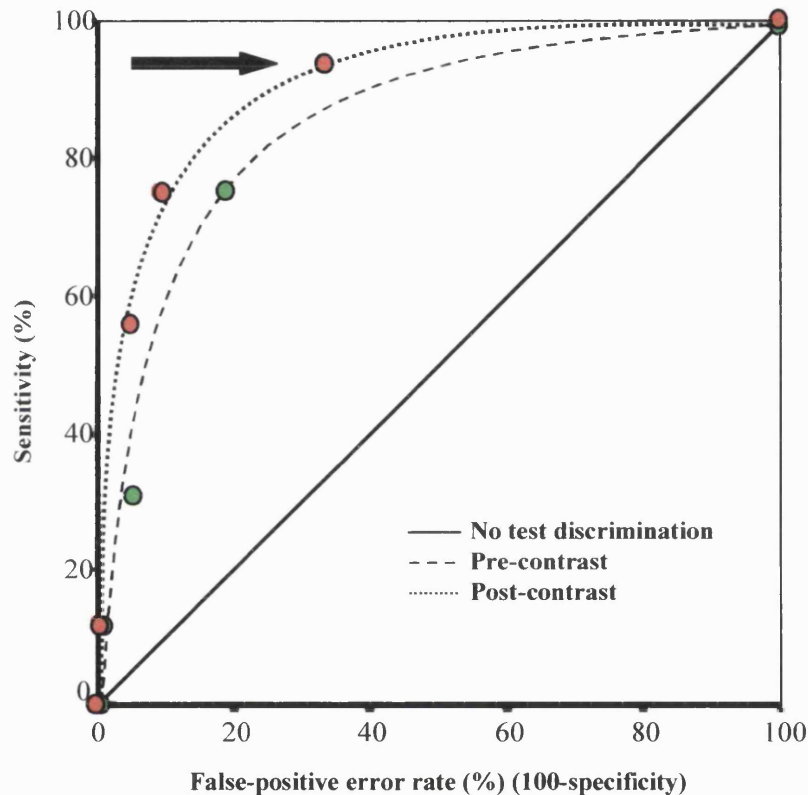
	<i>Cut-off point of radiological vascularity grade (bold)</i>					
	<b>&lt;0</b>	<b>0-1</b>	<b>1-2</b>	<b>2-3</b>	<b>3-4</b>	<b>&gt;4</b>
Pre-contrast:						
<i>True positive</i>	16	12	5	2	0	0
<i>False positive</i>	42	8	2	0	0	0
<i>True negative</i>	0	34	40	42	42	42
<i>False negative</i>	0	4	11	14	16	16
<i>Sensitivity (%)</i>	<b>100</b>	<b>75</b>	<b>31</b>	<b>12</b>	<b>0</b>	<b>0</b>
<i>Specificity (%)</i>	<b>0</b>	<b>81</b>	<b>95</b>	<b>100</b>	<b>100</b>	<b>100</b>
<i>False –positive error rate (%)</i>	100	19	5	0	0	0
Post-contrast:						
<i>True positive</i>	16	15	12	9	2	0
<i>False positive</i>	42	14	4	2	0	0
<i>True negative</i>	0	28	38	40	42	42
<i>False negative</i>	0	1	4	7	14	16
<i>Sensitivity (%)</i>	<b>100</b>	<b>94</b>	<b>75</b>	<b>56</b>	<b>12</b>	<b>0</b>
<i>Specificity (%)</i>	<b>0</b>	<b>67</b>	<b>91</b>	<b>95</b>	<b>100</b>	<b>100</b>
<i>False –positive error rate (%)</i>	100	33	9	5	0	0

Sensitivities and false positive error rates were plotted to obtain ROC curves for pre- and post-contrast Doppler US. The area under each ROC curve (figure 12.4), represents the diagnostic accuracy of each test for the detection of recurrence. Recurrence was detected with an overall diagnostic accuracy of 80 % pre- contrast (95 % c.i. 65.3-93.6)

Chapter 12: US in the Detection of Recurrence based on tumour angiogenesis and 90 % post-contrast (95 % c.i. 79.7-99.2). The difference in area between the 2 curves, was statistically significant ( $p=0.038$ ).

**Figure 12.4: Receiver-operator characteristic (ROC) curve pre- and post-contrast enhanced Doppler US:**

Sensitivities and false positive error rates for each vascularity grade cut-off plotted here, correspond to values in table 12.4. The post-contrast vascularity grade which provides highest sensitivity for breast recurrence (arrow) is the clinically optimal vascularity grade.



#### 12.3.4 Decision pathway analysis: possible implications of Doppler US on number of breast biopsies

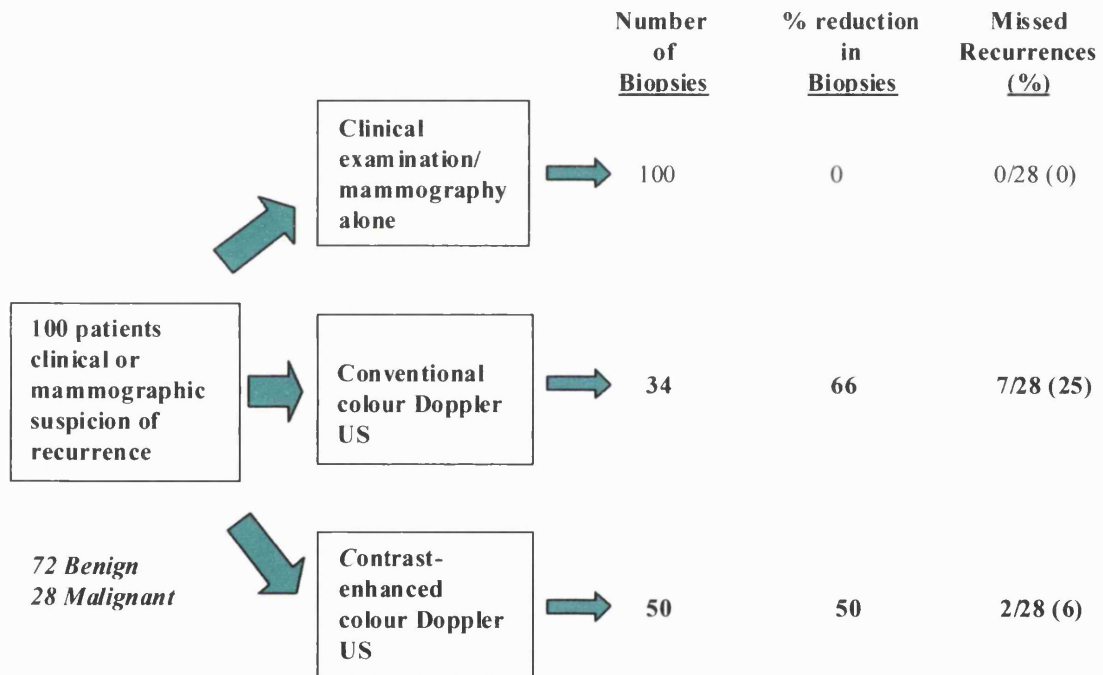
The clinically optimal vascularity grade cut-off point can be defined as that which would provide the highest sensitivity for the detection of recurrence. From the post-contrast data, it is evident that it is the 0-1 (vascular – avascular) cut-off point (figure 12.2). Although at this cut-off point there is a small reduction in specificity from 81% pre-contrast to 67% post-contrast, sensitivity increases from 75% pre-contrast to 94% post-contrast. This study has a power of 72.5% to detect an increase in sensitivity from 73% to 95%, with Levovist.



The prevalence of breast recurrence found in our study was 28% (16/58). A decision pathway analysis based on biopsies from a projected 100 patients with a suspicion of recurrence (figure 12.5), would yield 72 benign and 28 malignant biopsies. The overall reduction in breast biopsies would be 50% with contrast-enhanced Doppler US and 66% with conventional Doppler ultrasound. However, with conventional Doppler ultrasound, 25% of recurrences would be missed compared to only 6% with *Levovist*.

**Figure 12.5: Decision pathway analysis on a group of 100 patients with suspected breast cancer recurrence.**

With current assessment all patients would require breast biopsy. Based on the results of this study, the introduction of conventional colour Doppler US would result in a 65% reduction in number of breast biopsies. Although *Levovist* contrast enhanced Doppler US would result in more biopsies, less cases of recurrence would be missed compared with conventional colour Doppler US (3% versus 25%).



### 12.3.5 Comparison between MRI and Doppler US

Breast MRI was performed on 7 of 11 patients, recruited at UCL. The reported lesion characterisation based on MRI and US vascularity grades are shown in table 12.5. There was full agreement between MRI lesion characterisation and tissue diagnosis. US vascularity grade was moderate to high in patients with recurrence and avascular to moderate vascularity in those with benign scars.

**Table 12.5 Comparison of MRI and Doppler US findings:**

<i>Patient</i>	<i>MRI</i>	<i>Doppler US (vascularity grade)</i>	<i>Histology</i>
1	Patient excluded	Slight vascularity (1)*	DCIS
2	Patient excluded	Avascular (0)*	Benign
3	Strongly enhancing smooth lesions, recurrence	Moderate vascularity (2); high (3) post contrast	Recurrence within 4 lymph nodes
4	Appearances of recurrence	High vascularity (3); very high (4) post contrast	Recurrence
5	Patient excluded	Avascular (0); slight vascularity (1) post contrast	Benign
6	Patient declined	Avascular (0)*	Benign
7	Linear low signal – scar	Avascular (0)*	Benign
8	Local tumour recurrence	High vascularity (4)*	Recurrence
9	Dense fibrosis and radiation skin changes - no recurrence	Moderate vascularity (2)	Benign
10	No recurrence	Avascular (0); slightly vascular (1) post contrast	FNA benign
11	Appearances highly suspicious of extensive local recurrence; strong peripheral enhancement	Moderate vascularity (2); high vascularity (3)	Extensive recurrence

\* Same vascularity grade pre- and post-contrast enhancement

## 12.4 Discussion

Over the last 25 years, mastectomy has largely been replaced by breast conservation surgery in the treatment of small breast cancers. Whilst survival has been unaffected by this change in clinical practice, there has been a slight increase in local recurrence. Clinical examination and mammography are limited in their ability to detect recurrence, resulting in large numbers of unnecessary breast biopsies. In this study, 72% of patients had a benign biopsy.

An inexpensive, accurate and reliable method to distinguish breast recurrence from post-operative scar tissue, would be of great clinical value. Mammography relies on tissue density and distortion to distinguish recurrence from benign scar tissue. However, scarring can cause significant distortion and thus effectively mimic recurrence. Imaging a known physiological or prognostic marker, may improve detection rates. Our results suggest that MVD, a histological measure of angiogenesis, is significantly increased in cases of recurrence. Furthermore, vascularity as assessed by both conventional and contrast-enhanced colour Doppler US correlates with MVD. Thus, breast recurrence can be differentiated from scar tissue based on lesion vascularity. Furthermore, the results have shown that colour Doppler vascularity grade is a powerful discriminator of malignancy in suspected breast recurrence.

The ROC curves (figure 12.4) demonstrate that both conventional and contrast-enhanced colour Doppler US can discriminate recurrence from scar tissue, since both curves lie far to the left of a hypothetical straight line representing a test that infers no benefit. Comparison of the relative areas under the ROC curve (representing overall diagnostic accuracy) demonstrates that *Levovist* contrast-enhanced US is significantly better than conventional colour Doppler US in the detection of recurrence ( $p=0.038$ ). These results appear to be consistent with those of Kedar et al. (1996), in their evaluation of benign and malignant breast tumours. Kedar et al. (1996) also used paired ROC curve analysis but in their study, morphological characteristics of vessels were assessed rather than overall vascularity. They obtained a similar increase in diagnostic accuracy, from 80% to 90%, with *Levovist* contrast-enhancement.

*Chapter 12: US in the Detection of Recurrence based on tumour angiogenesis*

From the ROC curves (figure 12.4), it is evident that at the 0-1 cut-off point a high sensitivity can be achieved with a relatively high specificity, and this was called the 'optimal clinical cut-off' point. At this vascularity grade cut-off point, avascular lesions (grade 0) are distinguished from vascular ones (any grade > 0). This is arguably an easier distinction to make than ranking vascularity grade to several grades of intensity, particularly for the less experienced operators. Using the sensitivities and specificities for the detection of recurrence at the optimal clinical cut-off point and prevalence of recurrence in the study group (28%), a decision pathway analysis was constructed and applied to a hypothetical group of 100 women (figure 12.5). From this analysis, it is evident that the addition of conventional colour Doppler US could reduce the number of biopsies from 100 to just 34 (a reduction of 66%) but at the expense of missing 25% of local recurrences (7/28). The use of *Levovist* contrast-enhanced colour Doppler US would result in a smaller reduction in breast biopsies (from 100 to 50; 50% reduction), but at the expense of missing only 2 local recurrences (6%).

Magnetic resonance imaging (MRI), the best available imaging modality for the detection of breast cancer recurrence, also relies on tumour vascularity and vascular permeability (Buadu et al. 1996) as demonstrated by contrast enhancement (Heywang-Kobrunner et al. 1997). MRI has a sensitivity of 93-100 % and specificity of 83-88 % for the detection of local recurrence in small studies (Turkat et al. 1994; Mumtaz et al. 1997a). Thus, the sensitivity and specificity achieved at the optimal clinical post-contrast cut-off point in our study, is comparable to that for MRI (Turkat et al. 1994; Mumtaz et al. 1997a; Davis and McCarty, Jr., 1997).

Colour Doppler ultrasound is more readily available than MRI and it would have been useful to be able to compare these 2 imaging modalities directly. However, in this study, patients who had undertaken a contrast-enhanced scan in the preceding 24 hours or those given an investigational drug within 30 days of the study were excluded and only 7 patients underwent MRI. Furthermore, many breast cancer patients take part in several research studies and asking them to undertake an additional research investigation may be too much of a burden. It would therefore be preferable to compare the 2 investigations following full licence approval for *Levovist*, at which stage the 2 investigation could at least be performed during the same hospital visit.

## **12.5 Conclusion**

Angiogenesis, as measured by MVD is increased in tumour recurrence. Vascularity grade as detected by *Levovist* contrast-enhanced colour Doppler ultrasound, is a powerful discriminator of malignancy in suspected local recurrence. *Levovist* contrast-enhanced colour Doppler ultrasound is significantly better than conventional colour Doppler at detecting local recurrence. Contrast-enhanced colour Doppler ultrasound is inexpensive, patient friendly, quick and easy to perform by a trained clinician in an out-patients clinic. The implementation of colour Doppler ultrasound in the investigation of breast local recurrence may dramatically reduce the numbers of unnecessary breast biopsies.

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## **Section D: Conclusion and Future Directions**

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**Chapter 13:        Role of MRI and US in Surgical Management**

**Chapter 14:        Future Research**

# **Chapter 13: Role of MRI and US in Surgical Management**

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- 13.1 Breast Cancer Screening
- 13.2 Diagnosis of Breast Cancer
- 13.3 Detection of Additional Breast Lesions
- 13.4 Detection of Recurrence
- 13.5 Surgical Planning
- 13.6 Assessing Response to PMT
- 13.7 Prognostic Assessment

The role of surgery in the management of breast cancer has undergone a revolution over the last 100 year. From radical surgical extirpation of breast cancer during the Halstedian era of ‘en block resection’, it soon became evident that this disease was systemic even at the time of diagnosis. Enthusiasm for overzealous surgical resection subsided and we have moved to an era of surgery as one modality, in a multidisciplinary approach to the management of breast cancer. Nevertheless, surgery remains central to breast cancer management. The surgical oncologist maintains a leading role from the moment of clinical suspicion to diagnosis and patient follow-up. The role of breast imaging modalities should be seen in this light, since modern imaging modalities should assist the surgeon during the various stages of patient management.

The role of MRI in the surgical management of breast cancer is not clear since large prospective studies are lacking. Key issues in breast cancer management (table 2.1) and the potential qualities of MRI that would be of assistance in surgical practice (table 2.2), have been considered in chapter 2. In chapter 3, indications for breast ultrasound have been discussed. In light of the work presented in this thesis, the current role of MRI in the management of breast cancer will be considered here.

### ***13.1 Breast Cancer Screening***

Neither MRI nor US are indicated for screening women for breast cancer. Even the use of mammography as a screening tool has been questioned since recent randomised controlled studies have not demonstrated a survival advantage in the screened group (section 1.2.1).

### ***13.2 Diagnosis of Breast Cancer***

Triple assessment remains the primary mode of management of suspicious breast lesions. Ultrasound has a role in lieu of mammography in the assessment of young women and women on HRT. MRI has no practical role in the diagnosis of the indeterminate breast lesions, since at present, the need for breast biopsy cannot be obviated. Furthermore, studies to date have been mixed with respect to the detection of DCIS and mammography remains the investigation of choice.



MRI may be useful in detecting a primary in women presenting with axillary metastases and mammographically occult lesions. The two women presenting in this way (table 5.2) underwent yearly MRI scans and at 3 year follow-up, neither women was found to have a breast primary. These women were treated with tamoxifen and thus any subclinical lesion appeared to have regressed. Although MRI may be useful in this instance, it cannot reliably be used to detect breast tumours in the axillary tail of these women since they invariably undergo axillary node clearance. MRI cannot reliably distinguish residual or recurrent disease from post-operative scarring until 6-18 months post-operatively (section 2.4.3).

### ***13.3 Detection of Additional Breast Lesions***

In chapter 6, additional enhancing foci detected by MRI, were found to represent DCIS or invasive cancer foci. However, since breast MRI is still a research investigation, these cancer foci should be regarded as an experimental finding. There is strong circumstantial evidence to suggest that these foci are probably not prognostically significant but in chapter 6, it was suggested that MRI could be used to study the natural history of these lesions prospectively. In chapter 9, 5/16 patients who were assessed prior to PMT, were found to have additional enhancing foci, away from the primary tumour, but in 4 patients who underwent a further MRI scan on completion of chemotherapy, these lesions were no longer visible. Thus, additional enhancing foci probably regress with chemotherapy and any subclinical cancer foci should continue to regress with post-operative radiotherapy. The high sensitivity of breast MRI leads to the detection of additional cancer foci but this should not lead to overzealous resections or be interpreted as an indication for mastectomy.

### ***13.4 Detection of recurrence***

In chapter 12 of this thesis, colour Doppler US was shown to be a powerful discriminator of malignancy in suspected local recurrence. The addition of *Levovist*, an intravascular microbubble contrast agent, significantly improves sensitivity for the detection of recurrence (section 12.3.3). Unfortunately, a direct comparison with MRI was not possible since patients could not be given 2 sequential contrast agents within the studies performed in this thesis.

MRI is a useful investigation in the detection of local recurrence and this remains the commonest clinical indication for breast MRI (table 5.1). An accurate assessment of local disease extent is an advantage over breast US. A major drawback, is the high cost of an MRI. Technological advances and novel contrast-agents have led to major improvements in the spatial resolution of breast US and colour Doppler US may well compete with MRI for this indication.

### ***13.5 Surgical Planning***

The form of surgery required for a particular patient depends on overall clinical status (e.g.: general health, age), the specific biology of a tumour and the stage of the disease at the time of treatment. However, the ultimate clinical end-points of surgical management are to improve patient survival, quality of life or a combination of the two. A reduction in the extent of surgery required may be regarded as an improvement in a patient's quality of life.

MRI provides extremely accurate information on breast tumour size pre-operatively (chapter 11). However, at present the provision of additional information from breast MRI, does not significantly influence the extent of surgical resection, at our centre. Clearly, a different interpretation of the findings by different surgeons could result in larger resection or even unnecessary mastectomies.

Volumetric analysis of breast and tumour volume (chapter 8) by MRI, may provide a more objective assessment of the proportion of the breast that is involved by tumour thus assisting the surgeon in deciding whether the patient is suitable for breast conservation surgery. In chapter 8, it was found that only tumour volume was significant in the objective decision on suitability for wide local excision.

### ***13.6 Assessing Response to PMT***

Since clinical assessment of breast tumours by bidimensional product overestimates tumour size by a factor of 3 (chapter 9), MRI staging pre-PMT may improve the selection of suitable patients. Post-PMT, MRI is useful in assessing the extent of residual disease but not in those patients with microscopic disease and no visible tumour. MRI is not useful in lymph node staging. Interestingly after 2 cycles of chemotherapy, neither

MRI nor clinical examination, are able to predict the final response to PMT. It is thus important to continue treatment even in those patients with no early clinical response.

### ***13.7 Prognostic Assessment***

MRI provides a very accurate assessment of tumour size. The role of MRI and ultrasound in the provision of pre-operative prognostic information remains to be proven.

With respect to tumour angiogenesis, detailed histological angiogenic mapping showed that areas with high enhancement intensity also had a high concentration of microvessels. However, this qualitative correlation, did not translate into a quantitative one as there was no correlation between MVD and percentage enhancement intensity. With respect to the detection of recurrence, MVD was shown to be higher in recurrence as compared to scar tissue (chapter 12) although again, evidence for a correlation with US vascularity grade was weak.

In the thesis, it was shown that MRI and Doppler US are not just forms of expensive ‘imagery’, but that there are numerous areas in which they may be of assistance to the surgeon. Technological advances are increasing the number of imaging modalities available and it is thus important, from the outset, to assess each modality in terms of its impact on clinically significant end-points. Hard evidence in the form of large prospective trials, is urgently required and further research work will be considered in chapter 14.

## **Chapter 14: Further Research**

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Clear improvements in the diagnosis, staging and detection of distant metastases, have resulted from advances in breast imaging. However, adequately powered comparative studies and randomised controlled studies are required to assess the impact of MRI and US on clinically relevant end points such as survival, quality of life and extent of surgical resection. Furthermore, since the fields of MRI and US are expanding rapidly, it is important to maintain a link between surgeons and radiologists in order to develop the best modalities for clinically useful indications.

Some prospective studies are already underway such as the study on MRI for screening patients at high genetic risk (chapter 2) and results are eagerly awaited. Although it would create a new indication for MRI, it is unlikely that MRI would replace mammography in the near future.

In chapter 6 it was demonstrated that the high sensitivity of MRI leads to the detection of additional enhancing foci, away from the primary tumour, and that these represent cancer foci. To ascertain the value of MRI in clinical management in a future study, patients with enhancing foci could be randomised to either surgical excision of these foci along with the primary tumour, or excision of the primary alone and MRI follow-up. In this way we would establish not only the natural history of enhancing foci but also whether removing these foci is feasible by conservative surgery and whether this practice would ultimately influence local recurrence rates. This work is essential prior to embarking upon a policy of preventative surgical removal and would be assisted by further development of interventional MRI biopsy.

In chapter 7 it was demonstrated that a knowledge of breast MRI findings pre-operatively, does not significantly influence the extent of surgical resection at our centre. Conrad et al. (1999) demonstrated that if breast MRI findings are used in clinical decision making, it results in a higher mastectomy rate, although this was interpreted as beneficial. Thus in a future study, patients should be randomised to either pre-operative MRI in addition to triple assessment or triple assessment alone.

Work on the objective assessment of tumour resectability by using volumetric analysis (chapter 8) could be developed further, but would depend upon automation of, and faster, image analysis.

Comparative studies are also needed in order to assess the role of US in comparison to MRI. There are difficulties when attempting to run studies with both imaging modalities since 2 experimental contrast agents may not be administered to the same group of patients, and this situation has been encountered in this thesis. Furthermore, competition exists between different clinical trials for the same patient group. It may be possible to plan clinical studies using both modalities in parallel with other trials but access to imaging at very short notice would be essential.

With respect to the use of US in the detection of local recurrence (chapter 12), the next step should be to assess the impact of Doppler US on benign breast biopsy rate prospectively in a one stop follow-up clinic. The rate limiting step here may be to obtain funding. Such a study could be deemed of interest to the pharmaceutical industry, but instead, could be interpreted by them as a potential source of revenue.

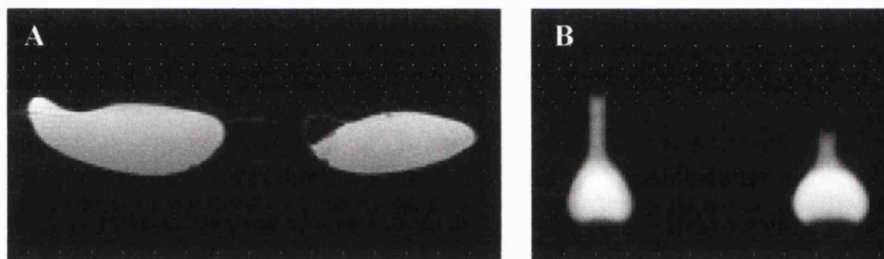
With respect to prognostic assessment of breast tumours pre-operatively, new contrast agents are currently being developed. Pure intravascular contrast agents for MRI may produce a correlation with MVD as well as other prognostic indicators. It would be important to compare high resolution MRI with dynamic MRI in this context. With faster image acquisition provided by modern scanners, it would be possible to perform 2 scans on the same patient without greatly lengthening scanning time. Contrast agents for US may selectively increase the reflectivity of blood while reducing artifacts such as blooming. The spatial resolution of US is improving but since the resolution of MRI is improving in parallel, it is likely that competition between the 2 modalities will remain.

## Appendix 1 Phantom Volume Measurements

The software used to measure MRI volume, was assessed for accuracy by scanning phantoms and then measuring volume by ROI analysis of individual slices, as discussed in section 8.2.3. The phantoms included 2 breast prostheses (200ml and 300ml) and 5 volumetric flasks containing water (100ml, 25ml, 20ml, 10ml and 5ml). Phantoms were scanned (figure A1) in the same breast coil and with the same scanning parameters used in chapter 8. Volume was measured 5 times for each phantom and results are shown in table A1. Actual volume was slightly over-estimated by MRI assessment. Percentage error rate was inversely related to actual volume and was under 5% for volumes greater than 5ml.

**Figure A1: Phantom volume measurement:**

The volume of breast prostheses (A) and volumetric flasks filled with water (B) was determined by ROI analysis of individual slices.



**Table A1: Comparison of actual and measured phantom volumes:**

Five volume measurements were performed on each phantom. Mean, standard deviation and percentage error are tabulated below.

<u>Actual volume</u> <u>(ml)</u>	<u>Measured on MRI</u> <u>(mean <math>\pm</math> sd)</u>	<u>Percentage</u> <u>Error</u>
300	301.45 $\pm$ 0.11	0.5
200	201.56 $\pm$ 0.23	0.8
100	102.22 $\pm$ 0.24	2.2
25	25.36 $\pm$ 0.06	1.4
20	20.41 $\pm$ 0.05	2.1
10	10.47 $\pm$ 0.07	4.7
5	5.30 $\pm$ 0.02	6.0

## Appendix 2 Comparison of Investigator's MVD with MVD of an Experienced Pathologist

In order to estimate the variation in MVD assessments by Chalkley point and Wiedner's vessel counts, 20 slides stained with endothelial markers were selected (10 stained with FVIIIIRAg and 10 stained with CD31).

The investigator and an experienced pathologist independently selected 10 vascular hot spots at low power and then counted at x 250 magnification. Both Chalkley and total vessel counts were carried out and the mean of 3 highest counts used to represent the MVD for each slide. The highest correlation was obtained with Chalkeley point counts (table A2).

**Table A2: Comparison of vascular counts between the investigator and an experienced pathologist:**

Mean and standard deviation (a) and Spearman's correlation coefficient (b) were used to compare Chalkley point and Weidner's vessel counts of the investigator with those of an experienced pathologist.

(a) Mean and standard deviation of vessel counts:

	n	Investigator	Pathologist
Chalkley point counts:			
Mean $\pm$ sd	20	6.5 $\pm$ 1.4	6.5 $\pm$ 2.1
Std. Error of mean		0.3	0.5
Weidner's vessel counts:	20		
Mean $\pm$ sd		22.6 $\pm$ 8.2	30.4 $\pm$ 9.7
Std. Error of mean		2.0	2.4

(b) Assessment of correlation using Spearman's correlation coefficient:

	n	Correlation coefficient	2-tailed p
Chalkley point counts	20	0.80	**0.000
Weidner's vessel counts	20	0.54	*0.026
Pathologist's counts	20	0.90	**0.000
Investigator's counts	20	0.68	**0.002

\* Significant at the  $p < 0.05$  level

\*\* Significant at the  $p < 0.01$  level



### Appendix 3 Comparison of Investigator’s Signal Intensity Measurements with those of an Experienced Medical Physicist

The investigator’s measurements of signal intensity in ROI were compared with those of an experienced medical physicist. Subtracted scans from the same 16 patients were independently reviewed. In the slice with the largest tumour cross section, a region of interest (ROI) was traced around the edge of the tumour. The mean tumour signal intensity and tumour cross-sectional area, were calculated. A statistically significant correlation was found between the measurements of the investigator and those of the medical physicist (table A3).

**Table A3: Comparison of signal intensity and surface area measurements between the investigator and an experienced medical physicist:**

Mean and standard deviation (a) and Spearman’s correlation coefficient (b) were used to compare the assessments of signal intensity and tumour cross-sectional surface area, by the investigator and physicist.

(a) Mean and standard deviation of vessel counts:

	n	Investigator	Physicist
Signal intensity:			
Mean ± sd	16	150.2 ± 10.7	144.3 ± 9.1
Std. Error of mean		2.7	2.3
Tumour surface area:	16		
Mean ± sd		559 ± 374	492 ± 307
Std. Error of mean		94	77

(b) Assessment of correlation using Spearman’s correlation coefficient:

	N	Correlation coefficient	2-tailed p
Signal intensity	16	0.68	**0.004
Cross-sectional area	16	0.86	**0.000

\*\* Significant at the  $p < 0.01$  level

## Appendix 4 Percentage Enhancement Intensity in Different Tissues

Using the pre and post contrast data sets studied in chapter 11, irregular ROIs were used to measure percentage enhancement intensities (EI) of the background outside the breast (background EI), breast fat tissue (fat EI), normal breast tissue (breast EI) and pectoralis major muscle (pec EI). A small circular ROI (87.2mm<sup>2</sup>) was used to measure percentage aortic enhancement (aortic EI). The highest percentage enhancement intensity was seen in the aorta (table A4).

**Table A4: Percentage enhancement intensity in different tissues:**

	<i>n</i>	<i>Mean ± sd (%)</i>	<i>Std. error of mean</i>
<b>Background EI</b>	26	0.93 ± 1.73	0.61
<b>Breast EI</b>	26	10.05 ± 5.08	1.69
<b>Fat EI</b>	26	4.75 ± 3.12	1.27
<b>Pec EI</b>	26	29.97 ± 16.90	5.63
<b>Aortic EI</b>	26	326.69 ± 76.22	16.25

# Meetings and Publications

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## Publications Related to This Thesis

1. **Douek M**, Vaidya J S, Lakhani S R, Hall-Craggs M A, Baum M, Taylor I. Can magnetic-resonance imaging help elucidate the natural history of breast cancer multicentricity? *The Lancet* 1998; 351: 801-802.
2. **Douek M**, Vaidya J S, Baum M, Taylor I. Magnetic-resonance imaging and breast cancer multicentricity. *The Lancet* 1998; 352: 652-653. (*letter*)
3. **Douek M**, Davidson T, Taylor I. Breast cancer imaging - what are the optimal modalities? *Eur J Surg Oncol* 1998; 24(6): 573-582. (*review*)
4. **Douek M**, Davidson T, Hall-Craggs M A, Lakhani S R, Baum M, Taylor I. Does pre-operative MRI influence the extent of surgical resection in conservative breast cancer surgery? *The Breast* 1999;8(2):84-88.
5. Winehouse J, **Douek M**, Holz K, Madjar H, W.Lees, Baum, M. Contrast-enhanced colour Doppler ultrasound can effectively reduce benign biopsy rates in suspected breast cancer recurrence. *Br J Surg* 1999; 86: 1198-1201.
6. Baum M, Chaplain M, Anderson A, **Douek M**, Vaidya J S. Does Breast Cancer Exist In A State Of Chaos? *Eur J Cancer* 1999; 35(6): 886-891.
7. **Douek M**, Hall-Craggs M A. Can the use of pre-operative MRI reduce local recurrence rates in patients with retroareolar breast cancer undergoing breast conservation surgery? *Radiology* 1999; 210(3): 880-881. (*letter*)
8. Winehouse J, **Douek M**, Lees W. ROC curve analysis in the evaluation of contrast-enhanced colour Doppler US. *Radiology* 1999; 212(3):911. (*letter*)
9. **Douek M**, Baum M. A surgical perspective of breast MRI *in Breast MRI in Practice*, Reading, UK: Harwood Academic Publishers, 2000: *in press*. (book chapter).

## Papers submitted for peer review

1. **Douek M**, Benjamin E, Davidson T, Hall-Craggs M A, Taylor I. Tubular carcinoma of the nipple: histological, radiological and MRI appearances. *Br J Radiol* 2000.

2. Douek M, Davidson T, Hall-Craggs M A, Stein R, Lakhani S R, Kissin M, Baum M, Taylor I. Is breast MRI superior to clinical examination in the assessment of response to primary medical therapy ? *Cancer* 2000

#### Published Abstracts

1. Douek M, Davidson T, Hall-Craggs M A, Benjamin E, Wilkinson I D, Davies M, Mumtaz H, and Taylor I. Contrast enhancement patterns in subtraction breast MRI correlate with tumour angiogenesis in breast cancer. *The Breast* 1997; 6(5):318.
2. Douek M, Davidson T, Hall-Craggs M A, Stein R, Taylor I. Assessment of response to treatment during chemotherapy for breast cancer. A new role for breast MRI ? *The Breast* 1997; 6(5): 319-320.
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20. **Douek M**, Winehouse J, Deng J, Holz K, Madjar J, Pezzella F, Lakhani S R, Lees W R, Davidson T, Taylor I, Baum M. Detection of breast cancer recurrence by contrast-enhanced Doppler ultrasound; implications for breast biopsy. *Eur J Surg Oncol* 1998; 24(6): 628.

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## **Meetings Attended**

### ***National Meetings***

1. BASO 54th Scientific Meeting, University of Aberdeen, Aberdeen 16-17 July 1997.  
(2 published abstracts)
2. Surgical Research Society Tripartite Meeting, Nottingham, 9-11<sup>th</sup> July 1997  
(1 published abstract)
3. 5th Nottingham International Breast Cancer Conference, East Midlands Conference Centre, University of Nottingham, Nottingham 17-19 September 1997.  
**Douek M**, Davidson T, Wilkinson I D, Hall-craggs M A, Benjamin E, Davey M, Mumtaz H, Taylor I. Subtraction and registration of contrast enhanced MR images for correlation with breast cancer angiogenesis. (unpublished)  
**Douek M**, Davidson T, Hall-Craggs M A, Stein R, Baum M, Taylor I. MRI in the assessment of response to neoadjuvant chemotherapy in advanced breast cancer. (unpublished)
4. British Breast Group (BBG) Meeting, Wetwood Hall, Leeds, 31st October 1997.  
**Douek M**, Vaidya J S, Lakhani S R, Blanchard K, Hall-craggs M A, Davidson T, Baum M, Taylor I. MRI in the detection of breast cancer multicentricity. (unpublished)  
Winehouse J, Baum M, **Douek M**, Lees W. A vascular study of malignant lymph nodes in breast cancer using echo-enhanced colour doppler and CD31 immunohistochemistry. (unpublished)
5. Royal College of Radiologists (RCR) Breast Group, Watershed Media Centre, Bristol 3-4 November 1997.

- Douek M**, Hall-Craggs M A, Davidson T, Wilkinson I D, Benjamin E, Taylor I. A classification of breast tumours based on subtracted contrast enhanced MRI. (unpublished)
- Douek M**, Davidson T, Hall-Craggs M A, Stein R, Baum M, Taylor I. MRI in the assessment of response to neoadjuvant chemotherapy in advanced breast cancer. (unpublished)
6. BASO / BACR Meeting, The Royal College of Surgeons of England, London 27-28th November 1997. (1 published abstracts)
  7. Annual Meeting of Associations of Surgeons of Great Britain and Ireland, Edinburgh International Conference Centre, Morrison Street, Edinburgh 13-15 May 1998.  
**Douek M**, Vaidya J S, Lakhani S R, Blanchart K, Baum M, Taylor I.  
MRI in the detection of breast cancer multicentricity. (unpublished)
  8. The British Association of British Surgical Oncology (BASO), 56th Scientific Meeting, Liverpool, 25th-26th June 1998. (2 published abstracts)
  9. The British Association of British Surgical Oncology (BASO), 56th Scientific Meeting, Liverpool, 25th-26th June 1998. (2 published abstracts)
  10. The Surgical Research Society (SRS), Royal College of Surgeons in Ireland, Dublin 2-3 July 1998. (1 published abstracts)
  11. Section of Surgery Paper Presentation, The Royal Society of Medicine, London 4<sup>th</sup> November 1998. *Awarded The Glaxo Travelling Fellowship for the best paper presentation.*
  12. Is breast cancer in a state of chaos ?, UCL Workshop series, Gustave Tuck Theatre, UCL, London. Delivered lecture entitled: New in-vivo imaging techniques to study tumour vasculature as a way of testing predictions of the mathematical model,
  13. British Association of Surgical Oncology Silver Jubilee Meeting, The Royal College of Surgeons, London 26-27 November 1998. (1 published abstract)
  14. Charles Grant Clark Presentation Meeting, Michael Hobsley Seminar Room, Department of Surgery UCL, London 4<sup>th</sup> June 1999. *Awarded Charles Grant Clark Prize for Surgical Research.*
  15. British Association of Surgical Oncology, Annual Meeting, Portsmouth 21-23 November 1999. *Presented paper at Ronald Raven Prize Session.*

***International Meetings***

16. 2nd International Conference EUSOMA (European Society of Mastology), Palazzo dei Congressi, Florence 18-22<sup>nd</sup> March 1997. (2 published abstracts)
17. The 22<sup>nd</sup> Congress of the International Association for Breast Cancer Research, Astir Palace Hotel, Vouliagmeni, Athens, Greece 25-28<sup>th</sup> September 1998. (4 published abstracts)
18. 1<sup>st</sup> European Breast Cancer Conference, Florence, Italy 29<sup>th</sup> September - 3<sup>rd</sup> October 1998. (4 published abstracts)
19. 1<sup>st</sup> Anglo-Israel Cancer Conference, Eilat, Israel 17-21<sup>st</sup> December 1998.  
**Douek M, Winehouse J, Deng J, Holz K, Madjar J, Pezzella F, Lakhani S R, Lees W R, Davidson T, Taylor I, Baum M.** Differentiation of scar tissue from breast cancer recurrence by contrast-enhanced Doppler ultrasound; correlation with angiogenesis. (unpublished) *Sponsored by Glaxo Travelling Fellowship.*



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### **Addendum: Immunohistochemistry for markers of blood vessel endothelium**

Immunohistochemical staining for markers of blood vessel endothelium was undertaken with 3 monoclonal antibodies (FVIIIIRAg, CD34, CD31). Whole blocks were sectioned at 5-10 $\mu$ , deparaffinized, hydrated and fixed onto glass slides (pressure cooker was used for CD31 and CD34; microwave was used for FVIIIIRAg). Endogenous peroxidase was inactivated by incubation with 3% hydrogen peroxide in distilled water for 5 minutes, followed by rinsing with Tris-buffered saline (TBS: 0.05mol/l Tris/HCl, 0.15mmol/l NaCl, pH 7.6 at 37°C) for 5 minutes. The primary antibodies were diluted at 1:2 for CD 31 (obtained from Prof David Mason, Oxford), 1:30 for CD 34 (QBEnd10; Bionostics, UK) and 1:50 for FVIIIIRAg (DAKO) in BSA (1% bovine serum albumin in Tris-buffered saline). Primary antibodies were added to the deparaffinized sections and incubated for 120 minutes at room temperature. The optimum concentration for each antibody was determined by assessing the microvessel staining after incubation of a control tumour specimen with a serial dilution of the primary antibody, over various time periods. Slides were then rinsed in TBS for 5 minutes.

Immunohistochemistry was undertaken using the avidin-biotin-peroxidase method (Strept ABCComplex/FDRP Duet Kit from, DAKO) and manufacturer's instructions. The secondary antibody was a biotinylated goat anti-mouse/rabbit antibody and slides were incubated with it for 30 minutes. Following further rinsing with TBS, the bound antibodies were detected using the avidin-biotin complex/horse-radish peroxidase (30 minute incubation) and finally visualised using 0.05% 3,3'-diaminobenzidine in Tris-HCl pH 7.6 plus 33  $\mu$ l of 30 volume hydrogen peroxide per 100 ml (10 minute incubation). Meyers haematoxylin was used for counterstaining of nuclei and coverslips were mounted with DPX (Merk, Poole, UK). All slides were stained in batches of 20-30 with both positive and no-first antibody negative controls. The entire batch was rejected if neither control was satisfactory.