

Diffusion Weighted and Diffusion Tensor MRI-derived Apparent Diffusion Coefficient and Fractional Anisotropy Values as Biomarkers for Treatment Response in Breast Cancer

A thesis submitted to Trinity College Dublin, The University of Dublin

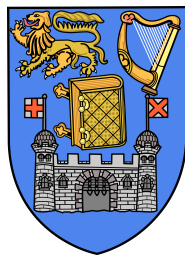
For the degree of M.D. in Clinical Medicine

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DECLARATION

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THESIS SUMMARY

Background

With increasing numbers of patients receiving neoadjuvant chemotherapy (NACT) for breast cancer and with multiple emerging drug agents, a reliable indicator of treatment response early in the course of chemotherapy is vital to facilitate tailored treatment for individual patients. Determination of response is unreliable with clinical assessment, and conventional imaging techniques such as ultrasonography and mammography are of limited use as they primarily detect changes in tumour size which may not become evident for several weeks after the initiation of chemotherapy. Functional MRI (including diffusion imaging) provides an opportunity to detect alterations in the cellular environment early in the course of chemotherapy. Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values are quantitative parameters derived from diffusion MRI.

Purpose

To evaluate if changes in ADC and FA values can predict early response in patients receiving NACT for breast cancer.

Methods

20 consecutive patients with invasive breast carcinoma underwent 3.0 Tesla MRI at 4 time-points: pre-treatment (TP0) and following the first (TP1), second (TP2) and final cycles of NACT. ADC and FA maps were generated for each patient at each time-point. Baseline and sequential data in responder and non-responder groups were compared to assess the potential of ADC and FA in predicting tumour response, as determined by Miller Payne grading of the surgical specimen.

Results

Tumour ADC in responders significantly increased at TP1 ($p<0.001$) and TP2 ($p<0.001$) over baseline, while a significant increase in tumour FA of responders was seen at TP2 ($p=0.005$) only. No statistical change occurred in tumour ADC or FA values in the non-responder group. The percentage increase in mean tumour ADC was significantly higher in the responder group compared to the non-responder group after the first and second cycles of chemotherapy ($p<0.001$). The optimal time point to discriminate responders from non-responders was after the first cycle of chemotherapy with a percentage increase cut off in tumour ADC values of 7.7%. Despite tumour FA significantly increasing in the responder group from TP0 to TP2, no significant percentage change in FA values was observed between responders and non-responders.

Conclusion

Changes in ADC values early in the course of treatment are useful for predicting response in patients receiving NACT for breast cancer. Although tumour FA significantly increased in the responder group after the second cycle of chemotherapy, FA values did not demonstrate efficacy in the early differentiation of responders from non-responders.

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DEDICATION

This thesis is dedicated to my parents, Patrick and Breda, and my fiancé, Mark.

ABBREVIATIONS

ADC	Apparent diffusion coefficient
AUC	Area under the curve
BCT	Breast conservation therapy
BMI	Body mass index
DCE	Dynamic contrast-enhanced
DCIS	Ductal carcinoma in situ
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
EPI	Echo planar imaging
FA	Fractional anisotropy
FOV	Field of view
HER2	Human epidermal growth factor receptor 2
IDC	Invasive ductal carcinoma
ILC	Invasive lobular carcinoma
MD	Mean diffusivity
MDT	Multidisciplinary team
MPG	Miller Payne grade
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NACT	Neoadjuvant chemotherapy
NCCP	National Cancer Control Programme
NMV	Net magnetization vector
NCRI	National Cancer Registry Ireland
RA	Relative anisotropy
ROC	Receiver operator curve
ROI	Region of interest
SNR	Signal-to-noise ratio
T	Tesla
TDLU	Terminal ductal lobular unit
TE	Echo time
TNM	Tumour node metastasis
TP0	Baseline MRI scan prior to treatment
TP1	MRI scan after the first cycle of chemotherapy
TP2	MRI scan after the second cycle of chemotherapy
TP3	Final MRI scan after the final cycle of chemotherapy
TR	Repetition time
VR	Volume ratio

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CHAPTER 1: INTRODUCTION

SECTION 1.1: BREAST CANCER

1.1.1 DEMOGRAPHICS AND RISK FACTORS

Breast cancer represents the second commonest malignancy worldwide and the most frequent cancer diagnosed in woman with an estimated 1.67 million new cases in 2012 (25% of all female cancers) [1]. In less developed regions, it is the most frequent cause of cancer death in woman accounting for 324,000 deaths in 2012 (14.3% of total cancer mortalities). In more developed regions, it is the second cause of cancer mortality in females, after lung cancer, with 198,000 deaths in 2012 (15.4%) [1].

In Ireland (2012), excluding non melanoma skin cancers, breast cancer accounted for 30.5% of invasive cancers (n = 2899) (Figure 1.1) [2]. As the second leading cause of cancer mortality it accounted for 704 deaths (18% of total cancer deaths). According to the National Cancer Registry Ireland (NCRI), the estimated lifetime risk of female breast cancer in Ireland is 1 in 10 [3]. Based on trends from 1994-2010, the NCRI has predicted a 55%-152% increase (depending on the method of projection used) in the number of female breast cancer up to 2040 [4].

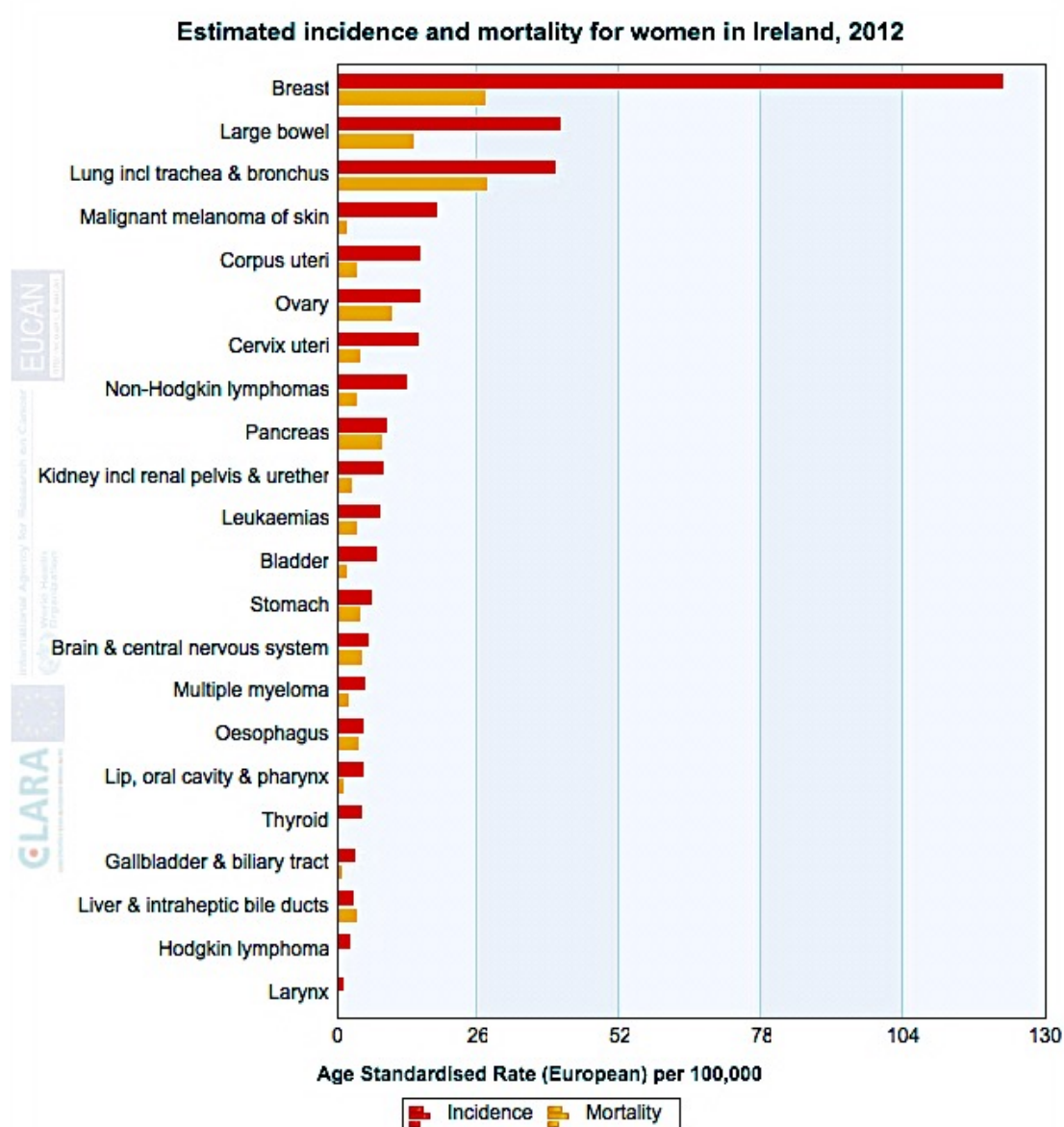


Figure 1.1 [5]: Bar chart demonstrating the cancer incidence and mortality per 100,000 of the Irish population in 2012 (European age-standardized rate).

There are several well recognized risk factors for the development of breast cancer. The risk of breast cancer is strongly related to age. In Ireland, between 2011 and 2013, 76% of female breast cancer cases were diagnosed in patients over 50 years of age [3]. Having one first degree relative (mother, sister or daughter) with breast cancer approximately doubles a woman's risk [6, 7]. This risk increases further

with additional affected first-degree relative or with affected relatives under 50 [6, 7]. Inherited genetic mutations increase the risk of breast cancer, most notably BRCA1 (57% increase) and BRCA2 (49% increase)[8]. Hormonal factors also play a role, in particular, increased exposure to oestrogen. Risk is therefore increased among women who experience early menarche or late menopause, have no children or fewer children than their peers, and who take oral contraceptives or hormonal replacement therapy [9]. Observational studies have repeatedly shown an association between alcohol consumption and breast cancer with an increased risk of approximately 10% per unit (10g) of alcohol consumed daily [10]. This risk increases linearly up to approximately 60g per day after which no further increased risk is observed. Obesity in post menopausal women is another risk factor with one meta-analysis reporting a 15% increased risk in post-menopausal woman who are overweight (BMI 25-29.9) or obese (BMI 30+) [11].

1.1.2 BREAST ANATOMY AND BREAST CANCER DEVELOPMENT

The breast is a modified apocrine gland, predominantly composed of adipose and glandular tissue. It is part of the superficial chest wall, overlying ribs 2-6 vertically and extending horizontally from the sternal edge to the mid-axillary line (Figure 2).

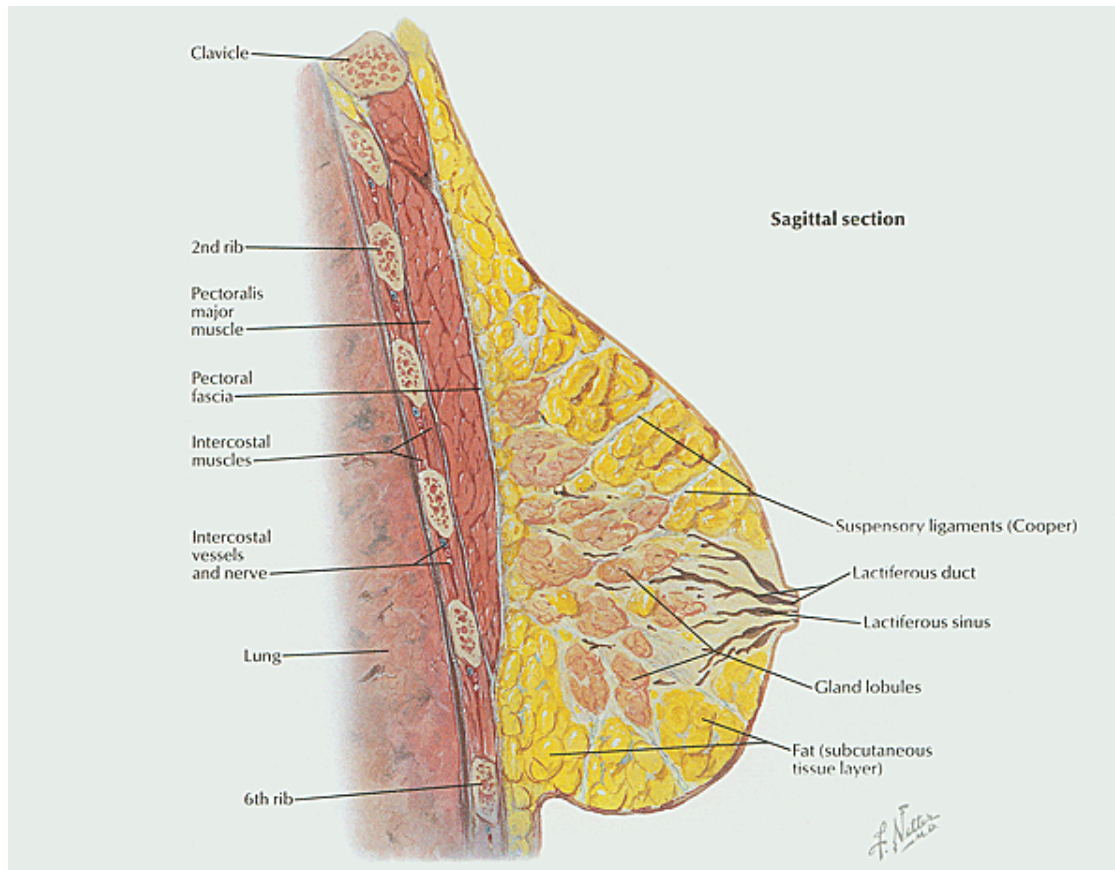


Figure 2 [12]: Illustration of a sagittal section through the mammary gland.

The glandular component is made up of approximately 15-20 independent lobes. Each lobe is comprised of clusters of acini called lobules. The acini are epithelial cells that produce milk. The terminal ductal lobular unit (TDLU) is the basic functional unit of the breast and consists of a lobule drained by a terminal duct, which has an intralobular and extralobular course (Figures 3 and 4).

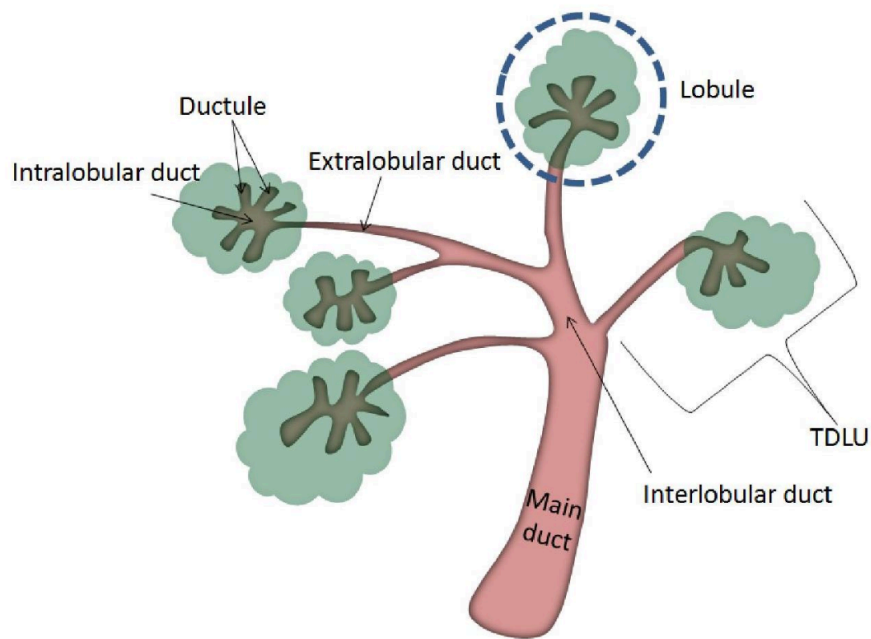


Figure 3 [13]: Illustration of the terminal ductal lobular unit which is the basic functional unit of the breast.

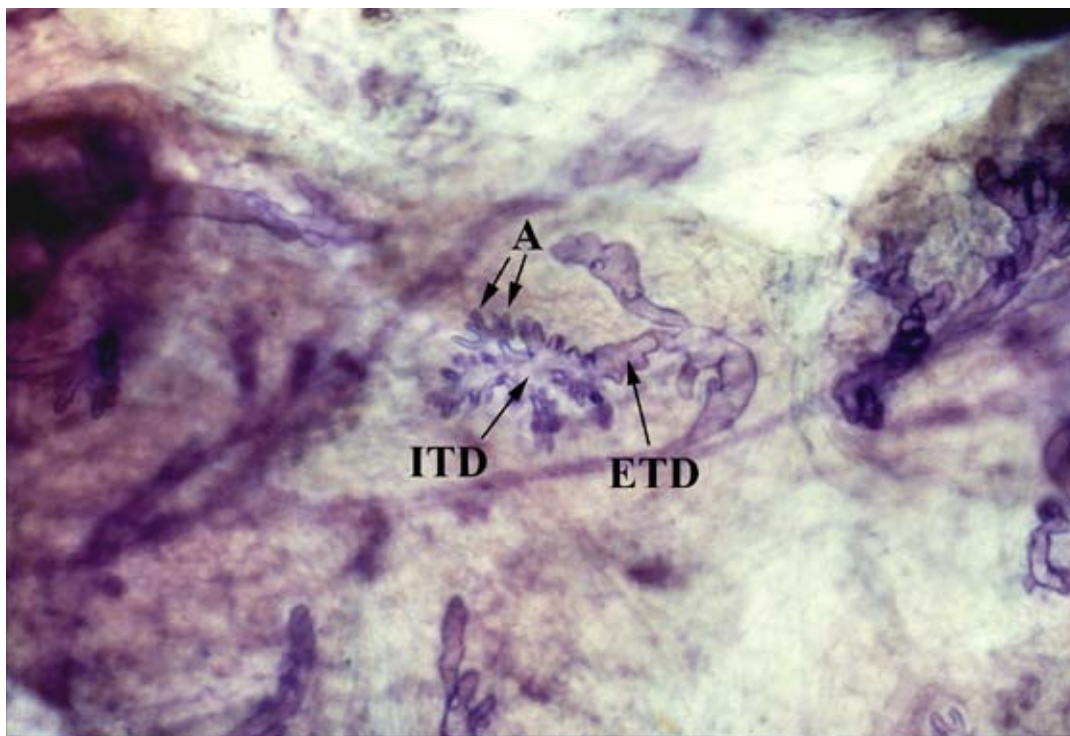


Figure 4 [14]: Slide of a TDLU (approx 1.5mm x 1mm). Arrows demonstrate acini (A), an intralobular terminal duct (ITD) and an extralobular terminal duct (ETD).

Intralobular terminal ducts drain milk from the lobules into extralobular terminal ducts which converge into main interlobular ducts which drain all the milk from that cluster of lobules. Interlobular ducts then drain into the main lactiferous ducts of which there are 15-20. These main ducts converge at the nipple areola complex. The mean diameter of the ducts as they open onto the nipple is 0.57mm [15]. Widening of the distal end of each of the main lactiferous ducts is known as the ampulla. The ampulla serves as a reservoir for milk just prior to it leaving the breast. The ductolobular system is composed of an inner layer of luminal cells and an outer layer of myoepithelial cells resting on a basement membrane [16]. It is postulated that most cancers and benign lesions arise in the terminal duct either inside or just proximal to the lobule.

Surrounding the ductolobular system is breast stroma. Interlobular stroma surrounds large ducts and TDLUs and consists mainly of adipose tissue but also fibroconnective tissue, blood vessels, lymphatics and nerves. Cooper's ligaments (named after Sir Astley Cooper who is credited with first studying the anatomy of the breast in 1840 [17]) are fibrous connections coursing between lobes from the dermis to the deep fascia overlying the pectoral muscles. They are largely responsible for maintaining the configuration of the breast and may become stretched with advancing years and fat deposition. Intralobular stroma is a less dense form of connective tissue that surrounds acini in TDLUs. The blood supply of the breast is via branches of the subclavian and axillary arteries and its lymph drains principally to the axilla.

Breast cancer is separated into two main categories; carcinoma in situ and invasive carcinoma. Ductal carcinoma in situ (DCIS) refers to the presence of

'malignant-appearing' cells within the ducts and terminal lobular units of the breast which have not breached the basement membrane [18]. The natural history of DCIS is unclear however it is considered a precursor of invasive disease [18]. Invasive carcinoma is generally divided into two main types; invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC), arising from the epithelial cells of the ducts or lobules respectively. While other invasive carcinoma subtypes exist, IDC is the most common type accounting for 70-80% of invasive lesions with ILC accounting for 5-10% [19]. The natural history of breast cancer involves progression from atypical hyperplasia, to in situ carcinoma, then invasive carcinoma, and finally to metastatic disease (Figure 5) [20, 21].

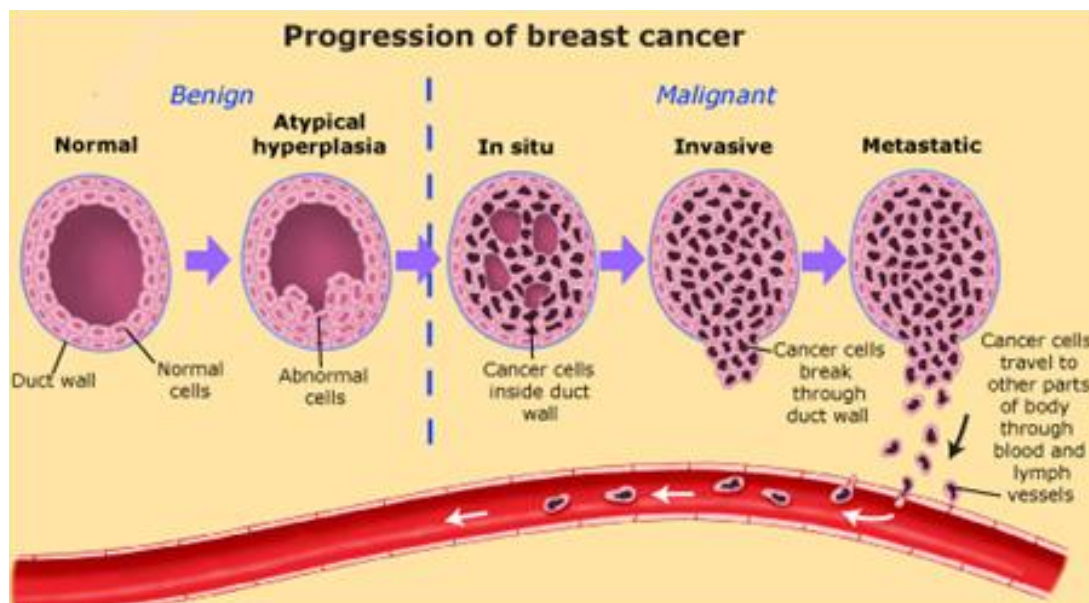


Figure 5 [22]: Illustration demonstrating the development of cancers in mammary ducts. Normal breast ducts are composed of a basement membrane and a layer of luminal epithelial and myoepithelial cells. In situ carcinoma refers to the presence of 'malignant appearing' cells in the ducts. Invasive carcinoma exists when these abnormal cells extend beyond the basement membrane. Spread of cancerous cells to areas other than the breast, via lymphatic or haematogenous routes, is termed metastatic disease.

1.1.3 DIAGNOSIS AND STAGING

Breast cancer can be diagnosed through screening or when a patient develops symptoms and/or signs of local or metastatic disease. The imaging methods most frequently used in the diagnosis of breast cancer include mammography, ultrasonography and magnetic resonance imaging. Ultimately, the diagnosis of breast cancer is based upon histological sampling.

Mammography employs x-rays that are attenuated based on the characteristics of the breast. Cancers on mammography generally manifest as masses or clusters of microcalcifications. The sensitivity and specificity of mammography are estimated to be approximately 78% and 99% respectively [23]. In extremely dense breasts, the sensitivity can reduce to as low as 48% as the attenuation of x-rays in normal dense breast tissue and cancers can be comparable [23]. Mammography is the imaging tool most frequently used for breast cancer screening. If an indeterminate or suspicious for cancer lesion is detected during screening, the patient is recalled for supplemental mammographic views and/or breast ultrasound to determine the need for biopsy. All women in Ireland presenting with a palpable breast lump will generally undergo mammography (if over 35 years of age) and ultrasound. Ultrasound insonates tissues with high frequency sound waves and records the returning echoes to produce images. It is predominantly used for further characterization of an abnormality detected on clinical examination or mammography. In addition, ultrasound is used for needle guidance during biopsy or aspiration procedures.

MRI is an imaging technique that uses a strong magnetic and pulsed radiowaves to create high quality images without the use of ionizing radiation. It has

a number of roles in the management of breast cancer acting as a valuable screening, diagnostic and staging tool. While the sensitivity of MRI for the detection of cancer is high, ranging from 88 – 100% [24-26], it is an expensive investigation with limited specificity [25] and thus MRI screening is reserved for high risk patients only. These include BRCA1 or BRCA2 gene mutation carriers and individuals with a life-time risk of developing breast cancer of 20–25% or higher (based on family history) [27]. The routine use of MRI is not recommended in the pre-operative assessment of biopsy proven invasive breast cancer. MRI is however indicated for evaluation of disease extent in patients with invasive lobular carcinoma, in patients with a discrepancy between clinical, mammographic and ultrasound findings, and in patients where breast density may preclude accurate tumour assessment [28].

The “stage” of a cancer is a measure of the extent of local and distant disease and is used to determine prognosis and to guide management. The internationally accepted system for the staging of breast cancer is the Tumour Node Metastasis (TNM) staging system proposed by the American Joint Committee on Cancer (AJCC) [29]. TNM is based on three criteria: characteristics of the primary tumour (T), status of regional lymph nodes (N) and the presence or absence of distant metastases (M), and from this disease stage (0 to IV) is calculated (Table 1).

Stage	Tumour size (T)	Lymph node involvement (N)	Metastases (M)
0	CIS	No	No
I	≤ 2cm	No	No
IIA	Tumour absent / ≤ 2cm	Regional lymph node involvement (nodes mobile)	No
	2-5cm	No	No
IIB	2-5cm	Regional lymph node involvement (nodes mobile)	No
	>5cm	No	No
IIIA	Tumour absent/ ≤ 2cm/ 2-5cm/ > 5cm	Regional OR internal mammary lymph node involvement	No
IIIB	Any size with direct extension to chest wall or skin	Regional OR internal mammary lymph node involvement	No
IIIC	Any size with direct extension to chest wall or skin involvement	Infraclavicular, supraclavicular or internal mammary node involvement (with regional lymph node involvement)	No
IV	Any size	Any node	Yes

Table 1: TNM staging system for breast cancer provided by the AJCC. Disease stage is based upon the characteristics of the primary tumour (T), the extent of lymph node involvement (N) and the presence or absence of metastases (M).

In the absence of distant metastases, breast cancer is classified as early stage or locally advanced disease. Early stage includes stage I, stage IIA and a subset of stage IIB disease (T2N1), while locally advanced disease includes stage IIB disease

(T3N0) and patients with stage IIIA to IIIC disease [30]. Approximately 5% of patients will have stage IV disease (distant metastatic disease) at initial presentation [30].

1.1.4 TREATMENT

The management of breast cancer should involve a multidisciplinary team including specialists in nursing, radiology, histopathology, surgery, radiation oncology and medical oncology. Treatment delivered by the MDT has been associated with a reduction in breast cancer mortality [31]. The treatment of breast cancer includes the management of local and systemic disease and, in Ireland, is guided by the National Cancer Control Programme (NCCP) guidelines [28]. The type of treatment a patient receives depends on a large number of variables including the stage of disease at presentation, the molecular and pathological characteristics of the tumour, the patient's functional status and the patient's family history/gene status. The treatment of local disease includes surgery and radiotherapy or both while treatment options for systemic disease include chemotherapy, endocrine therapy, biological therapy in combinations.

Surgery generally involves breast conservation (i.e. lumpectomy and a surrounding margin of normal tissue) or mastectomy. Patients also undergo sentinel lymph node biopsy and/or ipsilateral axillary lymph node clearance if involved. Breast conservation therapy (BCT) refers to breast conservation surgery followed by moderate dose radiation therapy to reduce the chance of local recurrence. The aim of BCT is to provide a survival equivalent to mastectomy whilst maintaining a cosmetically acceptable breast. Randomized clinical trials directly comparing BCT with mastectomy have shown equivalent survival [32], but BCT is associated with

decreased morbidity and improved body image when compared to mastectomy [33, 34].

Factors indicating mastectomy over BCT include:

- A large tumor size in relation to the breast.
- Multicentric disease or diffuse malignant appearing microcalcifications on mammography.
- A prior history of radiotherapy to part of the affected breast.
- Pregnancy (contraindication to radiotherapy).
- Persistently positive resection margins after breast conserving surgery despite re-excision.

During radiation therapy, high energy x-rays are directed to the area where cancer might reside after surgery. The high energy beam induces damage to nuclear DNA leading to irreversible loss of the reproductive integrity of the cell and eventual cell death. The beam is not selective and causes damage to normal cells as well as malignant cells, however, it preferentially impacts cancer cells as these are more rapidly dividing and often have impaired DNA repair system. Radiotherapy in the adjuvant setting results in a significant reduction in local recurrence rates and improves the overall survival [32, 35]. Radiation therapy can also reduce tumour bulk prior to surgery and can be used for palliative measures.

Systemic treatment for breast cancer can be administered in the adjuvant, neoadjuvant or palliative settings. Chemotherapy involves systemic treatment with cytotoxic drugs which cause cell death by apoptosis, either by directly interfering with DNA or by targeting the key proteins required for cell division [36].

Unfortunately, they also exert their effects on normal cells, particularly those with a

high turnover. The nausea and vomiting associated with the administration of chemotherapy is often a direct effect of the drugs or their metabolites on the chemoreceptor trigger zone and the vomiting centre located in the medulla [37]. However, many of the side effects encountered following the administration of chemotherapy are as a result of cytotoxic effect on normal cells [36]. Common adverse effects include nausea, extreme fatigue, hair loss and myelosuppression. These side-effects vary according to the agent, the dose, the route and schedule of administration and are patient specific. Hence, personalised patient care is important, allowing the administered agent and dose to be tailored to each individual depending on patient tolerance and disease response.

Oestrogen and progesterone can promote the growth of tumours that express oestrogen and/or progesterone receptors. Endocrine therapy is indicated in patients with hormone positive breast cancer. This form of therapy blocks the actions of these hormones to inhibit cancer growth. Tamoxifen is a drug that acts as an oestrogen receptor antagonist while aromatase inhibitors are agents that decrease the production of oestrogen in the body. Tamoxifen, for a duration of 5-10 years, is an accepted standard for the treatment of pre-menopausal women with hormone-positive breast cancer while tamoxifen and/or an aromatase inhibitor should be considered for post-menopausal women [28].

Biological therapy is another form of systemic treatment. Trastuzumab (Herceptin) is the most commonly used biological agent for breast cancer and is indicated in HER2 positive disease. HER2 positive cancer cells have a gene mutation that makes an excess of a protein called human epidermal growth factor receptor 2, which promotes the growth of cancer cells. Approximately 20-30% of breast tumours

demonstrate HER2 overexpression [38]. HER2 positivity is associated with a more aggressive disease and poorer prognosis. The addition of Trastuzumab to the standard chemotherapy regimen has been shown to improve outcomes for HER2 positive breast cancer patients [38]. The most clinically significant side effect of Trastuzumab is cardiac myocyte injury which can lead to cardiac dysfunction [38].

SECTION 1.2: NEOADJUVANT CHEMOTHERAPY FOR BREAST CANCER

1.2.1 INDICATIONS AND BENEFITS

Traditionally, chemotherapy has been given to patients with operable breast cancer after surgery and 5-yearly meta-analyses of the worldwide experience with adjuvant chemotherapy showed significant improvements in progression-free and overall survival [39]. Since the 1980's, interest has grown in administering chemotherapy prior to definitive surgery and it is now a standard treatment for patients presenting with locally advanced breast or inflammatory breast cancers [28]. The 2015 NCCP guidelines recommend that any patient who is a candidate for adjuvant systemic therapy should be considered for neoadjuvant (pre-operative) chemotherapy. Thus, it should be considered as part of a multimodal treatment approach for patients with stage IIa, IIb, and III breast cancer [28]. The chemotherapy regimens recommended in the adjuvant setting are appropriate to consider in the neoadjuvant setting [28].

One of the benefits of neoadjuvant chemotherapy (NACT) is that it can increase the proportion of patients suitable for breast conservation surgery. Numerous trials have shown that NACT can allow breast conservation surgery in

some patients for whom mastectomy was initially the preferred option for local-regional control [40-44]. By down-staging tumours, NACT may also convert a previously unresectable tumour to an operable one [45, 46]. Response to NACT also serves as a useful prognostic tool. There is a clear correlation between the pathological response of the primary tumour and long-term patient outcome in terms of both disease free and overall survival. Patients with a complete pathological response (i.e. the absence of residual invasive tumour cells after NACT and surgery) have a more favourable long-term outcome than those with residual invasive cancer on pathological examination [47, 48]. NACT also allows patients time to undergo genetic testing if there is a suspicion of an underlying BRCA 1 or BRCA 2 mutation, which if present may influence a decision for unilateral or bilateral mastectomy over lumpectomy.

A Cochrane review concluded that NACT is comparable to adjuvant (post-operative) chemotherapy in terms of disease free and overall survival but identified a higher loco-regional recurrence rate for patients receiving NACT [49]. In three of the studies included in this review, a substantial number of patients received exclusive radiotherapy without surgery post chemotherapy. If these studies were excluded, the difference in loco-regional recurrence between patients receiving NACT and those receiving adjuvant chemotherapy was not significant. This finding emphasizes the importance of surgery post NACT even in patients who demonstrate a complete clinical and radiological response.

1.2.2 METHODS OF ASSESSING RESPONSE TO NACT FOR BREAST CANCER

Current methods to assess chemotherapy response of the primary tumour include palpation, ultrasonography, mammography and MRI. No formal guidelines exist regarding the ideal response assessment strategy during NACT for patients with breast cancer. In our institution, a clinical examination of the affected breast and ipsilateral axilla is performed at two weekly intervals during administration of NACT. Ultrasonography and mammography are performed after completion of NACT to assess for residual tumour and to guide surgical treatment. Imaging studies of the primary tumour are not performed during the course of NACT unless disease progression is suspected based on clinical examination.

Chagpar et. al reported only a moderate correlation between tumour size estimates on palpation, mammography and ultrasonography with pathologic tumour size in 189 patients post NACT [50]. Conventional breast MRI is superior in both the initial staging of breast cancer [51] and for estimating disease response [52-54]. However, both overestimation [55, 56] and underestimation [54, 55] of residual disease by conventional breast MRI have been reported which can have a negative impact on the appropriate management of individual patients. Contributing factors to overestimation include the presence of sclerosis, necrosis or reactive inflammation within the tumour bed or the presence of accompanying ductal carcinoma in situ [57]. In addition, small foci of disease identified on MRI may not be detected on gross examination of the specimen if the sectioning interval is greater than the size of the foci. Potential causes of underestimation of residual disease include change in tumour vascularity post NACT manifesting as decreased or delayed enhancement [58] or the effect of partial volume averaging in the case of

disseminated residual disease. Therefore, a complete tumour response on MRI post NACT does not obviate the need for surgery. More recently, there has been a focus on the use of MRI for early response assessment during NACT (the subject of this thesis). Early response assessment is defined as response to NACT after the first or second cycle of chemotherapy. As conventional imaging techniques such as ultrasonography, mammography and standard MRI primarily detect changes in tumor size which may not be apparent for several weeks after the initiation of chemotherapy, their use is limited. In addition, newer drugs such as anti-angiogenic and molecular target agents may provide therapeutic benefit by mechanisms other than size reduction. As it is known that functional tumor changes occur prior to alterations in size [59-61], newer methods that assess changes at the cellular and physiologic level might provide more accurate response assessment early in the course of chemotherapy. From this perspective, MRI is considered a superior choice for early response assessment because of its potential to detect and quantify functional and biochemical properties of tumours with spectroscopic, dynamic contrast-enhanced and diffusion-weighted techniques.

1.2.3 FUNCTIONAL MRI IN THE ASSESSMENT OF TREATMENT RESPONSE (DCE AND SPECTROSCOPY)

Functional MRI encompasses a spectrum of techniques that gives information about physiological and molecular processes. Quantitative monitoring to detect early response to NACT with MRI is performed using 3 major functional

imaging techniques: dynamic contrast enhanced MRI (DCE MRI), MR spectroscopy (MRS) and diffusion MRI.

DCE MRI:

Contrast enhanced MRI exploits the fact that breast cancers possess an increased microvasculature which is often poorly developed, resulting in leakage of the contrast agent through the vessel wall into the interstitium. Thus, malignant lesions demonstrate increased enhancement compared to normal tissues post gadolinium administration. DCE breast MRI can refer to 'standard' contrast enhanced breast MRI or 'ultrafast' DCE MRI. "Ultrafast" DCE MRI follows the passage of contrast enhancement through the tissue over time. By acquiring a rapid succession of images at baseline and at multiple time-points following gadolinium administration, a kinetic curve of time-dependent tissue enhancement can be generated. A malignant lesion typically demonstrates rapid pooling of contrast followed by early washout (type III kinetic curve) [62]. Parameters derived from DCE MRI, based on pharmacokinetic modeling, have shown potential for the monitoring of treatment response in patients receiving NACT for breast cancer. These pharmacokinetic parameters include K_{trans} (a measure of capillary permeability i.e. the leakage of contrast from the vascular space into the extravascular/extracellular space) and K_{ep} (a measure of the diffusion of contrast agent from the extravascular – extracellular space back into the vascular space). K_{trans} and K_{ep} are expected to be higher in tumours relative to normal breast tissue due to the preponderance of leaky capillaries secondary to tumour angiogenesis [63]. In a study of 28 patients receiving NACT for breast cancer, Ah See

et. al found that changes in K_{trans} and K_{ep} after two cycles of chemotherapy correlated significantly with clinical and pathological response, even though changes in lesion size were not significant at that time point [64]. Furthermore, in study of 68 patients, Pickles et. al reported a significant reduction in K_{trans} and K_{ep} between baseline and early treatment scans in the 'responder' patient group [65]. Yu et. al reported a significant correlation between early changes in tumour size and both K_{trans} and K_{ep} in 29 patients however these parameters were not significant response differentiators [66]. Manton et. al also found that pharmacokinetic parameters in 38 woman did not detect early response [67]. The changes in pharmacokinetic parameters during the administration of chemotherapeutic agents likely reflects alterations in the vascular supply of tumours as a result of cell death [63]. Whilst most of the above studies demonstrate the potential of pharmacokinetic parameters for therapy monitoring, contradictory findings warrant larger scale studies.

Spectroscopy:

MRS is a technique that provides information on the concentration of different proton containing metabolites in tissue. This technique can be exploited to detect the altered metabolic signature of cancer cells. Relative to normal breast tissue, breast cancers have been shown to have raised composite choline (tCho) concentrations. Choline containing compounds are implicated in the synthesis and metabolism of cell membranes and thus increases in total choline signal in breast cancer is likely related to the increased cell turnover [68]. In a pooled analysis of five clinical studies, Katz Brull et. al reported the sensitivity and specificity of ^1H -MRS for

detecting malignancy as 85% and 83% respectively [69]. Studies to date have shown promising results for the use of MRS in monitoring metabolic response to chemotherapy. Baek et. al compared changes in the tCho concentration with changes in tumour size during NACT between patients who achieved a pathological complete response and those who did not [70]. The authors concluded that patients who show a greater reduction in tCho compared with changes in tumour size are more likely to achieve a pathological complete response. Tozaki et. al found that changes in the choline signal after two cycles of NACT were more sensitive than changes in tumour size in predicting pathological response [71]. While these studies demonstrate that ¹H-MRS may have predictive power for therapy response assessment, the technique is faced with many challenges. The analysis of MRS data can be time consuming and there are many technical difficulties in assessing the very low concentration of tCho in vivo [57]. Despite some encouraging results, these limitations have hindered the clinical use of MRS to monitor early response in patients receiving NACT.

Diffusion MRI and its role in therapy response monitoring will be discussed in section 1.3.6.

1.2.4 PATHOLOGIC ASSESSMENT OF TREATMENT RESPONSE

Following the administration of chemotherapy, breast tumours undergo a reduction in size and vascularity as demonstrated by magnetic resonance imaging. Histologically, this manifests as fibrous tissue with a lymphocytic infiltrate, iron-

loaded macrophages and, when present, scattered foci of tumour cells [72]. The key feature of cellular damage in tumours responding to chemotherapy is an overall loss of tumour cellularity [73]. Even if tumours do not significantly decrease in size, they often demonstrate reduced cellularity [74]. Wang et. al described three types of residual tumours post NACT [75]. Type I comprised a solitary lesion in a fibrotic tumour bed. Type II involved multifocal lesions scattered throughout a fibrotic tumor bed. Type III comprised a main residual focus of tumour with at least one satellite lesion visible in the fibrotic cancer bed. Type I shrinkage pattern is the commonest, occurring in approximately 60% of patients with residual cancer post NACT [75]. Type III occurs in approximately 5% of patients and extra caution should be taken in these cases as despite the surgical margins being negative, remaining satellite lesions can lead to local recurrence [75].

The clinical and complete pathological response of the primary tumour to NACT has been shown to be an important prognostic indicator for patient outcome in terms of both disease free and overall survival [47, 48]. In particular, Wolmark et. al reported a 9 year survival rate of 85% for patients with a complete pathological response following NACT for breast cancer [47, 48]. However, clinical and radiological methods of assessment of end of treatment response have been shown to both underestimate and overestimate residual tumour burden [50, 55]. Histopathology provides an accurate assessment of treatment efficacy on the basis of the extent of residual tumour and regressive changes within tumour and is the gold standard for assessment of response. Unfortunately, there is currently no standard method of

assessing pathological response to primary chemotherapy in patients with breast cancer. Several studies have attempted to provide criteria for pathologic response after treatment [74]. In general, all of the studies recognize a category of pathologic complete response and a category of little or no response. One such system is the Miller Payne grading system, a five-point histological scoring system that estimates chemotherapy response based on a comparison of tumor cellularity between the pre-treatment core biopsy and the resected surgical specimen. The Miller-Payne grading system is as follows:

- Grade 1: No change or some alteration to individual tumour cells but no reduction in overall cellularity.
- Grade 2: A minor loss of tumour cells (up to a 30% reduction) but overall the tumour cellularity remains high.
- Grade 3: Between an estimated 30% and 90% reduction in tumour cellularity.
- Grade 4: A marked disappearance (more than 90%) of tumour cells such that only small clusters or widely dispersed individual cells remain.
- Grade 5: No invasive malignant cells identifiable in sections from the site of the tumor (complete pathological response).

Ogston et. al reported a significant correlation between the Miller Payne grade of response and 5-year disease-free survival and overall survival with a highly significant survival advantage in those patients achieving a complete histological response [73].

The Miller Payne grading system for assessment of pathological response is now widely accepted for use in both clinical and research settings [76-78].

SECTION 1.3: DIFFUSION WEIGHTED AND DIFFUSION TENSOR MRI

1.3.1 BASICS OF MRI

Almost all MRI techniques, including DWI, rely on water hydrogen nuclei to generate signal in order to form an image. Hydrogen nuclei consist of a single proton which possess a positive charge. These protons are constantly spinning around their own axes. Owing to the laws of electromagnetic induction, a moving unbalanced charge induces a magnetic field around itself. Thus, each hydrogen nucleus acts like a tiny bar magnet. The magnetic field for each proton is known as a magnetic moment. In a normal environment, the magnetic moments of hydrogen nuclei point in a random direction, thus they produce no overall magnetic effect. When these nuclei are placed in a static external magnetic field (B_0), their magnetic moments line up either with (parallel) or against (antiparallel) the field. Each proton lined parallel with B_0 cancels the effect of a corresponding proton lined antiparallel with B_0 . The preferred state of alignment is parallel because it requires less energy. Consequently a tiny excess of protons align with the magnetic field. This excess produces a net magnetic effect called the net magnetization vector (NMV) aligned in the longitudinal direction, parallel to B_0 (Figure 6).

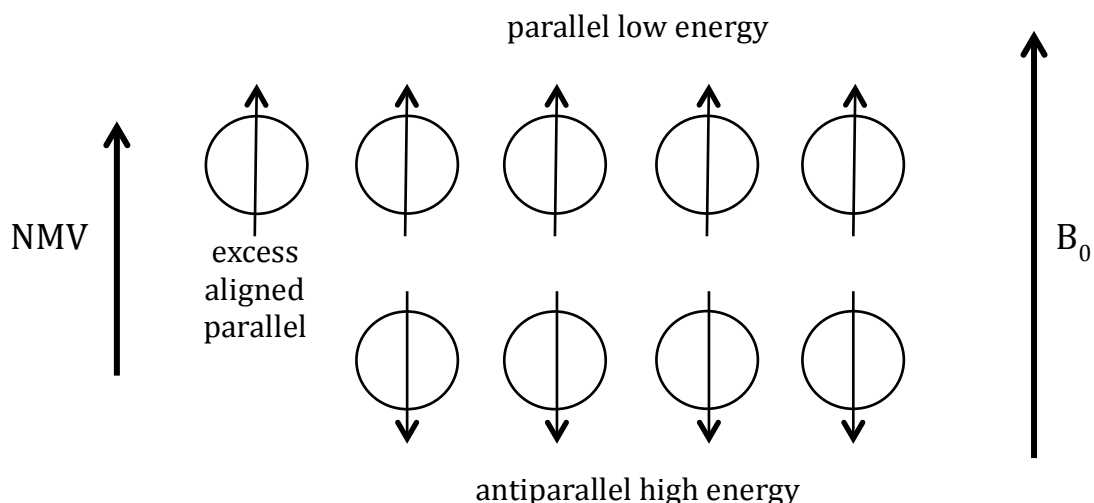


Figure 6 [79]: Illustration demonstrating the alignment of hydrogen nuclei in an applied magnetic field. A small excess of protons will exist in the low energy state compared to the high energy state resulting in a NMV aligned parallel to the main field (B_0).

The influence of the external magnetic field also causes the magnetic moments of individual protons to spin in a circular path around B_0 , known as precession (Figure 7). The speed at which the magnetic moments precess is called the precessional frequency and is determined by the Larmor equation (Equation 1).

$$\omega_0 = B_0 \times \gamma$$

Equation 1: Where the precessional frequency is denoted by ω_0 , the strength of the external magnetic field is denoted by B_0 (expressed in tesla) and the gyromagnetic ratio is denoted by γ .

The gyromagnetic ratio is the precessional frequency of a specific nucleus at 1 tesla (hydrogen = 42.57MHz/T). Because the protons in a static external magnetic field do not precess in phase with each other, i.e. their magnetic moments lie at different

places on the precessional path, they cancel each other out and do not contribute to the NMV.

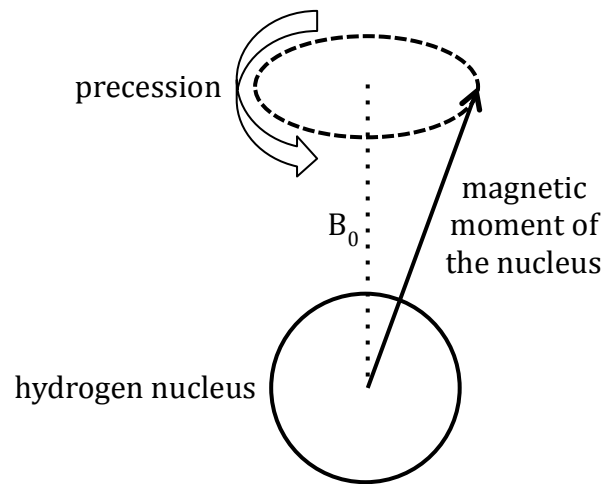


Figure 7 [79]: Precession; when placed in an external magnetic field (B_0), the magnetic moments of individual hydrogen nuclei spin in a circular path (dashed line) around the applied field.

With the patient in the scanner and possessing longitudinal magnetization, a radiofrequency pulse is applied at the same frequency as the precessing hydrogen nuclei, causing them to resonate. This results in some protons gaining energy and moving to the antiparallel state causing a reduction in the longitudinal magnetization. In addition, the radiofrequency pulse causes the protons to move in phase with each other i.e. their magnetic moments move to the same place on the precessional path (Figure 8). This results in the NMV having a transverse component which passes across a receiver coil, inducing a voltage in it and producing MR signal (Figure 9).

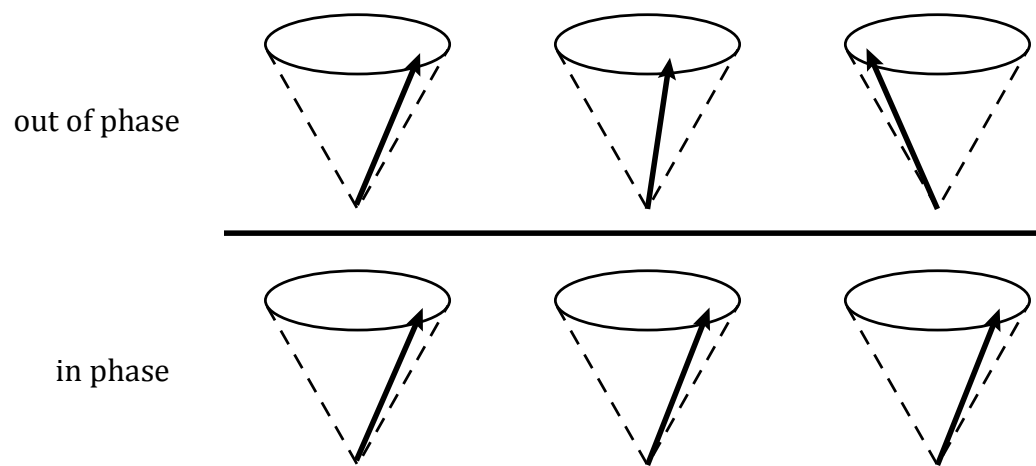


Figure 8 [79]: In a static external magnetic field, the magnetic moments of individual protons precess out of phase with each other i.e. they lie at different places on the circular precessional path. Following the application of a radiofrequency pulse at the same frequency as the precessing nuclei, the magnetic moments move into phase with each other i.e. they lie at the same place on the precessional path.

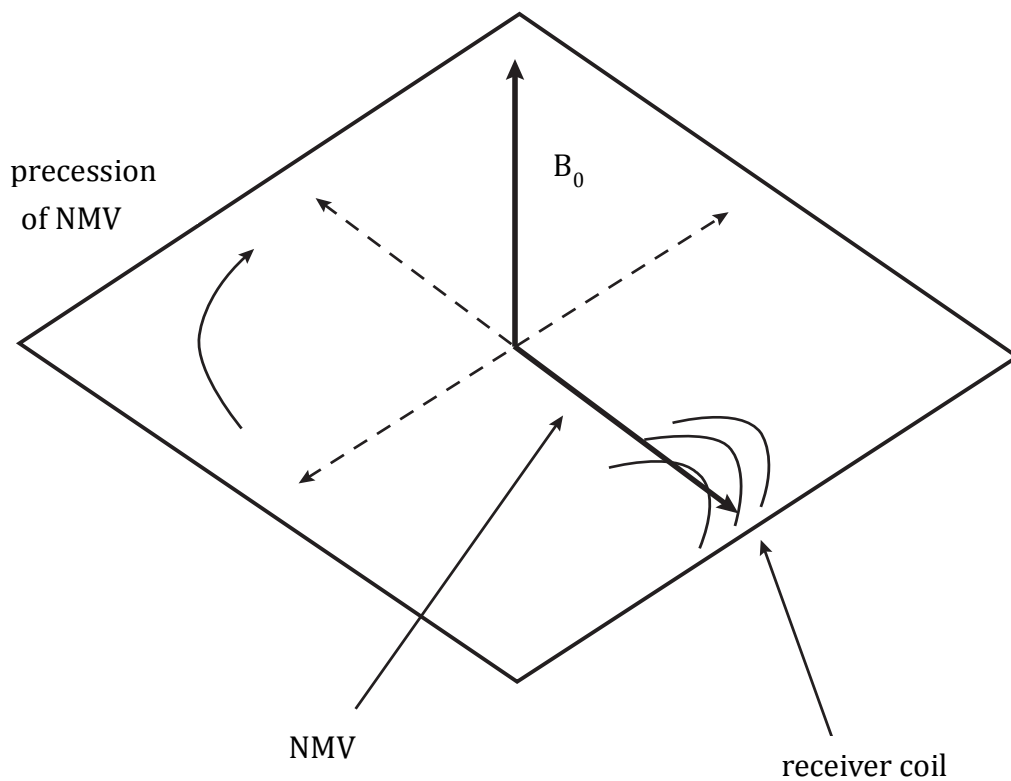


Figure 9 [79]: The transverse component of the NMV is detected by a receiver coil, inducing a voltage in it which produces the MR signal.

When the radiofrequency pulse is removed, the signal induced in the receiver coil begins to decrease because the coherent component of NMV in the transverse plane diminishes. This is as a result of tissue relaxation and external magnetic field inhomogeneities.

Image contrast is determined by parameter selection (extrinsic contrast mechanisms) and the properties of the tissues being imaged (intrinsic contrast mechanisms). The T1 and T2 times of a particular tissue are intrinsic contrast parameters that are inherent characteristics of the tissue being imaged. The T1 time is defined as the time it takes for 63% of the longitudinal magnetization to recover. The T2 time is defined as the time it takes for 63% of the transverse magnetization to decay. The magnetization in each tissue relaxes at different rates. Consequently, tissue relaxation times, namely, the spin-lattice (T1) and spin-spin (T2) relaxation times form the fundamental basis of soft tissue contrast and anatomic imaging with MRI. Differences in the proton densities (number of hydrogen protons in the tissue) also impacts on intrinsic contrast mechanism. In a T1 weighted imaging sequence, image contrast between tissues is primarily determined by differences in T1 values between the tissues, while in a T2 weighted sequence, image contrast is predominantly due to the differences in the T2 decay of times of tissues. A proton density weighted image is an image whose contrast is predominantly due to differences in the proton density of the tissues.

An MR sequence, also known as a pulse sequence, is the particular MR image acquisition technique selected for a particular examination. It consists of a

series of radiofrequency pulses and gradients applications with defined intervening time periods. One of the most basic pulse sequences is a conventional spin echo pulse sequence which can be used to produce T1-, T2- or proton density-weighted images. In a spin echo pulse sequence, a 90° excitation radiofrequency pulse is followed by a 180° refocusing pulse. The 180° pulse flips the dephasing nuclei through 180° causing them to rephase. Voltage induced in the receiver coil after the 180° pulse produces a signal known as an 'echo'. These echoes form the MR signal and are subsequently interrogated and spatially located to produce images. Spatial encoding of the signal is accomplished through the use of gradient pulses created by gradient coils. The term K space is a concept that refers to an area where data collected from the MR signal is stored. It stores information about the frequency of a signal and where it comes from in the patient. This data is then subjected to a mathematical process called a Fourier transform to generate the final image.

Basic pulse sequence parameters include the echo time (TE) and the repetition time (TR). The TE is the time between a radiofrequency excitation pulse and the collection of the signal. The TR is the time from the application of one radiofrequency pulse to the application of the next. A gradient echo pulse sequence differs from a spin echo sequence in that the initial excitation pulse is less than 90° and no gradient is used to rephase the magnetic moments of the nuclei. While gradient rephrasing is less efficient than radiofrequency rephrasing, it is faster and therefore gradient echo sequences have shorter TEs and TRs than spin echo sequences, resulting in a shorter scan time. Echo planar imaging (EPI) is one of the

fastest MRI acquisition sequences and has many applications including diffusion imaging, perfusion imaging and cardiac imaging. Unlike conventional MRI sequences that acquire one k space line at each phase encoding step, EPI collects all the data required to entirely fill k space from a single echo train. To achieve this, multiple echoes are generated by either 180° rephrasing pulses (termed spin echo EPI) or by gradients (termed gradient echo EPI). The echoes generated are then phase encoded by a different slope of gradient to fill all the required lines of k space.

Of note, the unit of measurement used to quantify the strength of a magnetic field in an MRI machine is the Tesla (T). Whilst most clinical MRI scanners operate at a strength of 1.5 Tesla, a 3 Tesla MRI operates at twice the normal field-strength and generates greater signal-to-noise ratio, which is a major determinant in generating an improved quality image.

1.3.2 PRINCIPLES OF DWI

DWI is based on molecular diffusion (first described by Einstein in 1905 [80]) and refers to the concept that molecules in a fluid are in constant random motion (so called Brownian motion), at a rate described by the diffusion coefficient (D), as a result of agitation by thermal kinetic energy. In a glass of water, the motion of molecules is completely random and is limited only by the boundaries of the container. In contrast, the motion of water molecules in the cellular microenvironment is impeded by intra and extracellular compartments as well as tissue cellularity [81]. In other words, the degree of water diffusion in biologic tissue

is inversely correlated to the degree of cellularity of the tissue and the integrity of cell membranes [82]. Therefore, the diffusion of water molecules is more restricted in tissues with high cellular density and intact cellular membranes, such as tumours. The cell membranes act as barriers to molecular diffusion in both the intra- and extracellular spaces. Conversely, in microenvironments with fewer cells and disrupted cell membranes, molecular diffusion is less restricted. This is due to the increased extracellular space available for diffusion and the ability of molecules to freely transgress the defective membranes to move between the intra- and extracellular spaces (Figure 10). DWI uses motion sensitizing gradients to characterize the arbitrary motion of water molecules within a given tissue without interfering with the diffusion process itself.

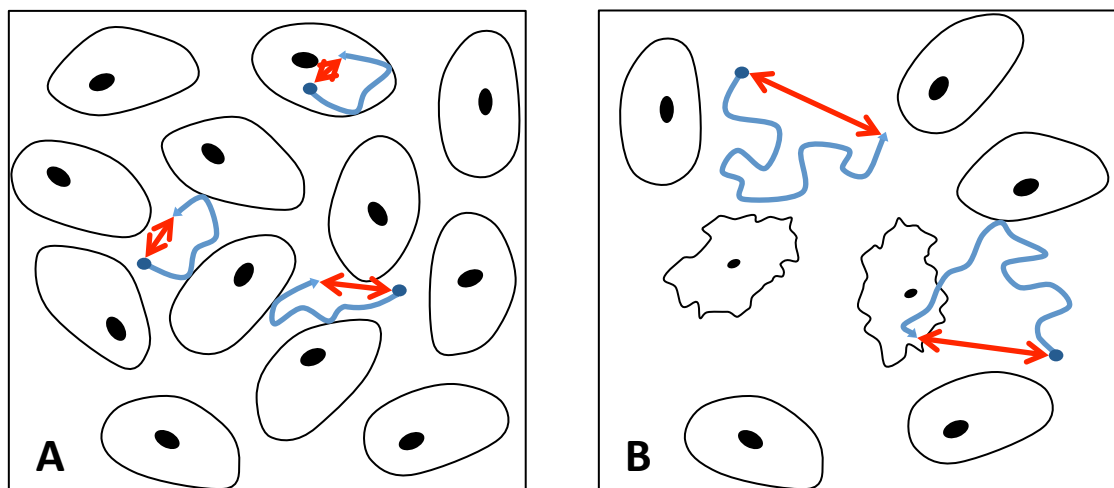


Figure 10: Illustration comparing water diffusion in two types of tissue (A and B). Tissue A has a high cellular density with intact cell membranes. Tissue B has a relatively lower cellular density with defective cell membranes. Note the increased net distance (indicated by red arrows) travelled by molecules (blue circles with arrows) in tissue B where random motion is less impeded by cells and cellular membranes.

While early diffusion measurements were made in biological tissues using nuclear magnetic resonance in the 1960s and 1970s, it was not until the 1980s that the basic principles of DWI were introduced and applied to the human brain [83-85]. In the early 1990s, DWI gained widespread recognition for the detection of cerebral infarction and it is now an established tool in neuroimaging for the detection of early stroke and other neurologic diseases. More recent advances in MR technology such as echo-planar imaging, high amplitude gradients, multi-channel coils and parallel imaging have been instrumental in the application of DWI outside of the brain [82]. These advances, in particular parallel imaging, have led to a significant reduction in DWI acquisition time, resulting in substantially less motion artifact, thus enabling higher-quality DW images to be obtained [82]. In addition, as these sequences are relatively short, they can be added to the standard imaging protocols without substantially increasing total examination time.

1.3.3 DWI TECHNIQUE

DWI is typically performed using a T2- weighted spin-echo EPI sequence with the addition of two symmetric motion-probing gradient pulses. This approach is based on methods described by Stejskal and Tanner in 1965 [86]. As discussed in section 3.1, EPI is an MR technique with a rapid acquisition time, thus reducing likelihood of motion induced artifact. The motion sensitizing gradients are applied on either side of the 180° rephasing pulse (Figure 11). The application of the first gradient pulse induces a phase shift in proton precession. When a second reverse gradient is applied, particles that are in the same location will experience no net

phase shift, while particles that have moved will end up with a phase shift (Figure 11). Signal attenuation therefore occurs in normal tissues with random motion and high signal appears in tissues with restricted diffusion.

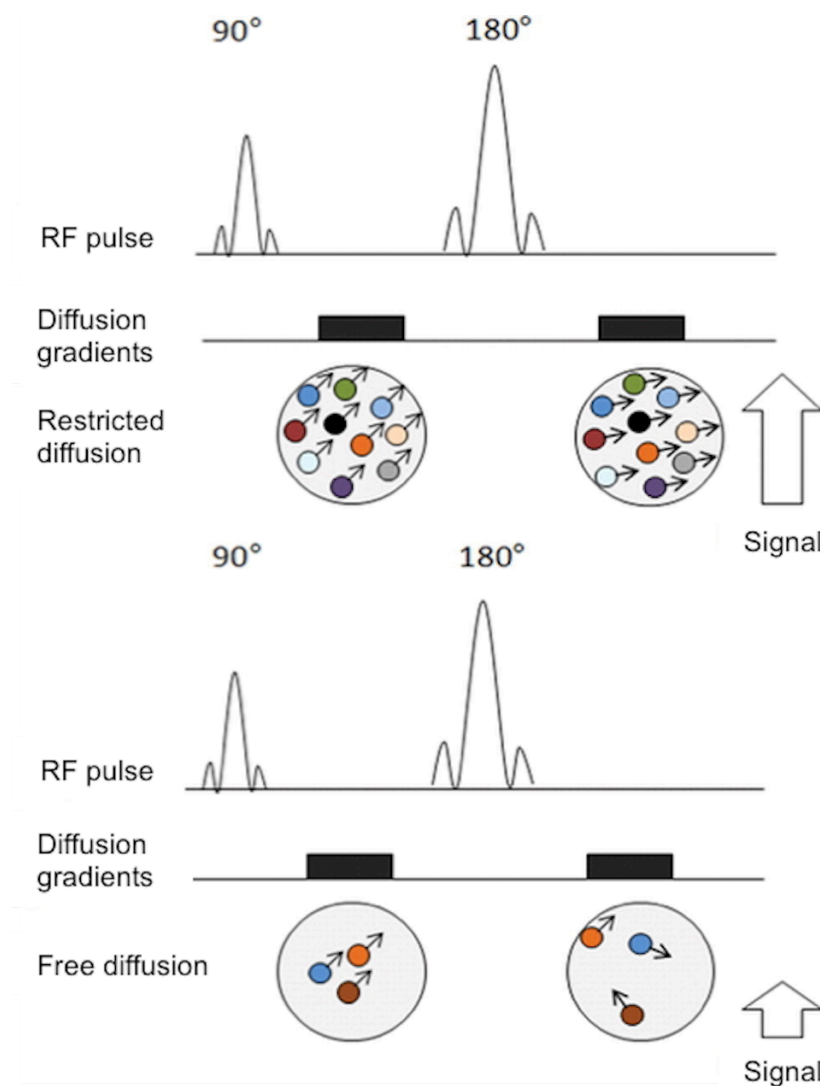


Figure 11 [87]: Pulse sequence diagrams illustrating how a diffusion weighted sequence involves the application of two symmetric motion-probing gradient pulses either side of the 180° rephasing pulse. The gradient pulses are designed to cancel each other out if spins do not move, whilst moving spins experience phase shift. Restricted diffusion (top) manifests as increased signal, whereas free diffusion (bottom) translates into signal attenuation.

The sensitivity of diffusion weighted imaging to diffusion depends on the strength of the gradients applied (amplitude and duration) as well as the time spacing between them. The combination of these factors generates the b value (expressed in seconds per square millimeter), an index of the degree of diffusion weighting. The higher the b value, the more sensitive the sequence is to diffusion restriction. In order to produce a diffusion weighted image, the ease with which water can diffuse should be assessed in at least three orthogonal directions (X, Y and Z).

1.3.4 ADC

The ADC is a quantitative parameter derived from DWI that reflects the rate of water diffusion in a tissue. ADC values (expressed in mm^2/s) are calculated from acquisitions performed with at least two different b values. If the logarithm of signal intensity for each b value is plotted, the slope of a line through the points defines the ADC value (Figure 12) (Equation 2).

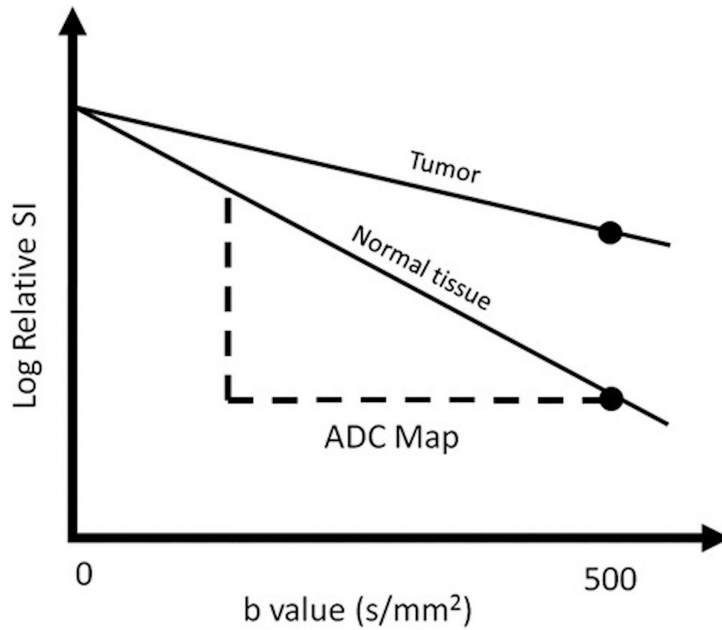


Figure 12 [87]: The ADC value is the slope of the line that is superimposed on the plot of the logarithm of relative signal intensity (y-axis) versus b value (in this case, 0 and 500)(x-axis). The slope of the tumour line is less than that of the line representing normal tissue, which will appear as an area of reduced signal on the ADC map.

$$ADC = (-1/b) \ln(S_b/S_0)$$

Equation 2 [88]: Where S_b is the signal intensity of a selected pixel for the image acquired with the higher b value and S_0 is the signal intensity in the same pixel of the image acquired with the lower b value.

More accurate ADC values are obtained from more than two diffusion-weighted images using different b values but each additional b value increases the acquisition time. ADC values are displayed on pixel-by-pixel maps (ADC maps), which are automatically generated from an MRI workstation. The ADC maps are commonly displayed in greyscale with tissues that have higher ADC values appearing bright and tissues that have lower ADC values appearing dark. ADC maps have poor anatomical

detail and should be interpreted along with other MR images including b-value diffusion-weighted images, higher- resolution anatomic images and, if available, contrast-enhanced images. The ADC value of a lesion can be acquired by drawing a region of interest with the lesion on an ADC map.

DWI is inherently T2-weighted. This means that lesions with long T2 relaxation values that do not have restricted diffusion (eg. fluid filled cysts) may appear bright even on high-value diffusion weighted images, a phenomenon called T2 shine-through. The ADC map eliminates any confounding T2 contribution and is therefore used to distinguish between restricted diffusion and T2 shine-through. Tissues with restricted diffusion will appear bright on all b value images and hypointense on the ADC map whilst T2 shine-through will manifest as bright on high b value images and the ADC map.

1.3.5 PRINCIPLES OF DTI

DTI is an extension of DWI that in addition to the magnitude of diffusion also provides information about the directionality of water diffusion. Diffusion is termed isotropic if the motion is equal in all directions (e.g. as would occur in a glass of water). The cellular microstructure of tissue complicates diffusion as numerous cellular barriers create individual compartments within tissue and these barriers are influenced by disease processes and therapeutic interventions [89]. Because of this, the diffusion of water molecules in tissue is said to be anisotropic, i.e. not equal in all directions [90]. In brain white matter, diffusion anisotropy is hypothesized to

originate from the specific organization of bundles of myelinated axons running in parallel [90].

The investigation of diffusion anisotropy in the brain started with the proposal of the diffusion tensor for use in MRI by Peter Basser in 1994 [91]. Since then, DTI has gained popularity in several clinical applications, principally in investigation of white matter architecture and integrity in normal and diseased brains. Investigational applications include multiple sclerosis [92], autism [93], traumatic brain injury [94], schizophrenia [95] and ageing [96]. DTI is also used for neurosurgical planning and a prospective study has shown that addition of preoperative DTI to neuro-navigation improved tumor resection and survival while decreasing neurologic morbidity [97].

As mentioned previously, DWI is acquired by the application of motion sensitive gradients in at least 3 orthogonal directions. Diffusion tensor MRI is an extension of DWI that employs gradients in at least six directions to provide information about the degree and directionality of water diffusion in tissues. In order to accurately depict the magnitude and orientation of anisotropy, the diffusion process is modelled as a 3-dimensional ellipsoid, called a tensor. The tensor is usually described in terms of three eigenvectors with corresponding eigenvalues (λ_1 , λ_2 , and λ_3). If the eigenvalues are significantly different from each other, diffusion is said to be anisotropic. The eigenvector corresponding to the largest eigenvalue, termed the principal eigenvector, defines the main direction of diffusion of water molecules in that voxel. If the eigenvalues are equal, there is no principle diffusion direction and

diffusion is said to be isotropic. The geometric model of isotropic diffusion is represented as a sphere (Figure 13) [98].

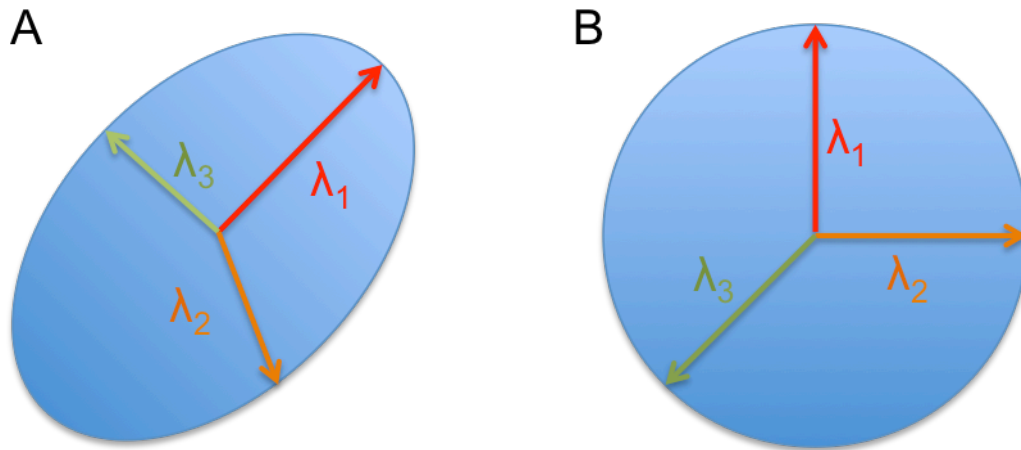


Figure 13: The geometric model of anisotropic diffusion is an ellipsoid (A) defined by 3 main eigenvectors, each with a determined eigenvalue (λ_1 , λ_2 , and λ_3). While the eigenvectors define the orientation of the anisotropic diffusion ellipsoid, the degree of directional preference (defining how extended or non-spherical the ellipsoid is) is defined by the eigenvalues of the tensor. The eigenvector associated with the largest eigenvalue denotes the predominant orientation of diffusion. If diffusion is isotropic (occurring equally in all directions), the eigenvalues will be equal and the geometric model representing this will be a sphere (B).

1.3.6 DTI-DERIVED PARAMETERS INCLUDING FA

Several quantities related to diffusion can be calculated from the tensor using software designed for this purpose. Eigenvalues (λ_1 and λ_2 and λ_3) can be determined for the three main directions of the ellipsoid and used to characterize the relative probability of movement of water molecules in each direction [98]. However, other indices (e.g., mean diffusivity, FA) derived from these “raw” values are far more frequently used for characterizing the tissue microstructural

organization [89, 99].

Mean diffusivity is similar to ADC and reflects the isotropic or average degree of diffusion in a tissue. It is therefore related to cell density, size and parenchyma permeability. MD is computed as one-third times the sum of the eigenvalues of the diffusion tensor (Equation 3). Note that in clinical imaging ADC maps may be measured using fewer diffusion gradients than needed for the tensor.

$$MD = (\lambda_1 + \lambda_2 + \lambda_3) / 3$$

Equation 3 [100]

FA measures the degree of anisotropy or directionality of diffusion in a tissue. Its name comes from the fact that it measures the *fraction* of the diffusion that is *anisotropic*. In other words, FA reflects the extent to which the organization of the tissue determines a preferential directionality of movement of water molecules [99]. It has no information about the orientation of the tensor; it is rotationally invariant. FA is calculated as the normalized variance of the three eigenvalues (Equation 4). It is a scalar value ranging from 0 (absolutely isotropic, $\lambda_1 = \lambda_2 = \lambda_3$) to 1 (fully anisotropic, $\lambda_2 = 0, \lambda_3 = 0$).

$$FA = \sqrt{\frac{3}{2}} \sqrt{\frac{(\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

Equation 4 [100]: Where $\bar{\lambda}$ denotes mean of the three eigenvalues.

FA values can be displayed on a pixel-by-pixel map (FA map) which is a gray-scale display of FA values across the image with brighter areas being more anisotropic than darker areas.

Relative anisotropy (RA) is an alternative metric used to describe anisotropy but this is not commonly utilized as FA images have a higher signal-to-noise ratio and superior noise immunity [101]. The volume ratio (VR) has also been used as a sensitive measure of anisotropy [102]. FA is sensitive to low values of diffusion anisotropy while VR is sensitive to high values of diffusion anisotropy [103]. RA is linearly scaled for different levels of anisotropy [103]. Neither RA nor VR are the subject of this thesis.

An important point to note is that parameters derived from diffusion imaging are not absolute and can vary depending on multiple factors including field strength, the number of diffusion gradients and the voxel size [104]. Another potential cause for variations in measurements is inter-scanner differences with studies demonstrating slight variability in FA values derived from different scanners, even with scanners of the exact same model [105, 106]. Nevertheless, longitudinal studies evaluating the reliability of DT-derived parameters with the same imaging system at different time-points demonstrate good reproducibility [107, 108].

1.3.7 APPLICATIONS OF DWI AND DTI IN THE BREAST

Englander and colleagues first described DWI of the breast in 1997 [109] and since then numerous studies have explored the clinical utility of this technique.

DWI is increasingly recognized as a promising quantitative method in the differentiation of breast lesions, with malignant lesions generally exhibiting lower ADC values than benign lesions [110-112]. This finding is attributed to the increased cell density of many cancers. In addition, ADC of malignant lesions has been shown to increase during chemotherapy, presumably reflecting a reduction in tumor cellularity as a result of apoptotic and/or necrotic cell death, with concomitant loss of cell membrane integrity, thus allowing water molecules to diffuse more freely [113]. Crucially, several studies report significant increases in tumor ADC after the first cycle of chemotherapy [61, 114-118], with increases more pronounced in responding than non-responding patients [61, 114, 116, 117]. While these findings support the use of ADC as a biomarker of treatment response, a small number of studies dissent and have not found tumor ADC to be predictive of early disease response following chemotherapy [67, 119, 120]. Pre-treatment tumour ADC values have also been evaluated to assess their potential in predicting response. Although lower tumor ADC at baseline correlate with a better response to NACT in some studies [116, 121-123] again, this finding is not universal [114, 119, 124, 125].

There is a small but growing number of studies of DTI in breast tissue, initially as proof of concept studies in the normal breast but more recently focusing on DTI in differentiating between benign and malignant breast lesions [126-142]. A measurable level of anisotropic water diffusion has been demonstrated in normal breast fibro-glandular tissue [126, 131, 134, 143]. This may be explained by the organization of the breast into a network of branching ducts and associated periductal fibrous stroma, resulting in preferential diffusion of water along or parallel to the ducts [131]. The hypothesis is that disruption of the breast architecture by

invading cancer cells should be reflected by changes in diffusion anisotropy. This hypothesis has been supported by several studies that have demonstrated lower FA values in malignant tissue compared to normal breast tissue [132, 135, 138, 139]. However, the use of FA values to differentiate between benign and malignant lesions has produced inconsistent results. While some studies report significantly lower FA values in benign breast lesions compared to malignant lesions [126, 135, 137, 140], others found no significant difference between the two [127, 128, 132, 138]. Two recent studies have also evaluated changes in FA following NACT at 1.5T in breast cancer with varied results [144, 145].

SECTION 5: PROJECT AIMS

A reproducible, objective, non-invasive biomarker of early tumour response to NACT in patients with locally advanced breast cancer is vital to support the emerging paradigm of individually tailored treatments. The importance of this study lies in the potential for differentiating responders to NACT from poor or non-responders by changes in ADC and FA values early in the course of treatment. Ultimately this information might be incorporated into the treatment algorithm with a view to modifying the chemotherapy regimen or performing surgery sooner for poor responders who could avoid the unnecessary toxicity associated with ineffective treatment. According to Schegerin et al. [146], if the use of imaging to track treatment response leads to chemotherapeutic regimen changes that result in a notable benefit for patients, neoadjuvant imaging is cost-effective: 'As long as the initial chemotherapy was less than 90% effective, most imaging systems would be cost effective, and if the cure rate of the disease could be increased as little as 1%

through a change to alternate therapy, then the cost effectiveness of the system would be acceptable.'

ADC values derived from DWI have shown potential as a biomarker for early treatment response in breast cancer but challenges remain before consideration of universal introduction to clinical practise. There is no published data in the literature as to whether DTI at 3.0T can detect chemotherapy induced changes in the breast tumour micro-environment, manifested as alterations in FA values. For these reasons, the primary aim of our study is to determine whether FA values can predict response in patients undergoing NACT for locally advanced breast cancer as well as to evaluate the importance of ADC values as a biomarker for early treatment response.

Secondary aims of the study include:

1. Identifying baseline FA and ADC values of malignant and normal tissue in the breast.
2. Evaluating the performance of baseline FA and ADC values in predicting response by correlating these values with the histopathological response.
3. Evaluating the performance of 'end of treatment' FA and ADC values in predicting response by correlating these values with the histopathological response.

MRI tumour volume calculation, pre and post chemotherapy, will also enable assessment of the performance of baseline tumour volume and end of treatment tumour volume change in predicting response.

CHAPTER 2: MATERIALS AND METHODS

SECTION 2.1: PATIENTS AND STUDY DESIGN

All research was performed in accordance with the Declaration of Helsinki, and the local research ethics committee granted written approval for this prospective study. All participants gave written informed consent. Participants were recruited from the breast oncology service over a one-year period. The following inclusion criteria were used:

- Clinical, radiological and histological diagnosis of stage II or stage III invasive breast cancer.
- NACT planned prior to surgery.
- Absence of contra-indication to magnetic resonance imaging.
- Normal renal function as defined by serum creatinine measurements.
- ECOG performance status 0-2.
- Patient age > 18years.
- Capable of providing written informed consent.

Exclusion criteria were as follows:

- No surgery planned.
- Contraindication to MRI.
- Known allergy to MR contrast.
- Impaired renal function.
- ECOG performance status 3-5.
- Pregnancy.
- Age <18years.

Our aim was to recruit 20 patients in total. This sample size was chosen for several

reasons. Similar studies looking at ADC values alone as a biomarker of treatment response in breast cancer achieved statistically significant results with sample sizes of 8, 14 and 15 patients [115, 118, 147]. Scanning 20 patients at 4 timepoints was considered an acceptable trade-off between funding constraints, patient tolerance and requirement to complete studies within a one-year period.

MRI was performed at 4 time points: prior to treatment (TP0), and after the first (TP1), second (TP2) and final cycles of chemotherapy (TP3). The chemotherapy regimen consisted of four cycles of doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) administered every two weeks followed by four cycles of paclitaxel (130 mg/m^2) administered every two weeks. HER2+ patients ($n=7$) received paclitaxel (80 mg/m^2) and trastuzumab (2 mg/kg) weekly for 12 weeks after the initial 4 cycles of doxorubicin and cyclophosphamide.

SECTION 2.2: MRI DATA ACQUISITION

Images were acquired on a 3T Achieva system (Philips Medical Systems, The Netherlands) with the use of a four-channel phased-array bilateral breast coil (MammoTrak SENSE Breast coil, Philips Medical Systems, The Netherlands) (Figure 1). Patients were examined prone with the breasts gently cushioned to reduce motion artifact and to maximize comfort. Care was taken to minimize skin folds within the coil, near the axilla and under the breast (e.g. abdominal fat) as skin folds can cause inhomogeneous fat suppression that would affect image quality [148].



Figure 1: Image demonstrating the Philips MammoTrak SENSE breast coil in a 3T Phillips Achieve MRI.

At baseline (TP0), a standard clinical protocol, including T1- and T2-weighted sequences, was performed in addition to diffusion weighted and diffusion tensor sequences, followed by dynamic contrast-enhanced (DCE) imaging. Diffusion imaging was performed prior to the administration of the contrast agent, not only to negate any possible effects the presence of contrast agents may have had on water diffusion within the tumour, but also to prevent any T2 shortening resulting from the contrast agent. DCE imaging involved administration of a bolus intravenous injection of 0.1 mmol per kg bodyweight of gadobenate dimeglumine (Gd-BOPTA, Multihance; Bracco Imaging SpA, Milan, Italy) followed by volumetric T1-weighted imaging with fat saturation. The purpose of DCE imaging at baseline was to delineate tumour extent and identify any areas of tumour necrosis that may interfere with diffusion

measurements. DCE imaging also provided an opportunity to calculate tumour volume. As lesion size was greater than 2.5cm in all patients, DCE imaging was not performed at TP1 and TP2 as it was deemed unnecessary for tumour identification. Contrast was also administered after the final cycle of chemotherapy (TP3) to allow re-evaluation of tumour size post treatment.

Diffusion weighted imaging was performed over the symptomatic breast in a sagittal plane using a single-shot echo planar imaging (EPI) sequence in three orthogonal diffusion encoding directions (x, y, and z). The following imaging parameters were used: b = 0, 100, 400, 800 s/mm², TR = 7986 ms, TE = 40 ms, FOV = 230 x 175 x 118 mm, acquisition matrix = 116 x 86, slice thickness = 3 mm, gap = 0.3 mm, in-plane voxel size = 2x2 mm², EPI factor = 59 and number of averages = 2. A spectral adiabatic inversion recovery (SPAIR) technique was used for fat suppression.

Diffusion tensor imaging was performed over the symptomatic breast in a sagittal plane using a single-shot echo planar imaging (EPI) sequence with 30 encoding directions. The following imaging parameters were used: b = 0, 700 s/mm², TR = 8000 ms, TE = 59 ms, FOV = 230 x 170 mm, acquisition matrix = 76 x 56, slice thickness = 3 mm, gap = 0, in-plane voxel size = 3 x 3 mm², EPI factor = 39 and number of averages = 3. A spectral inversion recovery (SPIR) technique was used for fat suppression.

SECTION 2.3: ASSESSING THE EFFECT OF A TUMOUR MARKER CLIP ON DIFFUSION DATA

In our institution, a titanium clip with a hydrogel coating (HydroMARK,

Devicor Medical Products, Inc., Cincinnati, USA) is placed at the center of the index lesion in all cases prior to NACT. The titanium clip can be easily identified on mammograms while the hydrogel coating aids in visualisation of the marker with ultrasound. Marking the tumour prior to neoadjuvant chemotherapy allows detection of the tumour on follow up studies. This is particularly useful in patients that have a complete pathological response to chemotherapy where the prior tumour site may be difficult to pinpoint. Visualisation of the clip in the surgical specimen also reassures the surgeon/pathologist that the lesion of interest has been removed.

There is a paucity of data of in the literature regarding the effect of tumour marker clips on diffusion parameters. In order to assess this, we embedded a hydrated tumour marker clip in a tumour-mimicking poly-vinyl alcohol cryogel phantom (Figure 2). The tumour-mimicking target was then surrounded by an agarose-based gel, which provided a uniform background for the phantom. DWI of the phantom was performed and an ADC map was generated. The titanium clip created a magnetic susceptibility-induced artifact. The ADC value of the clip in the phantom ($1.7 \times 10^{-3} \text{ mm}^2/\text{s}$) was higher than the ADC value of surrounding tissue ($1.4 \times 10^{-3} \text{ mm}^2/\text{s}$) (Figures 3 and 4). We concluded that the presence of a marker is a source of error when calculating ADC values and should be avoided when drawing regions of interest around tumour.

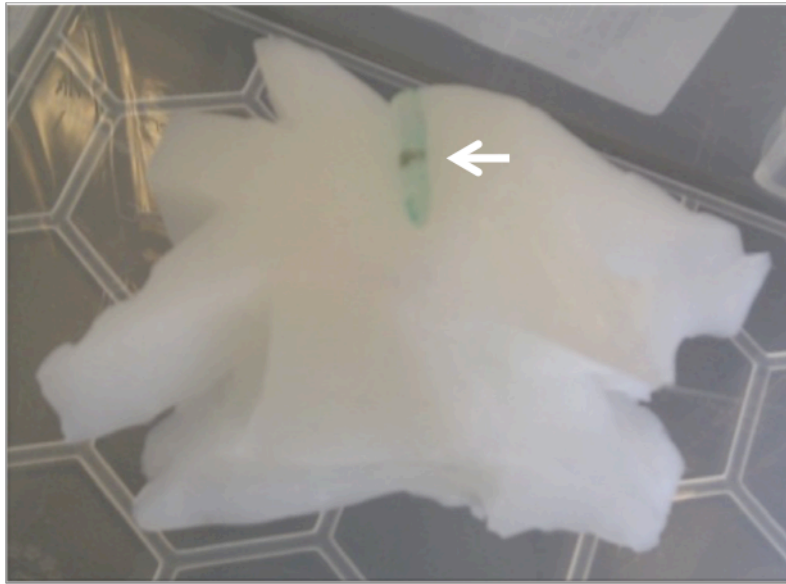


Figure 2: Image demonstrating the hydrated clip (arrow) embedded in the tumour-mimicking phantom before the addition of the background tissue mimicking gel.

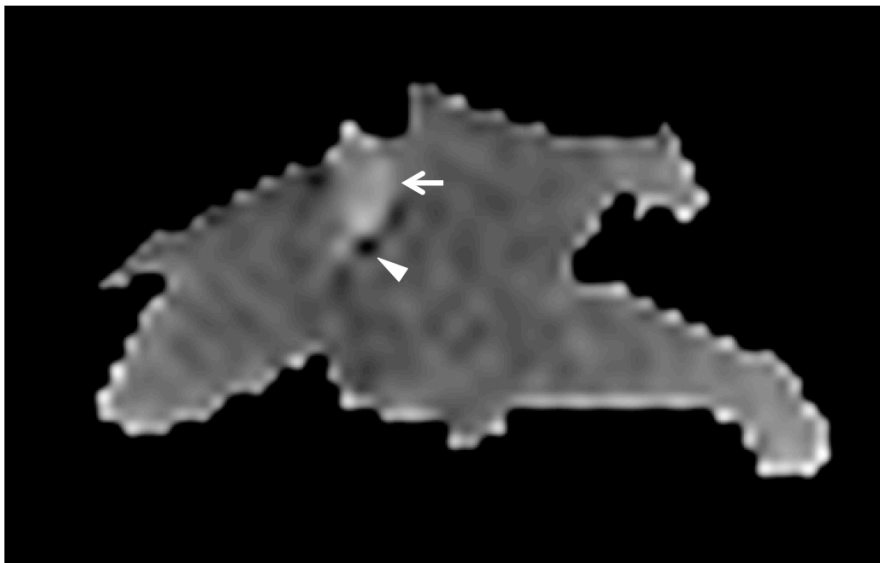


Figure 3: ADC map of the tumour mimicking phantom containing the marker clip. The higher ADC value of the gel component of the clip is demonstrated as increased signal in this region (arrow). The magnetic susceptibility-induced signal loss caused by the titanium component (arrowhead) is also evident.

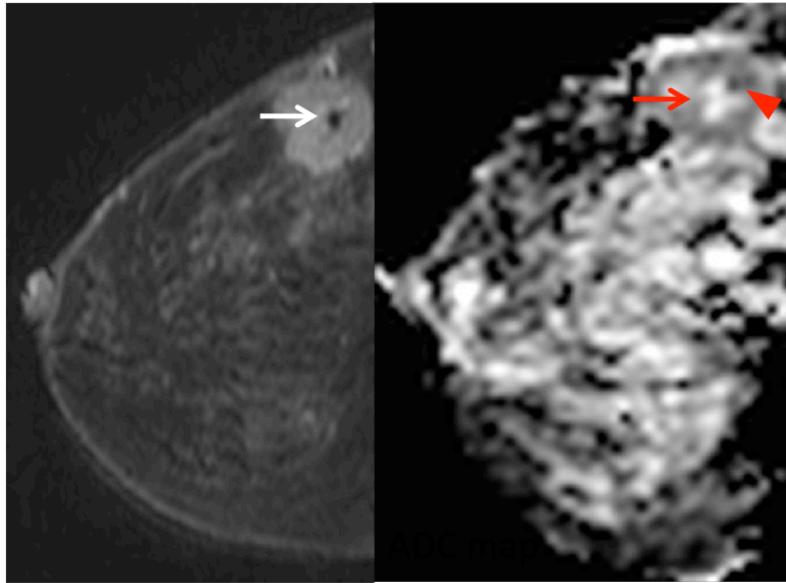


Figure 4: The image on the left is a post contrast image of the breast demonstrating an enhancing tumour in its upper aspect. A focal non-enhancing area within the tumour is in keeping with the clip site. The image on the right is the corresponding ADC map demonstrating an area of increased ADC (red arrow) created by the clip, which is out of proportion to its actual size. A tiny focus of signal loss posterior to this (arrowhead) represents susceptibility artifact created by the titanium component of the clip. These areas were avoided when drawing regions of interest within the tumour.

SECTION 2.4: TREATMENT RESPONSE ASSESSMENT

At the conclusion of NACT, all patients underwent surgery.

Histopathologic analysis of surgical specimens was performed by a specialist breast pathologist. Response to treatment was determined by Miller Payne grading of the surgical specimen (section 1.2.4., a five point histological scoring system that estimates tumour response to chemotherapy based on the reduction in tumour cellularity compared to pre-treatment baseline on core biopsy). For the purpose of this study, patients were divided into responders (Miller Payne grades 3-5, 31-100% reduction in tumour cellularity) or non-responders (Miller Payne grades 1 or 2, $\leq 30\%$ reduction).

SECTION 2.5: DATA ANALYSIS

2.5.1 ADC AND FA VALUES

Post-processing of data was performed using vendor-specific software (Extended MR WorkSpace, Philips). Quantitative ADC and FA maps were generated from DW and DT data respectively. When generating ADC maps, b-values of 100, 400 and 800 s/mm² were used; the b value = 0 s/mm² images were intentionally omitted. At very low b values (<100 s/mm²), the ADC predominantly reflects larger distances of water movement likely to represent movement within micro vessels. The use of higher b values suppresses this 'perfusion' effect in vessel-rich areas (such as tumours) and the ADC value can more accurately represent true extracellular water diffusion [149]. When generating FA maps, b values of 0 and 700 s/mm² were used. The DW and DT data were collected consecutively, in order to minimize the potential for misregistration between data-sets due to patient motion, thereby allowing similar regions of interests (ROIs) to be placed in each data set.

ROIs were manually drawn on all slices on which tumour was visible on the ADC and FA maps at each imaging time point. ROI placement was closely guided by tumour extent on the corresponding highest available b-value images and DCE images at baseline (Figure 5). Care was taken to exclude areas of necrosis and artifact created by tumour marker clip placement based on their appearance on T1-and T2-weighted and DCE sequences. Mean tumour ADC and FA values were calculated from the corresponding parametric maps for each ROI. On the baseline ADC and FA maps, ROIs were also drawn in disease-free fibro-glandular tissue in anterior, central and

posterior regions in the breast, which were averaged to give ADC and FA values for normal breast parenchyma for each patient.

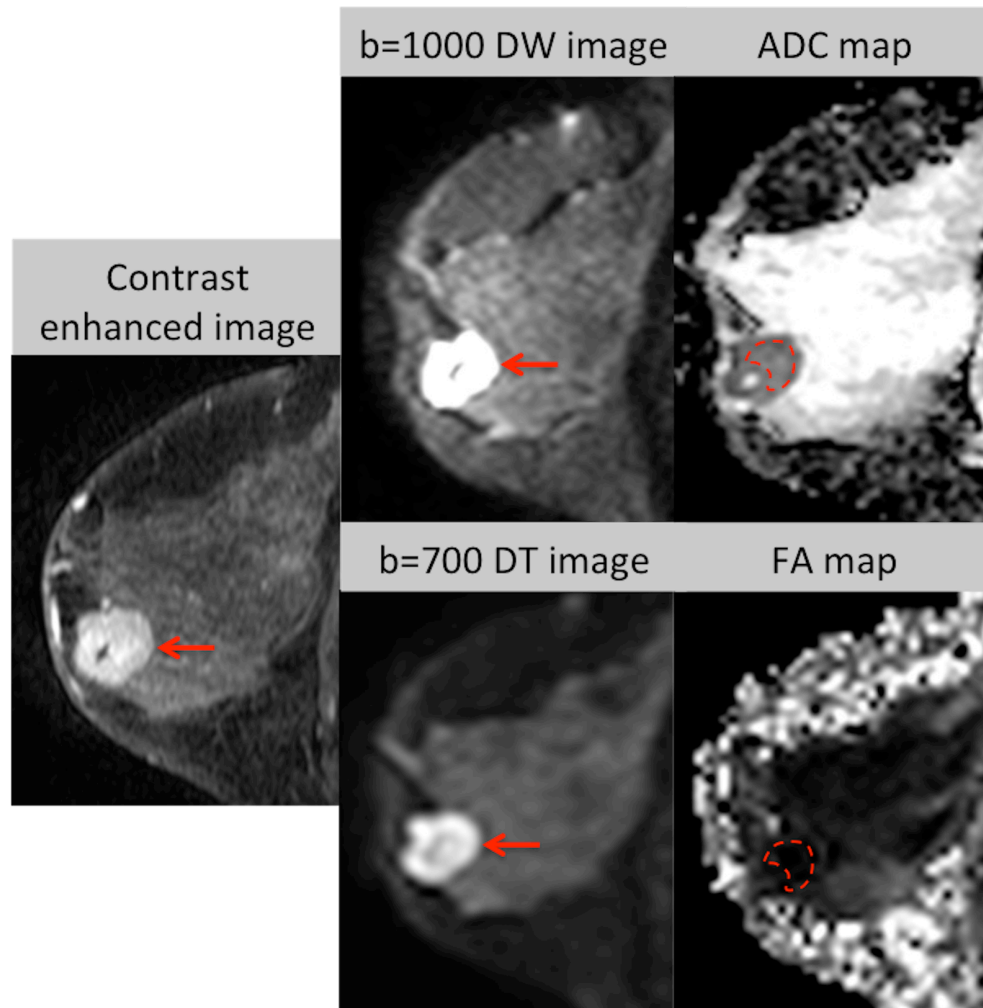


Figure 5: On DCE imaging, the tumour (arrow) is seen as an enhancing mass in the lower part of the breast. On diffusion weighted and diffusion tensor b value images, the tumour (arrow) is seen as an area of increased signal. ROI placement (dashed line) on the corresponding ADC and FA maps was closely guided by tumour extent on the DCE and b -value images. Care was taken to avoid artifact caused by the tumour marker clip.

2.5.2 TUMOUR VOLUME

Breast tumour volumes were obtained from the contrast-enhanced

images performed at baseline (TP0) and following the final cycle of chemotherapy (TP3) using software provided by Phillips (Extended MR WorkSpace, Philips). This software allows calculation of volume through the use of a semi-automated segmentation algorithm based on thresholding of the enhancement curve, a method previously described by Partridge et al. [65]. First, a set of maximum intensity projections were created from the contrast enhanced images. The projections are generated in the lateromedial, craniocaudal, and anteroposterior directions and allow the tumour extent to be visualised in all dimensions. Next, a volume of interest was defined by encircling the desired area of inclusion on two orthogonal projections. Voxels containing enhancing blood vessels or other interfering regions adjacent to the tumour, such as pathological skin thickening and noise, were also removed at this point. The software calculates contrast enhancement on a voxel by voxel basis for all voxels contained within the selected volume. Tumour voxels were segmented from normal breast tissue by using a threshold excluding voxels with less than 80% enhancement within 3 minutes of contrast administration. This threshold was reduced to 40% on the post chemotherapy scan to account for lower contrast uptake by tumour after NACT. Tumour volumes were then generated from the summing of the selected voxels.

2.5.3 STATISTICAL ANALYSIS

The mean and standard deviation of the ADC and FA values were calculated for each tumour at each of the four time points. Changes in parameter values between baseline (TP0) and TP1, baseline and TP2, and baseline and TP3,

were calculated as absolute differences and percentage change in the responder and non-responder groups. The % change in tumour ADC and FA values after each cycle, relative to the pre-treatment values, were calculated for as follows: $\% \Delta \text{ADC} = \frac{[(\text{ADC}_{\text{pt}} - \text{ADC}_{\text{b}}) / \text{ADC}_{\text{b}}] \times 100}{}$, where ADC_{b} is the baseline tumour ADC prior to chemotherapy and ADC_{pt} is tumour ADC post the first (TP1) or second (TP2) cycle of chemotherapy. Similar calculations were performed for FA tumour values. Changes in tumour volumes between baseline and TP3 were also calculated as absolute differences and percentage change in the responders and non-responders.

All statistical analysis was performed using Graphpad Prism V6 software. Two-way analysis of variance was applied to compare tumour ADC and FA values with those of normal fibro-glandular tissue. The changes between ADC and FA values at baseline and after the first and second cycles of NACT were tested using the non-parametric Wilcoxon signed rank test. Differences between responders and non-responders were compared using a Mann Whitney U-test. Values of $p \leq 0.05$ were considered significant. Box plots were drawn to examine the trend of ADC and FA values among the responders and non-responders. The horizontal line represents the median, the ends of the box represent the upper and lower quartiles and the vertical line shows the full range of values observed. When a statistically significant result was obtained, the ability of a parameter in differentiating between responding and non-responding patients was assessed by creating a receiver operating characteristic (ROC) curve and comparing the area under the curve (AUC).

CHAPTER 3: RESULTS

SECTION 3.1: PATIENTS

Twenty-one patients were enrolled over a one-year period; twenty patients were included in data analysis as one patient withdrew after the first scan. Eighteen patients had a diagnosis of invasive ductal carcinoma and two patients had a diagnosis of invasive lobular carcinoma. Patient characteristics are outlined in Table 1. Imaging was not performed on two patients at TP2 (one patient failed to attend and one was medically unfit for the scan). All baseline scans were performed within 7 days prior to treatment initiation while all post treatment scans (TP1 and TP2) were carried out between 7-14 days following chemotherapy administration. Pathologically, fifteen patients were classified as responders and five as non-responders. Of the responders, five had a complete pathological response (Miller Payne grade 5) with no residual cancer cells identified in the resected surgical specimen.

Patient	Age	Menopausal status	Type of cancer	Grade	ER/PR/HER2	Baseline MRI tumour size (mm)	Excised tumour size (mm)	MPG	R/NR
1	43	Pre	IDC	3	+/-/-	48	0	5	R
2	32	Pre	IDC	2	+/+/+	41	0	5	R
3	59	Post	IDC	3	+/+/-	28	0	5	R
4	36	Pre	IDC	3	+/+/-	25	0	5	R
5	43	Pre	IDC	3	-/-/+	48	0	5	R
6	59	Post	IDC	3	+/+/-	50	6	4	R
7	44	Pre	IDC	1	+/+/+	63	17	4	R
8	50	Post	ILC	2	+/-/-	32	12	4	R
9	39	Pre	IDC	2	+/+/+	45	20	4	R
10	46	Pre	IDC	2	-/-/+	41	3	4	R
11	29	Pre	IDC	3	+/+/+	70	4	4	R
12	50	Pre	IDC	3	+/-/-	32	7	3	R
13	59	Post	IDC	2	+/+/-	81	44	3	R
14	51	Post	IDC	3	+/+/-	38	25	3	R
15	52	Post	IDC	3	+/-/-	34	15	3	R
16	70	Post	ILC	2	+/+/-	27	26	2	NR
17	51	Post	IDC	2	+/-/-	44	25	2	NR
18	39	Pre	IDC	3	+/+/+	30	13	2	NR
19	43	Pre	IDC	2	+/+/-	65	50	2	NR
20	54	Post	IDC	2	+/-/-	91	80	1	NR

Table 1: Patient and tumour characteristics. IDC = intraductal carcinoma, ILC = intralobular carcinoma, ER = estrogen receptor, PR = progesterone receptor, HER2 = human epidermal growth factor receptor 2, MPG = Miller Payne grade, R = responder, NR = non-responder.

SECTION 3.2: PRETREATMENT ADC AND FA VALUES

At baseline, statistically significant lower ADC values were recorded for tumours (mean = $0.93 \times 10^{-3} \text{ mm}^2/\text{s}$, SD ± 0.08) compared to normal fibro-glandular breast tissue (mean = $1.75 \times 10^{-3} \text{ mm}^2/\text{s}$, SD ± 0.26) ($p < 0.001$) (Figure 1). ROC curve analysis to assess the ability of pre-treatment ADC in differentiating between tumour and normal breast tissue showed an excellent AUC (1.0) with a cut off ADC value of $1.13 \text{ mm}^2/\text{s}$ (sensitivity 100% and specificity 100%). No significant difference was observed between baseline tumour ADC values in the responder group (mean = $0.92 \times 10^{-3} \text{ mm}^2/\text{s}$, SD $\pm 0.08 \text{ mm}^2/\text{s}$) compared to the non-responder group (mean = $0.97 \times 10^{-3} \text{ mm}^2/\text{s}$, SD $\pm 0.08 \text{ mm}^2/\text{s}$) ($p = 0.266$) (Figure 2).

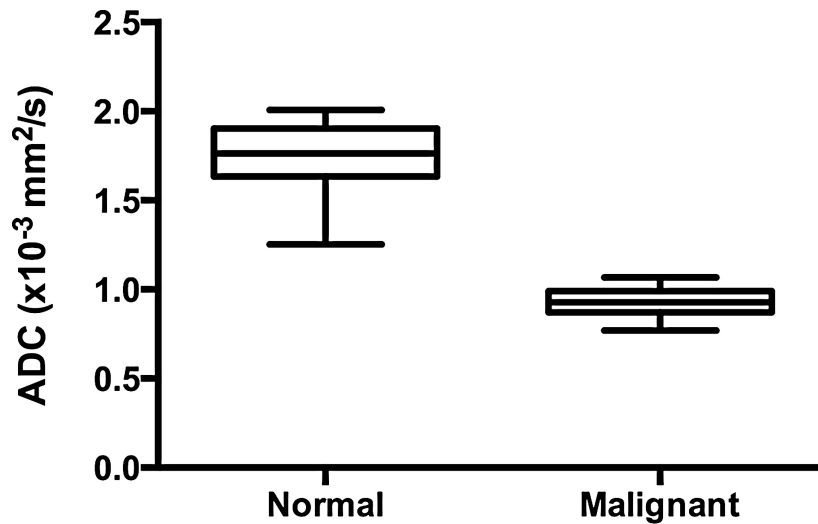


Figure 1: Box plot of ADC values in normal fibro-glandular tissue and malignant breast tumours. At baseline, tumour ADC values were significantly lower than those of disease free fibro-glandular breast tissue ($p < 0.001$).

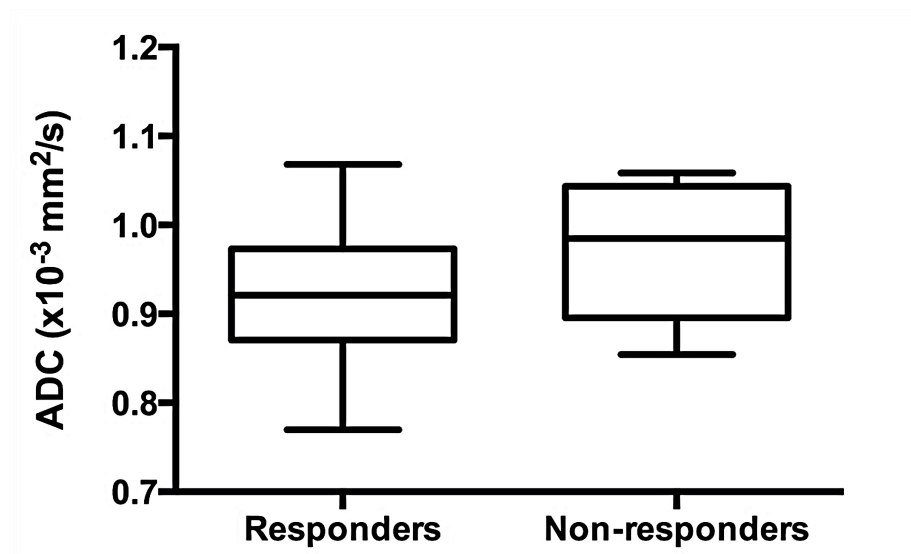


Figure 2: Box plot of baseline tumour ADC values in responder and non-responder groups. No significant difference was observed with baseline tumour ADC in the responder group compared to the non-responder group ($p = 0.266$).

At baseline, FA values of tumour (mean = 0.139, SD $\pm$ 0.04) and normal parenchymal tissue (mean = 0.14, SD $\pm$ 0.05) were not statistically different ($p = 0.542$)

(Figure 3). No significant difference was observed between baseline tumour FA

comparing the responder (mean= 0.139, SD $\pm$ 0.04) and non-responder groups (mean= 0.14, SD $\pm$ 0.03) ($p=0.931$) (Figure 4).

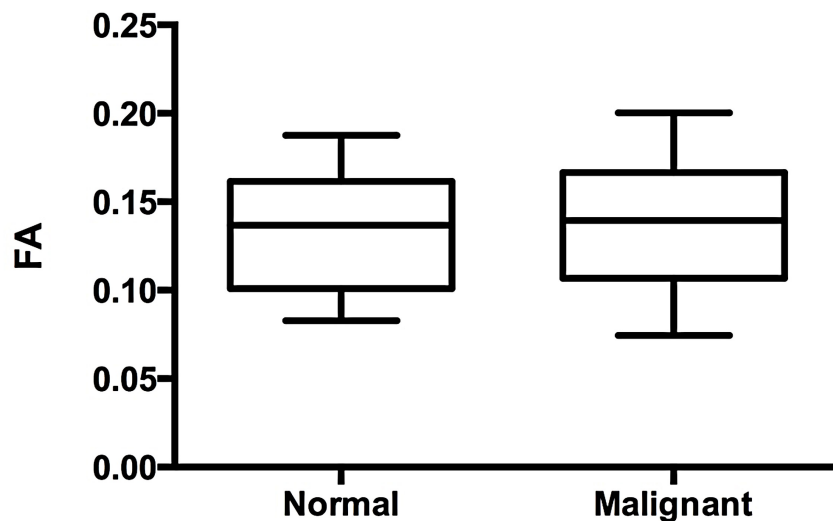


Figure 3: Box plot of FA values in normal fibro-glandular tissue and malignant breast tumours. At baseline, no significant difference was observed between FA values of tumour and disease free parenchymal tissue ($p=0.542$).

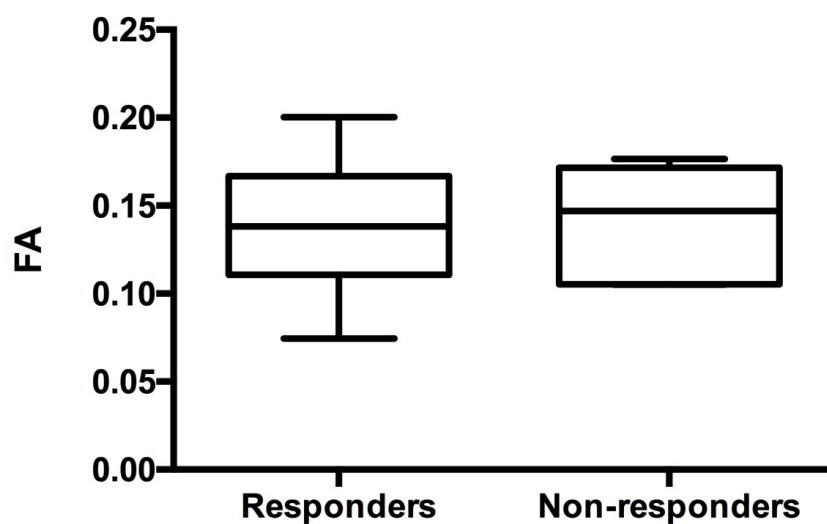


Figure 4: Box plot of baseline tumour FA values in responder and non-responder groups. No significant difference was observed between baseline tumour FA in the responder and non-responder groups ($p=0.931$).

SECTION 3.3: EARLY TREATMENT ADC AND FA VALUES

Compared with baseline values, pooled ADC values of patients in the responder group significantly increased at TP1 ($p<0.001$) and TP2 ($p<0.001$). The mean tumour ADC in the responder group increased from $0.92 \times 10^{-3} \text{ mm}^2/\text{s}$ to $1.1 \times 10^{-3} \text{ mm}^2/\text{s}$ at TP1 and $1.2 \times 10^{-3} \text{ mm}^2/\text{s}$ at TP2. Figure 5 shows the box plot of pooled ADC data of responders before therapy and after the first and second cycles of NACT while Table 2 demonstrates the ADC values for individual responders at these time-points. Pooled analysis of percentage change in ADC values of patients in the responder group also demonstrated a significant percentage increase in ADC values from baseline for both TP1 ($p<0.001$) and TP2 ($p<0.001$). Table 3 shows the percentage increase from baseline in tumour ADC values after the first (TP1) and second (TP2) cycles of chemotherapy in responders.

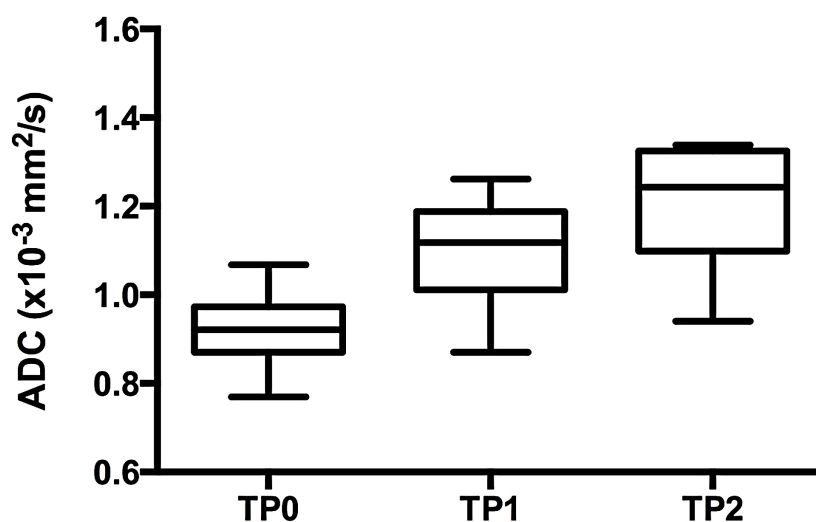


Figure 5: Box plot of ADC values of responders at TP0, TP1 and TP2. Tumour ADC values after the first chemotherapy cycle were significantly higher than the baseline values ($p<0.001$) and showed a further increase after the second cycle ($p<0.001$).

Responders	ADC ($\times 10^{-3}$ mm ² /s)			FA		
	TP0	TP1	TP2	TP0	TP1	TP2
1	0.89	1.16	1.31	0.153	0.176	0.248
2	0.87	1.16	DNA	0.141	0.147	DNA
3	0.93	1.01	1.12	0.13	0.157	0.213
4	0.92	1.19	1.34	0.19	0.244	0.298
5	0.99	1.09	1.16	0.138	0.174	0.175
6	0.86	1.01	1.3	0.2	0.196	0.306
7	0.87	1.1	1.32	0.102	0.121	0.125
8	0.95	1.22	1.37	0.196	0.197	0.201
9	1.05	1.26	1.34	0.123	0.116	0.153
10	1.07	1.18	1.27	0.114	0.118	0.122
11	0.83	0.91	0.94	0.144	0.142	0.119
12	0.77	0.87	0.97	0.167	0.135	0.169
13	0.92	1.19	1.22	0.098	0.105	0.089
14	0.97	1.12	1.09	0.111	0.106	0.152
15	0.89	1.08	1.1	0.075	0.08	0.128
Mean	0.92	1.1	1.2	0.139	0.147	0.177
± SD	0.08	0.11	0.14	0.037	0.044	0.067

Table 2: ADC and FA values of individual patients in the responder group at baseline (TP0) and after the first (TP1) and second (TP2) cycles of chemotherapy. (DNA – did not attend)

Responders	% change in ADC		% change in FA	
	TP1	TP2	TP1	TP2
1	30.7	47.7	15.3	62.3
2	33.3	DNA	4.7	DNA
3	8.3	19.6	20.9	37.1
4	28.6	44.6	28.0	56.5
5	9.8	17.0	26.1	26.8
6	17.6	51.6	-1.9	52.6
7	25.9	52.1	18.3	22.1
8	27.8	39.3	0.5	2.6
9	19.7	27.0	-5.5	24.4
10	10.5	18.8	4.3	7.4
11	10.6	13.9	-1.0	-17.0
12	13.1	25.4	-19.3	1.2
13	29.3	32.1	8.0	-8.6
14	14.8	12.2	-4.4	36.8
15	21.1	23.1	6.7	23.0
Mean	20.1	30.3	6.7	23.4
± SD	8.6	14.2	13.0	24.3

Table 3: Percentage changes from baseline in ADC and FA values of individual patients in the responder group after the first (TP1) and second (TP2) cycles of chemotherapy. (DNA – did not attend)

After the first and second cycle of chemotherapy, no statistically significant increase in ADC was observed in non-responders at either TP1 ($p=0.063$) or TP2 ($p=0.125$). Mean tumour ADC in the non-responder group increased from $0.97 \times 10^{-3} \text{ mm}^2/\text{s}$ to $1.02 \times 10^{-3} \text{ mm}^2/\text{s}$ at TP1 and $1.06 \times 10^{-3} \text{ mm}^2/\text{s}$ at TP2. Figure 6 shows the box plot of pooled ADC data of non-responders before therapy and after the first and second cycles of NACT while Table 4 demonstrates the ADC values for individual non-responders at these time-points. Pooled analysis of percentage change in ADC values of patients in the non-responder group demonstrated no significant percentage change in ADC values from baseline for either TP1 ($p=0.125$) or TP2 ($p=0.125$). Table 5 demonstrates the percentage change from baseline in tumour ADC values after the first (TP1) and second (TP2) cycles of chemotherapy in non-responders. Figure 7 shows $b=1000$ images and ADC maps from a responding and non-responding patient along with DCE images.

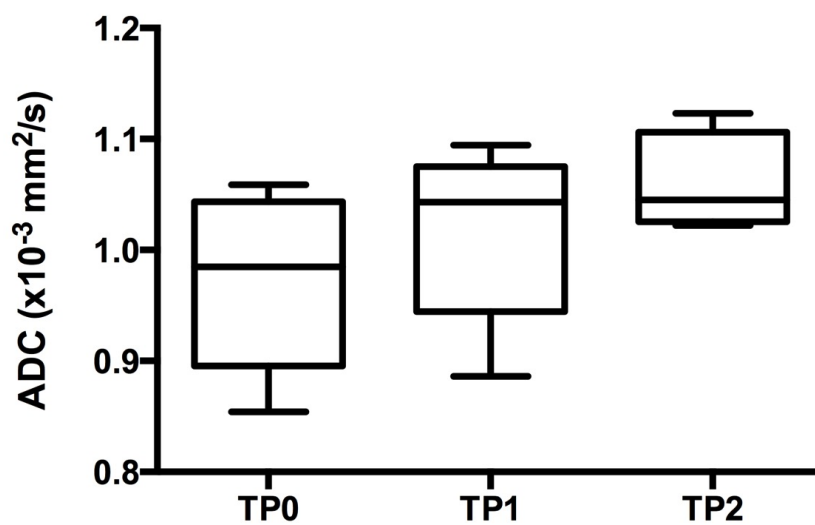


Figure 6: Box plot of ADC values of non-responders at TP0, TP1 and TP2. Compared with pre-therapy values, no significant change occurred in ADC values at TP1 ($p=0.125$) or TP2 ($p=0.125$).

Non-responders	ADC ($\times 10^{-3}$ mm ² /s)			FA		
	TP0	TP1	TP2	TP0	TP1	TP2
16	0.85	0.89	1.02	0.105	0.11	0.093
17	0.98	1.04	1.06	0.105	0.113	0.127
18	1.03	1.06	1.12	0.177	0.172	0.165
19	0.94	1	1.04	0.147	0.16	0.167
20	1.06	1.09	DNA	0.166	0.202	DNA
Mean	0.97	1.02	1.06	0.14	0.151	0.138
± SD	0.08	0.08	0.04	0.034	0.039	0.035

Table 4: ADC and FA values of individual patients in the non-responder group at baseline (TP0) and after the first (TP1) and second (TP2) cycles of chemotherapy. (DNA – did not attend)

Non-responders	% change in ADC		% change in FA	
	TP1	TP2	TP1	TP2
16	3.7	19.7	4.3	-12.0
17	5.9	7.1	7.7	20.9
18	2.7	9.2	-2.6	-6.6
19	7.1	10.5	9.0	14.0
20	3.4	DNA	21.2	DNA
Mean	4.6	11.6	7.9	4.1
± SD	1.9	5.5	8.7	15.8

Table 5: Percentage changes from baseline in ADC and FA values of individual patients in the non-responder group after the first (TP1) and second (TP2) cycles of chemotherapy. (DNA – did not attend)

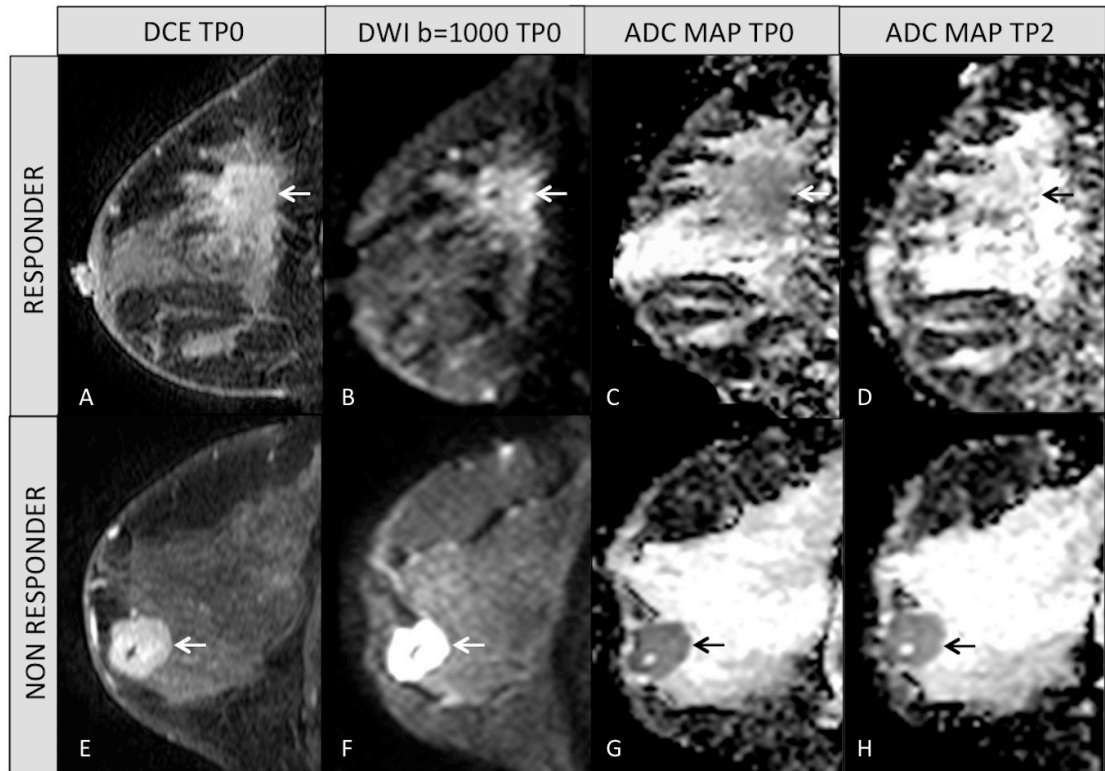


Figure 7: Sagittal images from a responder (above) and a non-responder (below). In the responding patient, dynamic contrast enhanced (DCE) imaging (A) demonstrates an ill-defined enhancing tumour (arrow) in the superior aspect of the breast. On the DW acquired $b=1000$ image (B), the tumour is seen as a localized area of high signal (arrow) while, on the ADC map (C), it appears low in signal (arrow) indicating diffusion restriction. Post 2 cycles of chemotherapy (D), the tumour region (arrow) appears less hypointense reflecting the 40% increase in the ADC value of this tumour. In the non-responding patient, DCE imaging (E) demonstrates a well-defined enhancing tumour (arrow) in the inferior aspect of the breast. On the DW acquired $b=1000$ image (F), the tumour is seen as a localized area of high signal (arrow) while, on the ADC map (G), it appears low in signal (arrow) indicating diffusion restriction. Post 2 cycles of chemotherapy (H), the tumour (arrow) has a similar appearance to its pre-treatment appearance (G) reflecting the smaller (7%) increase in ADC between the two treatment cycles.

In the responder group, tumour FA increased after the first and second cycle of chemotherapy. While this increase did not reach significance at TP1 ($p=0.095$), a statistically significant increase was demonstrated at TP2 ($p=0.005$). Mean tumour FA in the responder group increased from 0.139 to 0.147 at TP1 and to 0.177 at TP2. Figure 8 shows the box plot of pooled FA data of responders before

therapy and after the first and second cycles of NACT while table 2 demonstrates the FA values for individual responders at each time point. Pooled analysis of percentage change in FA values of patients in the responder group also demonstrated a significant percentage increase in FA values from baseline to TP2 ($p=0.004$) but the percentage increase at TP1 did not reach significance ($p=0.135$). Table 3 demonstrates the percentage change from baseline in individual tumour FA values after the first (TP1) and second (TP2) cycles of chemotherapy in responders. Figure 9 shows representative serial FA maps from a responder.

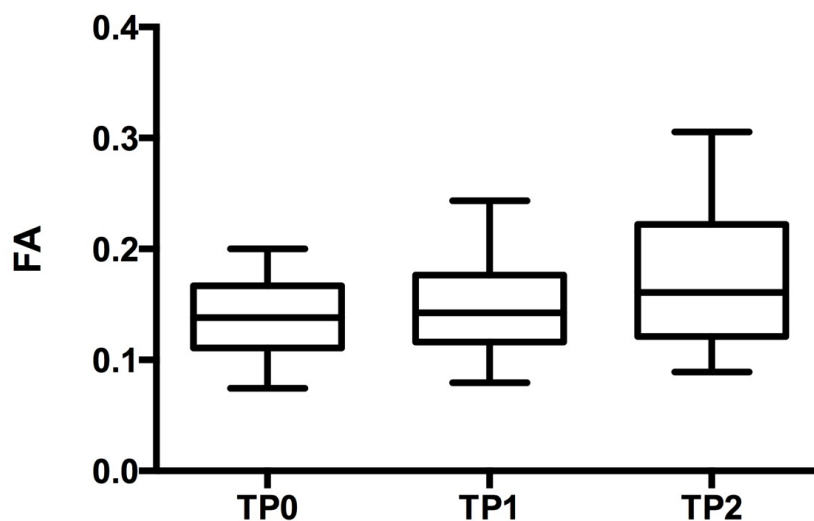


Figure 8: Box plot of FA values of responders at TP0, TP1 and TP2. Compared with pre-therapy values, tumour FA significantly increased at TP2 ($p=0.004$). No significant increase occurred between TP0 and TP1 ($p=0.135$).

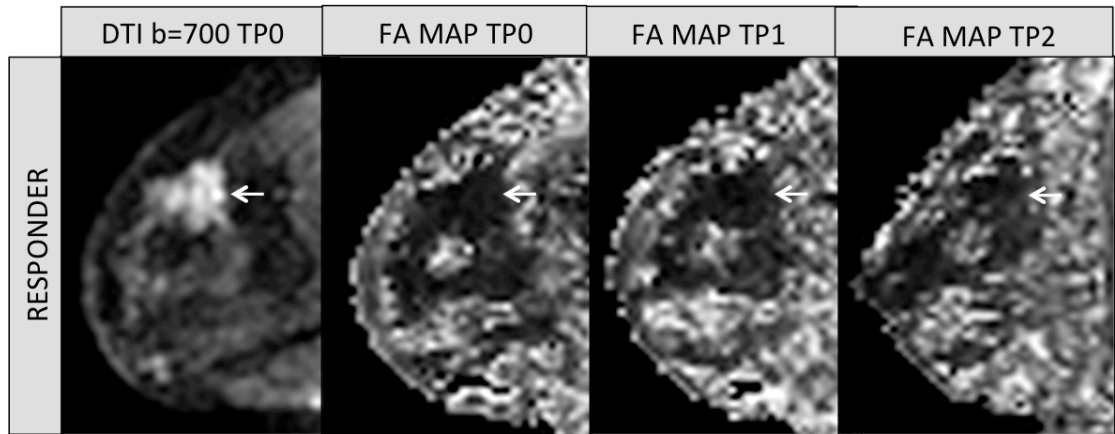


Figure 9: Sagittal DTI b=700 image and FA maps of a responder at TP0, TP1 and TP2. Tumour FA increased in the demonstrated responder by 6% between TP0 and TP1 and 23% between TP0 and TP2 reflected by the increase in signal at the tumour site (arrows) on the FA maps.

No significant change occurred in tumour FA in the non-responder group after the first ($p=0.188$) or second ($p=0.625$) cycles of chemotherapy. Mean tumour FA in the non-responder group initially increased from 0.14 to 0.151 at TP1. A subsequent decrease from baseline was then observed at TP2 with a mean tumour FA of 0.138. Figure 10 shows the box plot of pooled FA data of non-responders before therapy and after the first and second cycles of NACT while table 4 demonstrates the FA values for individual non-responders at these time-points. Pooled analysis of percentage change in FA values of patients in the non-responder group demonstrated no significant percentage change in FA values from baseline for either TP1 ($p=0.188$) or TP2 ($p=0.875$). Table 5 demonstrates the percentage change from baseline in tumour ADC values after the first (TP1) and second (TP2) cycles of chemotherapy in non-responders. Figure 11 demonstrates representative images from a patient with a suboptimal response to chemotherapy.

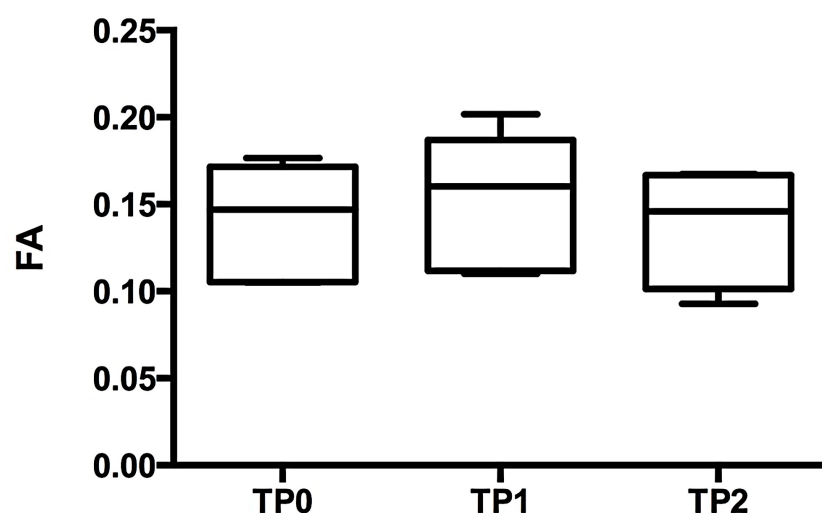


Figure 10: Box plot of FA values of non-responders at TP0, TP1 and TP2. Compared with pre-therapy values, no significant change occurred in FA values at TP1 or TP2.

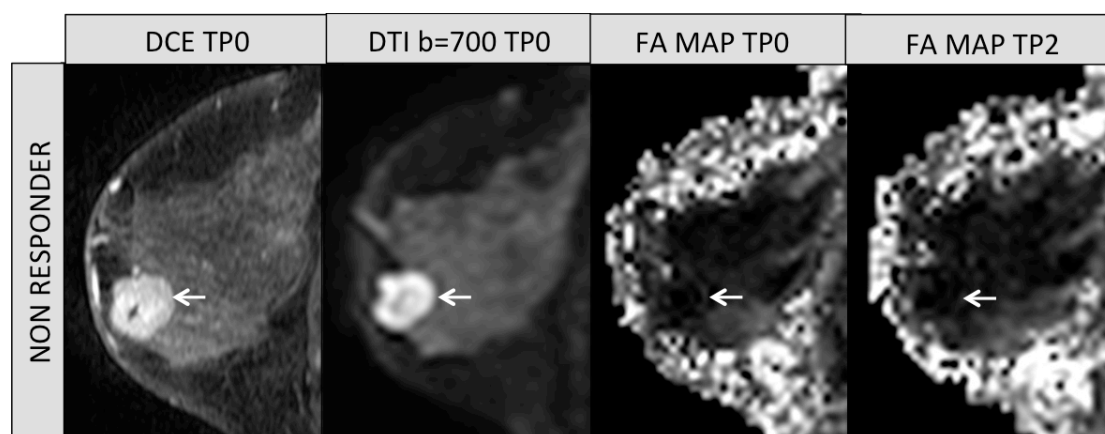


Figure 11: Sagittal DCE image, b=700 image and FA maps of a non-responder at TP0 and TP2. No significant change occurred in FA values of the non-responder (tumour indicated by arrows).

SECTION 3.4: EARLY RESPONSE PREDICTION

A comparison of the percentage change from baseline in ADC and FA values at TP1 and TP2, between responders and non-responders, was carried out to assess their potential to predict early tumour response. The percentage increase of tumour ADC values in the responder group from baseline to TP1 (mean = 20.1%, SD $\pm$

8.6%) was significantly higher than the increase observed for tumour ADC values of non-responders (mean = 4.6%, SD $\pm$ 1.9%) between the same time-points ($p < 0.001$) (Figure 12). Similarly, the percentage increase of tumour ADC values in the responder group from baseline to TP2 (mean = 30.4%, SD $\pm$ 14.2%) was significantly higher than those observed in non-responders (mean = 11.6%, SD $\pm$ 5.5%) between these time-points ($p = 0.012$) (Figure 13). A cut off value for the percentage change in ADC was calculated to predict response after the first and second cycles of chemotherapy. ROC analysis of the TP0 to TP1 ADC data gave a cut off value of 7.7% (sensitivity 100%, specificity 100% and AUC 1.0) while analysis of the TP0 to TP2 data gave a cut off value of 21.4% (sensitivity 100%, specificity 64% and AUC 0.91).

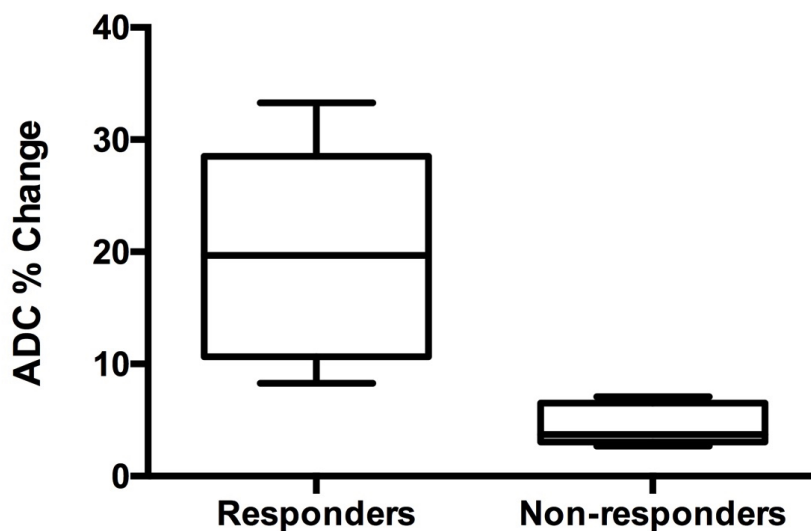


Figure 12: Box plot demonstrating the percentage change in ADC values from baseline to TP1 in responders and non-responders. The percentage increase of tumour ADC values in the responder group was significantly higher than the increase observed for non-responders ($p < 0.001$).

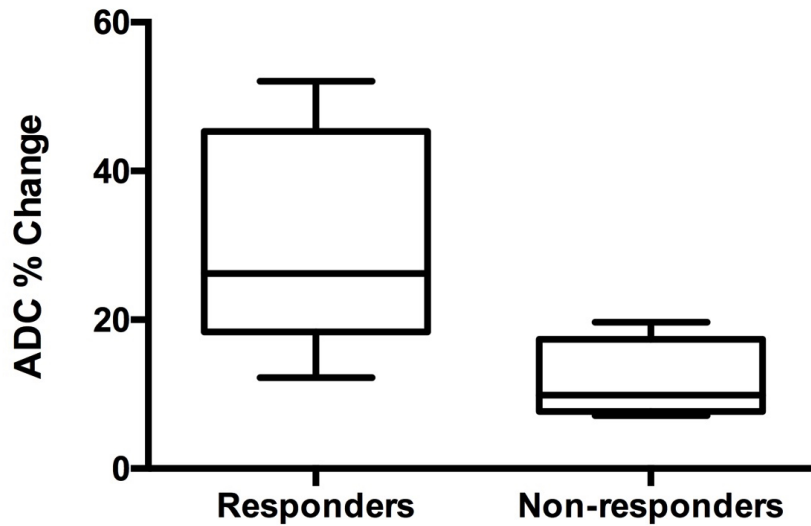


Figure 13: Box plot demonstrating the percentage change in ADC values from baseline to TP2 in responders and non-responders. The percentage increase of tumour ADC values in the responder group was significantly higher than the increase observed for non-responders ($p=0.012$).

The percentage change in tumour FA values of responders from TP0 to TP1 (mean = 6.7%, SD $\pm$ 13.0%) did not significantly differ from the change detected in the non-responder group (mean = 7.9%, SD $\pm$ 15.8) ($p=0.985$) (Figure 14). Despite tumour FA values in the responder group significantly increasing from baseline to TP2, no significant difference was observed in the percentage change of tumour FA values between the responder and non-responder groups from TP0 to TP2 ($p=0.116$) (Figure 15).

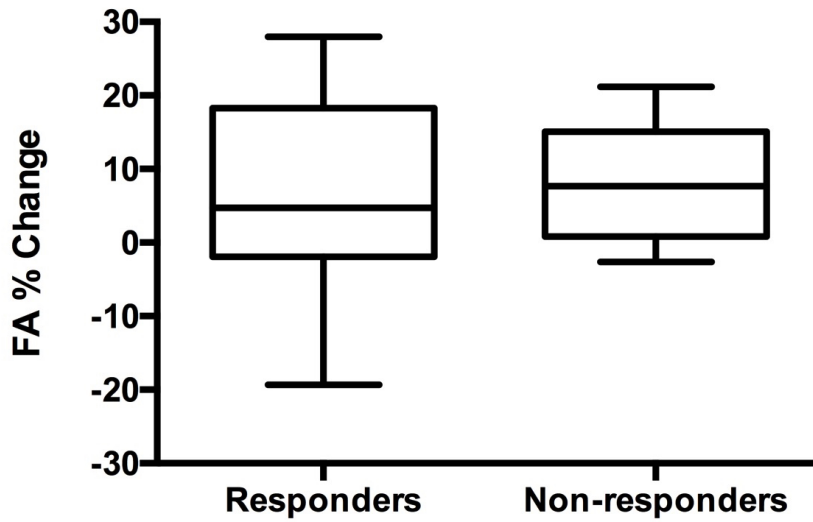


Figure 14: Box plot demonstrating the percentage change in FA values from baseline to TP1 in responders and non-responders. The percentage change of tumour FA values in the responder group did not significantly differ from the change observed for non-responders ($p=0.985$).

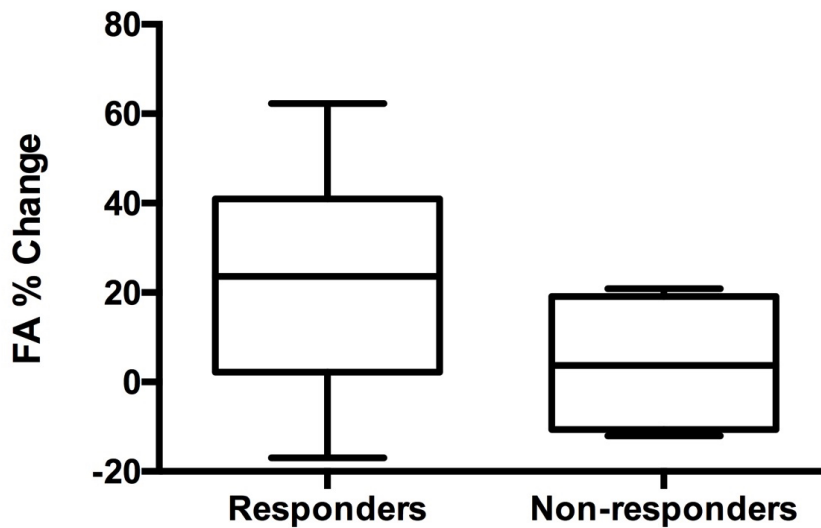


Figure 15: Box plot demonstrating the percentage change in FA values from baseline to TP2 in responders and non-responders. The percentage change of tumour FA values in the responder group did not significantly differ from the change observed for non-responders ($p=0.116$).

SECTION 3.5: POST CHEMOTHERAPY ADC AND FA VALUES

Due to the marked reduction in tumour size in 11 of the 15 patients in the responder group, ADC and FA values could not be determined for these cases on the final post chemotherapy scan (TP3) (Table 6). Tumour ADC and FA values could be determined for all patients in the non-responder group (n=4) at TP3 (Table 7). Tables 6 and 7 also demonstrate the percentage change in ADC and FA values of the responders and non responders, respectively.

Responders	TP3		% change from TP0-TP3	
	ADC	FA	ADC	FA
1			N/A	
2			N/A	
3			N/A	
4			N/A	
5			N/A	
6			N/A	
7			N/A	
8			N/A	
9	1.53	0.141	45.5	14.5
10	1.39	0.142	29.7	24.8
11	1.08	0.144	31.0	0.1
12			N/A	
13			N/A	
14			N/A	
15	0.93	0.137	4.1	72.3
Mean	1.23	0.141	27.6	27.9
± SD	0.27	0.003	17.2	31.2

Table 6: Tumour ADC and FA values, and percentage change in these values from baseline, of individual patients in the responder group after the final cycle of chemotherapy (TP3). (N/A – not applicable)

Non-responders	TP3		% change from TP0-TP3	
	ADC	FA	ADC	FA
16	1.05	0.133	22.6	25.9
17	1.08	0.139	9.8	32.6
18	1.12	0.187	9.3	6.2
19	1.09	0.205	16.3	39.8
20	1.13	0.303	6.9	82.2
Mean	1.09	0.194	13.0	37.3
± SD	0.03	0.069	6.4	28.0

Table7: Tumour ADC and FA values, and percentage change in these values from baseline, of individual patients in the non-responder group after the final cycle of chemotherapy (TP3).

No statistically significant difference was observed in tumour ADC values between the responder and non-responders groups at TP3 ($p=0.714$). In the responder group, a statistically significant increase was observed in ADC values ($p=0.014$) and in ADC percentage increase ($p<0.001$) between TP0 and TP3. However, a statistically significant increase was also demonstrated in ADC ($p=0.016$) and in ADC percentage increase ($p=0.008$) in the non-responder group after the final cycle of chemotherapy. While a greater increase was seen in the ADC values of the responder group (mean 27.6%, SD $\pm$ 17.2) compared to the non-responder group (mean = 13.0%, SD $\pm$ 6.4), this difference did not reach statistical significance ($p=0.286$).

There was no significant difference in tumour FA values between responders and non-responders at TP3 ($p=0.524$). In addition, no statistically significant difference was observed in FA values between TP0 and TP3 either in the responder group ($p=0.665$) or the non-responder group ($p=0.222$). A significant FA percentage change between TP0 and TP3 occurred in responders ($p=0.031$) that was not demonstrated in non-responders ($p=0.063$) however a comparison of the

percentage change of FA values between the two groups showed no significant difference to allow a distinction of response ($p=0.397$).

SECTION 3.6: TUMOUR VOLUME

At baseline, MRI-derived tumour volumes in the responder group ranged from 3.8 to 65.0 cm^3 (mean 13.1 cm^3 , SD ± 16.6), while in the non-responder group they ranged from 1.8 to 70.8 cm^3 (mean 23.4 cm^3 , SD ± 28.3). No significant difference was observed between the pre-treatment tumour volume of responders and non-responders ($p = 0.644$) (Figure 16).

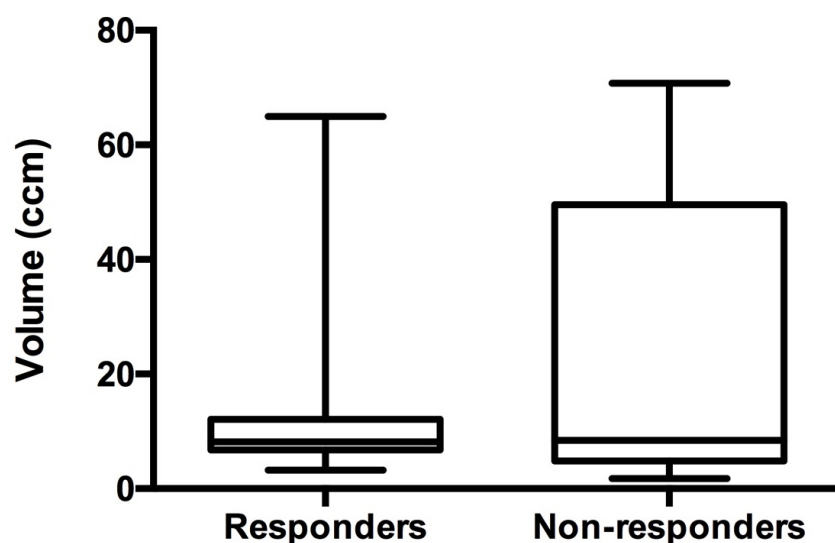


Figure 16: Box plot of tumour volumes in responder and non-responder groups at TPO (pre treatment). No significant difference was observed in baseline tumour volumes between the two groups ($p = 0.644$).

After the final cycle of chemotherapy, MRI estimated tumour volumes in the responder group ranged from 0 to 4.3 cm^3 (mean 1.1 cm^3 , SD ± 1.6) and in the non-responder group from 0.4 to 27.0 cm^3 (mean 6.3 cm^3 , SD ± 11.6). No significant

difference was observed between the final tumour volume of responders and non-responders ($p=0.124$) (Figure 17).

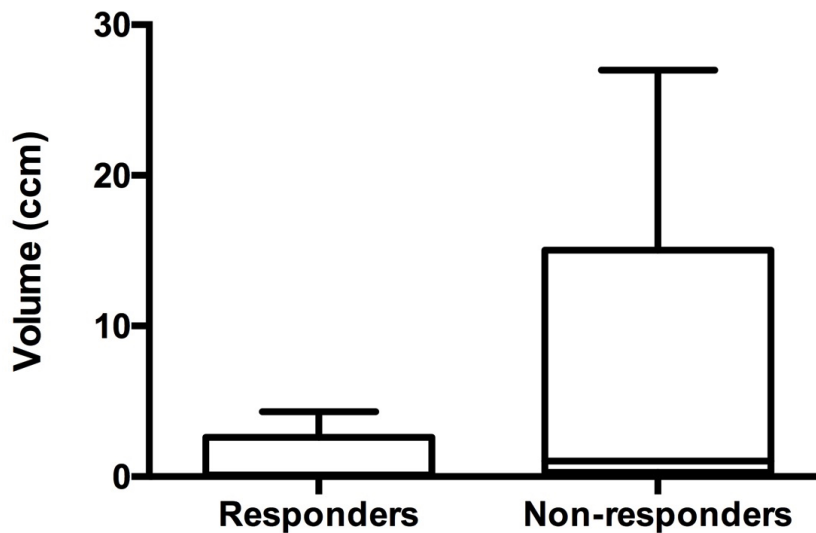


Figure 17: Box plot of tumour volumes in responder and non-responder groups at TP3 (post treatment). No significant difference was observed in the final tumour volumes between the two groups ($p = 0.124$).

Post chemotherapy tumour volumes in the responder group significantly reduced from pre-treatment volumes ($p<0.001$) (Figure 18) while a statistically significant reduction was not demonstrated in the non-responder group ($p=0.095$) (Figure 19). The percentage reduction in tumour volume of responders from TP0 to TP3 (mean = 92.0%, SD $\pm$ 13.0%) did not significantly differ from the reduction detected in the non-responder group (mean = 82.4%, SD $\pm$ 14.1) ($p=0.078$) (Figure 20). Conversely, when the patients who achieved a Miller Payne grade 3 response (30-90% reduction in tumour cells) were removed from the responder group, a statistically significant difference in percentage tumour volume reduction was then observed between responders (MPG 4&5) and non-responders (MPG 1&2) ($p=0.018$) (Figure 21).

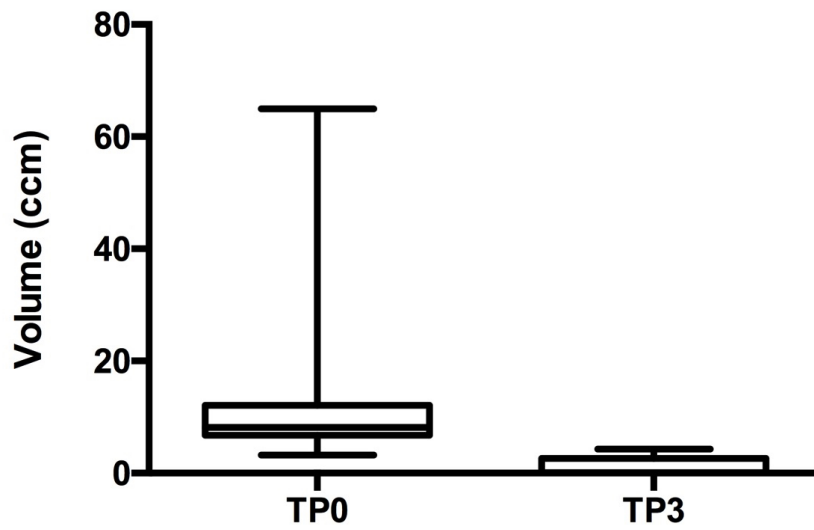


Figure 18: Box plot of tumour volumes of responders at TP0 and TP3. Tumour volumes in the responder group significantly reduced between the pre- and post-treatment MRI scans ($p < 0.001$).

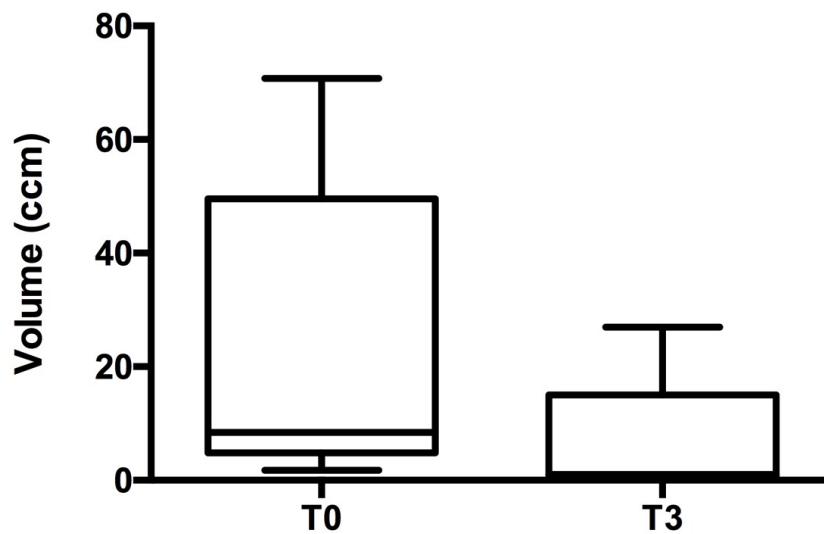


Figure 19: Box plot of tumour volumes of non-responders at TP0 and TP3. While tumour volumes in the non-responder group reduced between the pre- and post-treatment MRI scans, the reduction did not reach statistical significance ($p = 0.095$).

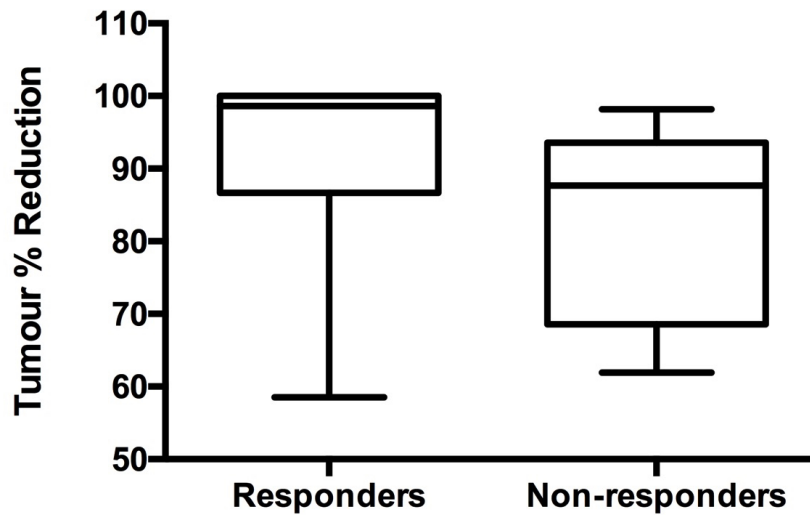


Figure 20: Box plot of percentage tumour reduction between TP0 and TP3 in responder and non-responder groups. No significant difference was observed between the percentage reductions in the two groups ($p=0.078$).

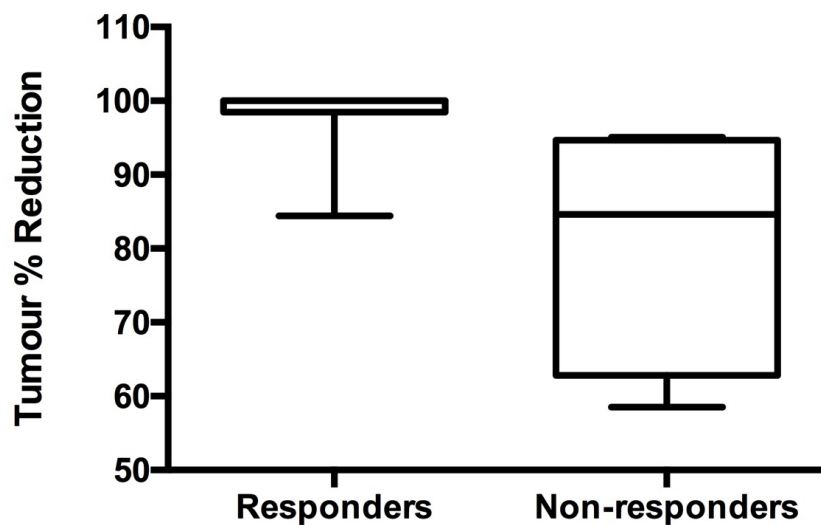


Figure 21: Box plot of percentage tumour reduction between TP0 and TP3 in responder (MPG 4&5) and non-responder (MPG 1&2) groups. When the MPG3 responders were removed from the responder group, a statistically significant difference was then observed between percentage tumour volume reduction in responders and non-responders ($p=0.018$).

CHAPTER 4: DISCUSSION

SECTION 4.1: PRE-TREATMENT ADC VALUES

4.1.1 NORMAL FIBROGLANDULAR BREAST TISSUE

Several studies have attempted to establish a normal range for ADC values of fibroglandular breast tissue. Reported mean ADC values of normal breast tissue vary widely from 1.33×10^{-3} to 2.09×10^{-3} mm²/sec (with maximum b-values ranging from 600 to 1000 sec/mm²) [150-154]. In our study, mean ADC of normal breast tissue (1.75×10^{-3} mm²/s, SD $\pm$ 0.26) fell within the reported range. Hormonal fluctuation can affect normal breast ADC values with studies demonstrating differences in ADC between the luteal and follicular phases of the menstrual cycle [155], and also between pre-menopausal and post-menopausal breast tissue [153]. Breast parenchymal ADC values have also been shown to increase with increasing mammographic density [156].

4.1.2 DIFFERENTIATING FIBROGLANDULAR BREAST TISSUE AND MALIGNANCY

Despite the large range of reported normal breast ADC values, almost all studies are in agreement that ADC values of malignant breast lesions are lower than those of normal breast tissue [61, 152, 154, 157]. Our results validate this well reported and consistent finding with a threshold for ADC of 1.13 mm²/s providing 100% sensitivity and 100% specificity in the differentiation of normal and malignant tissue. ADC has also shown to be useful in the differentiation of benign and malignant breast lesions. A meta-analysis of 13 studies evaluating the diagnostic performance of quantitative breast DWI in 964 lesions (615 malignant and 349

benign) demonstrated pooled sensitivity of 84% (95% CI: 82 - 87) and specificity of 79% (95% CI: 75 - 82) [158]. Malignant lesion mean ADC ranged from $0.87 - 1.36 \times 10^{-3} \text{ mm}^2/\text{s}$. Recommended threshold ADC cutoffs to differentiate benign from malignant lesions varied from 0.90 to $1.76 \times 10^{-3} \text{ mm}^2/\text{s}$.

Experimental evidence is lacking as to why malignant tumours have lower ADC values but it is probably related to increased cell density, disrupted tissue architecture and increased extracellular space tortuosity, all contributing to the reduced motion of water [159]. Increased cell density being a causative factor is further supported by studies demonstrating that breast tumour cellularity is inversely correlated to tumour ADC [110, 137].

4.1.3 RESPONSE PREDICTION

A number of studies have reported lower tumour ADC at baseline to be associated with improved treatment response [116, 121-123]. It is postulated that tumours with lower ADC values are better perfused and contain fewer areas of micro- and macro-necrosis, allowing chemotherapy to reach and affect a larger number of cells [121]. In the current study, no significant difference was identified between the baseline tumour ADC values of responders and non-responders which is in agreement with several other studies that did not find pre-therapy tumour ADC useful for response prediction [114, 119, 124, 125]. Three studies reported pretreatment ADC not capable of predicting response in the overall population of patients but found an ameliorated diagnostic performance was observed in the triple negative phenotype subgroup [160-162]. None of the patients included in our study had a triple negative breast tumour phenotype.

A meta-analysis of DWI in identifying breast cancer response to NACT, performed a subgroup analysis to assess pre-treatment ADC as a response predictor [163]. The pooled sensitivity from 6 studies of pre-therapy ADC to predict response was 90% (95% CI: 74 – 96) and the specificity was 63% (95% CI: 52 – 73). Pre- therapy ADC did not perform better than the change in ADC values during chemotherapy as a response predictor ($p=0.027$) which had a specificity of 80% (95% CI: 71 – 87).

SECTION 4.2: PRE-TREATMENT FA VALUES

4.2.1 NORMAL FIBROGLANDULAR BREAST TISSUE

Normal breast tissue has been shown to demonstrate a degree of anisotropic diffusion and although the explanation for this is unproven, it has been attributed to the unique structure of the breast where fibroglandular tissue is orientated radially along ducts and Cooper's ligaments [126, 131]. It is hypothesised that diffusion parallel to the walls of the ducts and lobules is close to that of free diffusion but perpendicular to the walls it is restricted [129]. Reported mean FA values of normal breast tissue vary widely from 0.09 to 0.5 (with maximum b-values ranging from 500 to 1000 sec/mm²) [126, 127, 131, 135, 142]. Mean FA of normal breast tissue in our study (0.14) fell within the reported range. Unlike ADC values, fibro-glandular composition [164] and hormonal fluctuations [130] have not been shown to influence the FA values of normal breast tissue.

4.2.2 DIFFERENTIATING NORMAL FIBROGLANDULAR BREAST TISSUE FROM MALIGNANCY

It is proposed that malignant transformation causes blockage of the ducts by proliferating neoplastic cells and loss of structured organization, resulting in a concomitant reduction of anisotropy [165]. Indeed, several studies support this hypothesis by demonstrating significantly lower FA values in malignant lesions relative to normal breast tissue [132, 135, 138, 139]. Nevertheless, there are conflicting studies that are in agreement with our own study, reporting no significant difference between the FA values of tumour and normal breast tissue. Anisotropy indices (including FA) are derived from various combinations of the diffusion eigenvalues λ_1 , λ_2 , and λ_3 of a diagonalized symmetric diffusion tensor. These anisotropy indices are normalized to reduce the sensitivity of the anisotropy measurement to experimental noise [166]. When the diffusion coefficients change markedly, the values of the normalized indices fail to serve as absolute anisotropy indicators, which may explain the conflicting results on the efficiency of FA to detect breast cancer [136]. Proposed reasons that may in part account for the variable findings are differences across studies in acquisition protocols, patient cohort, and lesion characteristics although these remain unproven.

The reported range of FA values for malignant breast lesions varies widely between 0.15 and 0.55 (with maximum b-values ranging from 600 to 1000 sec/mm^2)[127, 136, 140, 141, 167]. The mean FA value of tumours in our study is just below this range (0.139) while the mean FA of normal tissue in our study (0.14) falls within but towards the lower end of the reported range. This may be due to our acquisition protocol that used a relatively low echo time (TE), which reduces the time available for diffusion to occur. The choice of TE is a compromise between higher signal-to-noise ratio (SNR) (lower TE) and improved sensitivity to restriction of water

diffusion (higher TE) [136]. Furham-Haran et al. demonstrated a reduction in the average FA of normal breast tissue from 0.31 to 0.24 when the TE was reduced from 120msec to 90msec [136]. The TE time used in the current study was 59msec.

While not the focus of this study, it may be worth mentioning that there have also been conflicting reports regarding the ability of FA to differentiate benign and malignant breast lesions. While several reports are in agreement that FA is not useful in discriminating benign and malignant lesions [126-128, 132, 138], there are a small number of studies that refute this, demonstrating significant differences in the FA values of benign and malignant lesions [135, 137, 140].

4.2.3 RESPONSE PREDICTION

In the current study, pre- treatment FA tumour values were not found to be useful in the differentiation of responders from non-responders (mean of responders = 0.139, mean of non-responders = 0.14). This finding is in agreement with two other studies that have investigated the potential of FA to predict response in patients receiving NACT for breast cancer [144, 145].

SECTION 4.3: CHANGES IN DIFFUSION PARAMETERS WITH NACT

4.3.1 ADC VALUES AS AN EARLY RESPONSE PREDICTOR

Chemotherapy induces cell lysis, apoptosis, and necrosis with resultant disruption of cell membranes and decrease in cell density, thus allowing water molecules to diffuse more freely [113]. It is reasonable to expect such changes would be measurable prior to macroscopic changes in tumour size or morphology.

Therefore, it has been hypothesized that increase in tumour ADC values would precede changes in tumour volume during NACT and might reflect favorable treatment response. In support of this, several studies have shown increases in tumour ADC after the first cycle of chemotherapy which can differentiate responders from non-responders [61, 114, 116, 117]. Similar findings were demonstrated in our study with a significant increase in tumour ADC in the responder group after the first and second cycles of chemotherapy whilst no significant change occurred in the non responder group. In our study, the optimal timepoint to detect response was after the first cycle of chemotherapy. ROC analysis showed a percentage increase in tumour ADC of 7.7% had a sensitivity and specificity of 100% (AUC 1). After the second cycle of chemotherapy, the optimal percentage increase in ADC for predicting response was 21.4%, yielding 100% sensitivity and 64% specificity (AUC 0.91). While our findings and many others support the use of ADC as a biomarker of treatment response, a small number of studies have not found this association [67, 119, 120].

A recent meta-analysis concluded that quantitative DCE and DW MRI could predict the eventual response of breast tumours to NACT, with high specificity and sensitivity [168]. A random effects model, performed as part of the meta-analysis, examined differences between 10 studies in terms of patient population (patient age, tumour phenotype) and imaging protocol (1.5T versus 3.0T, temporal resolution of DCE-MRI, cycle of NACT). This highlighted a high degree of heterogeneity in published studies and found that the type of MRI performed and the time between the start of NACT and MRI significantly influenced both sensitivity and specificity. This high degree of heterogeneity may in part account for differences in published findings.

4.3.2 POST CHEMOTHERAPY ADC VALUES

In our study, no significant difference was observed in tumour ADC values between the responder and non-responders groups after the final cycle of chemotherapy. A statistically significant increase between pre and post treatment tumour ADC was demonstrated in both responders and non-responders and while a greater increase was seen in the ADC values of the responder group (mean 27.6% versus 13.0%), this difference did not reach statistical significance. When taking these results into consideration, it is important to be aware that ADC values were not acquired in 11 of the 15 patients in the responder group. In 5 patients who achieved a complete pathological response, no enhancing abnormality was identified on the DCE images and no area of diffusion restriction was identified at the previous tumour site on DWI. In 6 of the patients in the responder group, the volume of residual enhancing tumour was so minimal that accurate regions of interest could not be drawn on the corresponding ADC map. The small sample size in the TP3 responder group (n= 4) is likely to have affected our results. Previous studies have demonstrated a significant difference between post treatment tumour ADC values in responders and non-responders [123, 161]. An association has also been demonstrated between pre and post treatment ADC change and response with responders having a greater increase than non-responders [121, 169]. However, in at least two of these studies, when no residual tumour was visible after NACT, regions of interest were drawn on what was considered to be normal residual breast parenchyma at the site of the previously identified tumour [161, 169].

4.3.3 FA VALUES AS AN EARLY RESPONSE PREDICTOR

The present study evaluated FA as a biomarker of treatment response in a cohort of patients with breast cancer undergoing NACT. With previous studies demonstrating significant differences between FA values of normal breast tissue and malignancy, the hypothesis under investigation in the current study was whether disruption of normal breast architecture by tumour would be reflected by changes in diffusion anisotropy as more normal architecture was restored following chemotherapy. After one cycle of chemotherapy, a non-significant increase in FA was observed in the responder group that reached significance after the second cycle of chemotherapy. No significant increase was demonstrated in the non-responder group at either time point. Despite the significant rise in FA after two cycles of chemotherapy in the responder group, the percentage change in tumour FA did not significantly differ between the two groups, precluding its use as a response discriminator.

We identified one other study in the published literature investigating the potential of DTI-derived parameters for predicting response early in the course of treatment [145]. Wilmes et al. performed diffusion tensor MRI at 1.5T on 34 patients receiving NACT for breast cancer at three time points; pre NACT, after 3 cycles of chemotherapy and after completion of NACT (before surgery). This study demonstrated a weak association between early percent change in tumour FA and pathological complete response, and a significant correlation with early percent change in FA and final tumour volume change. The authors concluded that the significant correlation between early percent change in FA and final tumour volume suggests that FA may change with therapy and merits further evaluation.

Our study differs from Wilmes et al. in that our data was acquired with 3T MRI with 30 diffusion gradient directions (versus 1.5T MRI with 6 diffusion directions). Collecting the diffusion data with an increased number of directions reduces the sampling direction bias and provides SNR gain [170, 171]. We used a much earlier post-treatment time-point (TP1) in the hope that the rapid change in ADC value that has been previously reported as a predictor of response (and which was validated in our study) would be mirrored by a parallel early increase in FA value. Although mean FA values at TP1 (2 weeks after first cycle of therapy) had increased in our study, the result was not statistically significant. Also, the sequence of chemotherapy agents used (and therefore the timing of scans) differed between our and Wilmes's study. Furthermore, unlike Wilmes who calculated ADC values (or mean diffusivity) from DTI data, we used separate, dedicated acquisitions to measure ADC (DWI-derived) and FA (DTI-derived). One reason for the improved performance of ADC over FA as a biomarker of treatment may be related to the lower reported within-subject coefficient of variation of ADC (4.5%) compared to FA (11.4%)[131].

4.3.3 POST CHEMOTHERAPY FA VALUES

As was the case for ADC values at TP3, FA values were not acquired in 11 of the 15 responders in our study for the same reason of tumour invisibility. The limited number of included patients in responder group at TP3 (n = 4) should be taken into consideration when interpreting results regarding this time point. After the final cycle of chemotherapy, we found no significant difference between tumour FA values in responders and non-responders nor did we find a difference in FA values between TP0 and TP3 in the two groups. A significant FA percentage change between

TP0 and TP3 occurred in responders, which was not demonstrated in non-responders, but a comparison of the percentage change of FA values between the two groups showed no significant difference to allow a distinction of response. Despite performing an MRI after the final cycle of chemotherapy, Wilmes et al. did not refer to post treatment tumour FA values, focusing only on FA change early in the course of chemotherapy [145]. Furman-Haran et al. performed 1.5T MRI, pre and post NACT in 20 patients with breast cancer, to assess the ability of DTI-derived parameters to monitor treatment response [144]. They did not find FA to be a useful parameter in the differentiation of responders from non-responders at baseline or after completion of chemotherapy.

SECTION 4.4: TUMOUR VOLUME

DCE imaging was performed pre and post chemotherapy to delineate tumour extent and, in conjunction with the highest available b value image, assist in the accurate placement of tumour regions of interest on ADC and FA maps. DCE imaging was not acquired at TP1 and TP2 as it was deemed unnecessary for tumour identification. The acquisition of DCE imaging pre and post chemotherapy provided an opportunity to calculate tumour volumes at these time points, through the use of a semi-automated segmentation algorithm based on thresholding of the enhancement curve.

In a study of 162 patients receiving NACT for breast cancer, Hylton et al. reported final tumour volume and change in tumour volume from baseline to be a strong predictor of response and recurrence free survival [172]. In the current study, while post chemotherapy tumour volumes significantly reduced from pre-treatment

volumes in the responder group only, the percentage reduction in tumour volume of responders did not significantly differ from the reduction detected in the non-responder group. Nevertheless, when the patients who achieved a Miller Payne grade 3 response (30-90% reduction in tumour cells) were removed from the responder group, a statistically significant difference in percentage tumour volume reduction was then observed between responders and non-responders. The large range of percentage cellular reduction encompassed in the Miller Payne 3 category may mean that patients with a tumour cell reduction closer to that of the non-responders could have contributed to the failure of tumour volume change to reach significance in differentiating the entire responder cohort from non-responders initially.

SECTION 4.5: STUDY LIMITATIONS

The main limitation of this study is the small sample size, in particular the small number of patients in the non-responder group. Because of aforementioned higher within-subject coefficient of variation for FA over ADC (11.4% vs. 4.5%)[131], the small sample size may have disproportionately affected changes in FA. A further limitation is the histological cancer subtype was not homogenous, although most had invasive ductal carcinoma. Because of the small sample size, subgroup analysis of the distinct cancer subtypes was not possible.

The selection of a low TE reduced the time available to detect anisotropy as only part of the water molecules in most ducts will be fully restricted during the chosen time. Nevertheless, selecting an optimal echo time is a compromise between signal-to-noise ratio and sensitivity to restriction. We feel the chosen time allowed

sufficient time to detect diffusion anisotropy while providing a good signal to noise ratio to allow accurate calculation of FA values.

The measurements for normal fibro-glandular tissue were from disease free areas of the tumour-containing breast, a technical limitation as DWI and DTI owing to data acquisition in the sagittal plane, which excluded the asymptomatic breast from data acquisition. Nevertheless, we are confident that our ROIs of normal breast are appropriate as we paid close attention to contrast-enhanced and diffusion-weighted imaging to avoid areas with tumour.

CONCLUSIONS

Breast cancer is a heterogeneous disease that requires tailored treatment strategies. The development of targeted therapies relies on biomarkers that can be used to assess the effectiveness of agents, early and accurately. Functional MRI provides an opportunity to detect alterations in the cellular environment early in the course of chemotherapy.

Our results validate the well-reported and consistent finding that pre-treatment ADC values of tumour are significantly lower than that of normal fibro-glandular breast tissue, which has been attributed to high tumour cellularity within malignant lesions. Additionally, our findings concur with several previously published studies supporting the use of ADC as a biomarker of treatment response in breast cancer as we observed that tumour ADC significantly increased after the first and second cycles of chemotherapy in patients who ultimately had a good response to chemotherapy. This increase was statistically significant between responders and non-responders highlighting the potential of ADC to differentiate responders from non-responders early during treatment. The optimal time point to discriminate responders from non-responders is after the first cycle of chemotherapy with a percentage increase cut off in tumour ADC values of 7.7%.

Nevertheless, there are a small number of studies that have not shown an association between an increase in tumour ADC and treatment response. These disparate findings may be attributable to study design factors including differences in DWI acquisition and analysis, chemotherapy regimens, image timing and/or patient populations (including differing cancer subtypes). In order to rectify this, a large multi-institutional clinical trial (ACRIN 6698) is ongoing, which aims to determine if

changes in ADC values are predictive of pathological response in patients treated with NACT in the I-SPY 2 TRIAL.

DTI of the breast is a relatively new concept with a small but growing number of studies investigating its potential as a clinical tool. In the current study, no significant difference was demonstrated between FA values of normal breast tissue and tumours. A significant increase in FA values was observed in the responder group after the second cycle of chemotherapy that did not occur in the non-responder group. Nevertheless, the percentage change in tumour FA did not significantly differ between responders and non-responders, precluding its use as a response discriminator. Given the variability of results from studies focusing on DTI of the breast, further studies are warranted to investigate its' potential as a diagnostic and response-monitoring tool. Improvements in breast coil design, fat suppression techniques and EPI may enhance the performance of DTI of the breast. In conclusion, the present study reaffirms ADC as an important tool in the early differentiation of responders from non-responders in patients receiving NACT for breast cancer, but, whilst tumour FA significantly increased in the responder group after two cycles of chemotherapy, its value as an early response discriminator was not demonstrated.

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