Title
Diffusion-Weighted Magnetic Resonance Imaging in Monitoring Treatment Response in Patients with Locally Advanced Breast Cancer

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Publication Date
2010

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Diffusion-Weighted Magnetic Resonance Imaging in Monitoring Treatment Response in Patients with Locally Advanced Breast Cancer

by

Lisa Singer

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Bioengineering

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

AND

UNIVERSITY OF CALIFORNIA, BERKELEY
Acknowledgements

This dissertation was made possible through the help and support of so many people. My dissertation committee – Nola Hylton, Ruzena Bajcsy, Bonnie Joe, Mark Moasser, and Tejal Desai – supported me in my research. Other committee members were John Shepherd and Sabrina Ronen and I am thankful for their advice. My research advisor, Nola Hylton, welcomed me into the lab, provided helpful feedback and advice throughout the project, and helped the research move in new directions. I also want to thank the members of our lab, my professors at UCSF and Berkeley, and my mentors and advisors in the School of Medicine, MSTP, and Bioengineering. My first research projects helped me continue my interest in research and I want to thank my coworkers in my previous labs. I am also grateful for my undergraduate advisors and professors who supported my graduate school pursuits. I feel very grateful to have been able to work on this project.

Many chapters in this dissertation would not have been possible without the efforts of other researchers, and in addition to my research advisor, I want to acknowledge Savannah Partridge for her contribution to Chapter 3, as well as other projects; Jessica Gibbs for her contributions to Chapter 3; Catherine Klifa for her contributions to Chapter 7, as well as other image processing projects; Lisa Wilmes, Emine Saritas, Ajit Shankaranarayan, Evelyn Proctor, Dorota Wisner, Belinda Chang, Bonnie Joe, and Dwight Nishimura for contributions to Chapter 11.

For many of the studies analyzed in this dissertation, the imaging data was obtained through multicenter collaborations, led by ACRIN and other organizations. This research was made possible by the Department of Bioengineering, the Department of Radiology and Biomedical Imaging, and patients, physicians, and researchers in Breast Imaging and the Breast Care Center at UCSF. This research includes analysis of studies occurring in the lab, and at UCSF, since the 1990s, and would not have been possible without the patients who participated in these studies. This research was funded by the California Breast Cancer Research Program Dissertation Award, the Department of Bioengineering at UCSF, and the Medical Scientist Training Program. Funding was also provided by NIH R01 CA069587-13, NIH R01 CA116182-05, and past funding included the DAMD 17-96-C-6126.

I want to thank my friends and family. Many thanks to my mom and dad, and my brother and sister.

To everyone who helped me along the graduate school pathway, thank you!!
Diffusion-Weighted Magnetic Resonance Imaging in Monitoring Treatment Response in Patients with Locally Advanced Breast Cancer

Lisa Singer

Breast cancer is the most common non-skin cancer in women and the second leading cause of cancer death in women in the United States. Effective chemotherapy can potentially treat micrometastases as well as the primary tumor, reducing the risk of metastasis later in the lifespan and improving overall survival. Chemotherapy implemented prior to surgery (neoadjuvant chemotherapy) allows for the additional benefits of 1) potential tumor down-staging and the option of breast-conserving surgical methods and 2) the ability to assess the tumor's response to chemotherapy while the tumor is still present. Currently, it is difficult to predict tumor response to chemotherapy and effective methods of monitoring tumor response to treatment are needed.

Diffusion-weighted magnetic resonance imaging (DW-MRI) is sensitive to the random motion of water molecules, allowing for macroscopic detection of microscopic changes in cell density and cell content. This research focused on the use of DW-MRI in the monitoring of diffusion within the tumor during the course of treatment with neoadjuvant chemotherapy. In retrospective studies, the ability of DW-MRI measurements to predict patient outcomes was assessed. Problems related to the DW-MRI acquisition were identified and a technical solution was optimized for use in patients with breast cancer. Methods for improved DW-MRI processing were developed. Promising technical and processing innovations were evaluated in prospective clinical studies.

In retrospective studies, results from limited patient numbers suggested that DW-MRI measurements may predict long-term patient outcomes. Multiple barriers to DW-MRI analysis were identified, including image quality and the time needed for manual delineation of tumor regions of interest. Use of a new sequence resulted in improved image quality, quantitative differences in measurements related to the tumor apparent diffusion coefficient (ADC) distribution, and could allow for diffusion to be more accurately monitored throughout treatment. New segmentation methods were developed and could improve the efficiency of DW-MRI processing.

In this work, diffusion was identified as a marker of long-term patient outcomes and developments in image acquisition and image processing showed promise in further improving the ability to measure tumor diffusivity and monitor changes throughout treatment with neoadjuvant chemotherapy. Validation in large, prospective studies is needed.
# Table of Contents

Chapter Organization .......................................................................................................... 1

Part I: Background ........................................................................................................... 4

Chapter 1. Breast Cancer ................................................................................................ 5
1.1. Incidence .............................................................................................................. 5
1.2. Risk ....................................................................................................................... 6
1.3. Etiology ................................................................................................................ 9
   Unregulated growth .................................................................................................. 10
   Evasion of apoptosis ................................................................................................. 11
   Angiogenesis ............................................................................................................. 12
   Invasion and metastasis............................................................................................. 13
1.4. Breast Anatomy .................................................................................................. 14
1.5. Breast Cancer Diagnosis .................................................................................... 18
1.6. Breast Cancer Staging ........................................................................................ 19
1.7. Breast Cancer Treatment .................................................................................... 21
1.8. Chemotherapy Response .................................................................................... 24
1.9. Personalized Medicine for Breast Cancer .......................................................... 26
1.10. References ...................................................................................................... 28

Chapter 2. MRI Acquisition, Processing, and Data Analysis ....................................... 31
2.1. Introduction to Medical Imaging ........................................................................ 32
   Energy in Imaging ..................................................................................................... 33
   Resolution ................................................................................................................. 35
2.2. Basis of Magnetic Resonance Imaging .............................................................. 36
2.3. Steps in MRI Acquisition ................................................................................... 38
   Nuclear magnetization .............................................................................................. 38
   Excitation .................................................................................................................. 38
   Relaxation ................................................................................................................. 39
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal Measurement</td>
<td>44</td>
</tr>
<tr>
<td>Image Reconstruction</td>
<td>44</td>
</tr>
<tr>
<td>2.4. MRI Pulse Sequence</td>
<td>45</td>
</tr>
<tr>
<td>2.5. MRI Parameters</td>
<td>45</td>
</tr>
<tr>
<td>2.6. Contrast-enhanced MRI</td>
<td>50</td>
</tr>
<tr>
<td>Description</td>
<td>50</td>
</tr>
<tr>
<td>DCE-MRI Data Processing</td>
<td>52</td>
</tr>
<tr>
<td>2.7. Diffusion-Weighted MRI</td>
<td>57</td>
</tr>
<tr>
<td>Introduction to DW-MRI</td>
<td>57</td>
</tr>
<tr>
<td>History</td>
<td>57</td>
</tr>
<tr>
<td>Diffusion in Tumors</td>
<td>59</td>
</tr>
<tr>
<td>Measuring Diffusion with DW-MRI</td>
<td>62</td>
</tr>
<tr>
<td>Processing</td>
<td>65</td>
</tr>
<tr>
<td>2.8. Image Processing and Analysis</td>
<td>69</td>
</tr>
<tr>
<td>Regions of interests</td>
<td>69</td>
</tr>
<tr>
<td>Histogram Analysis</td>
<td>71</td>
</tr>
<tr>
<td>2.9. Statistics</td>
<td>75</td>
</tr>
<tr>
<td>2.10. Imaging Studies at UCSF</td>
<td>78</td>
</tr>
<tr>
<td>2.11. References</td>
<td>81</td>
</tr>
</tbody>
</table>

**Part II: Retrospective Study**

<table>
<thead>
<tr>
<th>Chapter 3. Diffusion as a Predictor of Treatment Response</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1. Introduction</td>
<td>86</td>
</tr>
<tr>
<td>3.2. Materials and Methods</td>
<td>89</td>
</tr>
<tr>
<td>Overview</td>
<td>89</td>
</tr>
<tr>
<td>Patients</td>
<td>89</td>
</tr>
<tr>
<td>Imaging</td>
<td>90</td>
</tr>
<tr>
<td>Image Processing and Analysis</td>
<td>92</td>
</tr>
<tr>
<td>Statistics</td>
<td>92</td>
</tr>
<tr>
<td>3.3. Results</td>
<td>93</td>
</tr>
<tr>
<td>Patient accrual and follow-up</td>
<td>93</td>
</tr>
</tbody>
</table>
Primary outcome: Recurrence free survival ............................................................. 97
Secondary outcome: Volumetric tumor response ............................................... 100
Selected patient cases ...................................................................................... 100

3.4. Discussion .................................................................................................. 102
3.5. Conclusions .............................................................................................. 105
3.6. References ................................................................................................ 106

Part III: Technical Developments ..................................................................... 108

Chapter 4. Fat Suppression: Quantitative and Qualitative Analysis of Fat Suppression in DW-MRI 109
4.1. Introduction ................................................................................................ 109
4.2. Materials and Methods .......................................................................... 111
Patients ............................................................................................................ 111
MR Imaging ....................................................................................................... 111
Image Processing .............................................................................................. 111
Image Analysis ................................................................................................. 112
Statistics .......................................................................................................... 114
4.3. Results ..................................................................................................... 115
Quantitative Analysis ....................................................................................... 115
Qualitative Analysis ......................................................................................... 119
4.4. Discussion ................................................................................................ 122
4.5. Conclusion ................................................................................................ 125
4.6. Acknowledgements: ............................................................................... 126
4.7. References ................................................................................................ 126

Chapter 5. Other Parameters: Sequence Type and Treatment Protocol .......... 127
5.1. Introduction .............................................................................................. 127
5.2. Materials and Methods .......................................................................... 129
Overview of research design .......................................................................... 129
Sequence type .................................................................................................. 129
Chemotherapy protocol .................................................................................. 132
5.3. Results ..................................................................................................... 134
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.3</td>
<td>Results</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>Comparison of new vs. manual technique</td>
<td>181</td>
</tr>
<tr>
<td>7.4</td>
<td>Discussion</td>
<td>183</td>
</tr>
<tr>
<td>7.5</td>
<td>Conclusion</td>
<td>189</td>
</tr>
<tr>
<td>7.6</td>
<td>Acknowledgements</td>
<td>190</td>
</tr>
<tr>
<td>7.7</td>
<td>References</td>
<td>190</td>
</tr>
</tbody>
</table>


| 8.1     | Introduction                                                         | 193  |
| 8.2     | Materials and Methods                                                | 195  |
|         | Patients                                                             | 195  |
|         | Imaging acquisition                                                  | 196  |
|         | Image processing and analysis                                         | 196  |
|         | Statistics                                                           | 198  |
| 8.3     | Results                                                              | 199  |
|         | Patient Accrual                                                      | 199  |
|         | Quantitative Analysis                                                | 199  |
|         | Qualitative Analysis                                                 | 203  |
| 8.4     | Discussion                                                           | 206  |
| 8.5     | Conclusions                                                          | 212  |
| 8.6     | Acknowledgements                                                     | 212  |
| 8.7     | References                                                           | 213  |

Chapter 9. Heterogeneity in Tumor Response to Treatment 215

| 9.1     | Introduction                                                         | 215  |
| 9.2     | Materials and Methods                                                | 218  |
|         | Patients                                                             | 218  |
|         | Imaging                                                              | 218  |
|         | Image Processing                                                     | 219  |
|         | Statistical Analysis                                                 | 221  |
Chapter 10. Tumor Segmentation: Comparison of Different Methods for Defining Breast Cancer Regions on DW-MRI

10.1. Introduction .................................................................................................. 236
10.2. Materials and Methods ................................................................................. 237

Patients .................................................................................................................... 237
Imaging ................................................................................................................... 238
Image Processing .................................................................................................... 239
Image Analysis ........................................................................................................ 239
Method Comparisons and Statistics: ....................................................................... 244

10.3. Results .......................................................................................................... 244

Patient accrual ......................................................................................................... 244
Quantitative Analysis .............................................................................................. 244
Qualitative Analysis ................................................................................................ 248
Case studies ............................................................................................................. 250

10.4. Discussion ..................................................................................................... 252
10.5. Conclusion .................................................................................................... 258
10.6. References .................................................................................................... 258

Chapter 11. High-Resolution DW-MRI of Invasive Breast Cancer

11.1. Introduction .................................................................................................. 261
11.2. Materials and Methods .................................................................................. 264
Appendices........................................................................................................................................ 303

Appendix 1: Breast Cancer Staging............................................................................................... 304

Appendix 2: Patients with Locally Advanced Breast Cancer Treated with Neoadjuvant
Chemotherapy and Scanned with MRI in Clinical Studies at UCSF................................. 306

Appendix 3: MR Parameters as Predictors for 3-year RFS....................................................... 307

Appendix 8.1. Histogram Analysis, Predictors of Recurrence-free Survival ...................... 310

Appendix 8.2. Histogram Analysis, Univariate Cox Regression ............................................. 313
Chapter Organization

The prevalence of breast cancer (Chapter 1) and the fact that current treatments are not always successful (Chapter 1) motivate this research (Figure 1). This research assesses and improves the ability of a particular type of imaging to predict response to treatment for breast cancer. Advances in image acquisition and image processing (Chapter 2) form the basis of the research method. Methods in statistics (Chapter 2) are used to evaluate these advances.

This research began with retrospective analysis of a pilot study at our institution (Chapter 3). Together with results from studies at other institutions, these prior studies suggest that diffusion-weighted MRI (DW-MRI) could be useful in predicting response to neoadjuvant chemotherapy.

Despite the fact that DW-MRI may be useful in predicting treatment response, not all studies have shown DW-MRI to be valuable. In the studies that have shown a value in DW-MRI, including the pilot study at our own institution, the ability to accurately measure diffusion is limited by available technology in imaging and image processing. This research sought to evaluate and expand available methods in image acquisition (Chapters 4-6) and in image processing (Chapter 7-10) for the purpose of monitoring response to neoadjuvant chemotherapy. Application of these new processing methods to prior studies has led to identification of associations between diffusion and patient outcomes that were previously not known. Applications of these acquisition and processing methods to future studies will allow for more complete assessment of
diffusion in patients and prospective validation of diffusion as a biomarker for treatment response. In Chapters 11-12, prospective clinical evaluation of a new DW-MRI acquisition method is presented.
Figure 1. Flowchart of chapter organization.
Part I: Background

The purpose of this work was to investigate the ability of diffusion to predict response to neoadjuvant chemotherapy in patients with locally advanced breast cancer. This research direction is motivated by the fact that breast cancer is not always successfully treated, and the epidemiology, etiology, and diagnosis and treatment of breast cancer are described in Chapter 1. In this work, diffusion is measured using magnetic resonance imaging and methods in image processing and methods in image acquisition, image processing, and statistics are described in Chapter 2. Underlying this work are principles in biology, medicine, physics, engineering, statistics, and related fields and these principles are introduced in Part I. These concepts both motivated and facilitated this research.
Chapter 1. Breast Cancer

1.1. Incidence

Breast cancer is a leading cause of death in the United States and around the world. Despite advances in the diagnosis and treatment of breast cancer, it is the most common non-skin cancer in women in the United States (Figure 1-1). It the second most common cause of cancer-related death in women, and the most common overall cause of death in women aged 45-55 (O'Leary, Tabuenca et al. 2008). One in eight women are expected to have breast cancer during their lifetime; when in situ breast cancers are considered in this statistic, the estimate increases to one in six (Costanza and Chen 2010).
Figure 1-1. Cancer Incidence. The most common types of cancer in men and women are listed. Estimates from (Jemal, Siegel et al. 2009).

1.2. Risk

Many factors are implicated in the etiology of breast cancer. Risk factors for disease can be considered modifiable or non-modifiable. Modifiable risk factors can be changed, allowing a person’s risk to be reduced; non-modifiable risk factors cannot be changed.

The most significant risk factors for breast cancer are non-modifiable: age and gender. Increased age and the female gender are associated with higher risk; however,
younger women develop breast cancer and in the United States, and approximately 1910 cases of breast cancer are diagnosed annually in men (Gradishar 2010).

In addition to age and gender, other non-modifiable risk factors are related to a person’s genetic make-up. Mutations in certain genes are associated with breast cancer risk. These inherited risks include mutations in the \textit{BRCA1}, \textit{BRCA2}, \textit{PTEN}, \textit{CHEK2}, and \textit{p53} genes. While having these mutations is not currently modifiable, the conferred risk can be modified with risk-reducing therapies. This is especially the case in \textit{BRCA1} and \textit{BRCA2} mutation carriers, where mastectomy may be suggested as a means to remove breast tissue, thereby reducing risk of breast cancer. Removal of ovaries (oopherectomy) may also be recommended, as this reduces estrogen production significantly in pre-menopausal women. Increased and earlier frequency of breast cancer screening provides a means to detect breast cancer earlier, thereby reducing the risk of breast cancer being diagnosed when it is also already late stage.

Earlier menarche, nulliparity, and use of hormone replacement therapies are also associated with increased risk. Some breast cancers grow in response to estrogen and this relationship between cancer and estrogen is an explanation for why these factors are associated with increased breast cancer risk. Hormone use is considered a modifiable risk factor. Other modifiable risk factors may be related to environmental exposures, although many of these risks are still being investigated. The relationship between alcohol and risk of breast cancer incidence is though to be linear: each 10 gram increase in daily alcohol use is associated with a relative risk of 1.11 for hormone receptor positive cancers (Mukamal 2010).
Another factor associated with breast cancer risk is breast density. Higher density is associated with increased risk; the highest breast density category is associated with a 2-6 times increased risk. High breast density impairs radiologists’ ability to interpret screening mammograms and identify dense masses beneath dense overlying tissue. High density may also be associated with increased risk because it may be associated with increased total breast tissue. Diet and exercise modify body fat, which impacts breast density; however, lowering breast density would require increasing body fat and density may therefore not be practically modifiable through lifestyle choices. Risk-reducing therapies such as tamoxifen may reduce breast tissue, lowering density; however, whether breast density can be modified to reduce risk is not yet known.

Taking all of these risk factors into account, as well as family history and personal medical history, an individual’s risk for breast cancer can be predicted. Many different prediction models are available, with some taking into account different factors than others. The Gale Model is a prediction model that is widely used in the United States and takes into account mainly personal history of hormone use and estrogen exposures (menarche, parity, menopause), and maternal family history of breast cancer; however, it does not account for other relatives with breast cancer. Other models such as the Claus and Frank models take into account more of a family’s history of breast cancer (Fletcher 2010). Models such as BRCAPRO are also available to predict the probability that someone has a mutation in the BRCA genes; BRCAPRO also utilizes familial models including the Claus and Frank (Fletcher 2010). BRCAPRO may be appropriate for someone considering genetic testing. Different models may lead to different risk calculations for the same individual. It is therefore necessary to make sure that the model
being used is appropriate for the individual. Recommendations for risk-reducing therapies and increased screening are often made based on whether someone’s risk is increased above a threshold amount as compared to the average individual. Because most treatments in medicine have pros and cons, risk should be carefully assessed with an appropriate model before risk-reducing therapies are implemented.

1.3. Etiology

Most cases of breast cancer are sporadic and not associated with a known inherited mutations or positive family history. When breast cancer occurs sporadically, it results from similar events that cause it to occur in those with a genetic mutation: genetic instability, loss of controls on cell growth, unregulated growth, and invasion of tissue leading to the mass that is identified as a breast cancer. Breast cancers, and all cancers, are thought to have six main properties: loss of anti-growth signals, gain of pro-growth signals, unlimited potential to replicate, evasion of apoptosis, angiogenesis, and invasion and metastasis (Hanahan and Weinberg 2000) (Figure 1-2). The six main properties of invasive cancer are discussed in more detail below. Not all cancers will acquire all six properties, and they will not necessarily be acquired in the above order.
Figure 1-2. Properties of Cancer. The central properties of cancers are shown. Diagram based on (Hanahan and Weinberg 2000).

The human body is carefully controlled, from organs, to tissues, to cells. The cell is the smallest functioning unit in the human body. The life of a cell is regulated by signals in the environment and by proteins within the cell. In cancer, the careful regulation of these proteins and signals is disrupted, manifesting as different properties that are characteristic of cancer. Cancer begins with molecular events: mutations in vital genes, encoding vital proteins that ensure normal cellular functioning.

**Unregulated growth.** Some proteins are important in promoting cell growth; others are important in inhibiting cell growth. The genes that encode the growth-promoting proteins are oncogenes; the genes encoding growth-inhibiting proteins are tumor suppressor genes. In cancer, the growth-promoting genes are often upregulated or become mutated such that they are no longer sensitive to their inhibitors and are therefore
more active (gain of pro-growth signals). The tumor suppressor genes are inactivated and unable to slow cell growth (loss of anti-growth signals). When oncogenes are activated and tumor suppressor genes are inactivated, the proteins they encode for may not function properly and when they cannot perform their intended functions, the careful regulation of the cell is disrupted, allowing for the unregulated cell growth and the unlimited potential to replicate that is hallmark of cancer.

Particular oncogenes (HER-2, EGFR) and tumor suppressor genes (p53, BRCA1, BRCA2, PTEN, Rb) are known to be implicated in breast cancer (Osborne, Wilson et al. 2004); similarly, particular mutations have been associated with other types of cancer (Table 1-1). When a cell has more mutations, it is less and less able to function normally, paving the way for accumulation of additional mutations.

**Table 1-1. Mutations in other types of cancer**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Associated mutation</th>
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</thead>
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<tr>
<td>Pancreatic cancer</td>
<td>SMAD 4</td>
</tr>
<tr>
<td>Small cell lung cancer</td>
<td>L-MYC</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>APC, K-RAS</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>PTCH</td>
</tr>
<tr>
<td>T cell acute lymphoblastic leukemia</td>
<td>NOTCH</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>RB</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>CYCLIN D</td>
</tr>
</tbody>
</table>

**Evasion of apoptosis**

During normal cellular functioning, cells divide, and this division requires DNA synthesis, during which mutation can occur. Mutations typically don’t have a deleterious effect on a person’s survival because the mutation is either repaired, or if it cannot be repaired and is potentially harmful to the cell, it may trigger apoptosis, or cell death. When a patient is treated with a chemotherapy drug that causes DNA damage, this
damage similarly sparks apoptosis, leading to cell death and subsequent tumor shrinkage. Cancers are successful at surviving despite mutations because of mutations in the apoptotic pathway, allowing cancer cells to develop mechanisms to avoid apoptosis. Just as oncogenes favor cell growth and tumor suppressor genes suppress it, two sets of proteins favor and suppress apoptosis. These proteins are pro-apoptotic and anti-apoptotic proteins, respectively.

**Angiogenesis**

Initially, cancers rely on diffusion of nutrients and oxygen for sustenance. Oxygen diffuses from the blood into the cancer cells, allowing the cells to continue to divide and grow. Once the cancer is further than .2 mm from a blood vessel, it is too far away to obtain adequate oxygen via diffusion (Weinberg 2007). Similarly, once a cancer grows to a certain size, some of its cells, often at the center, may be more than .2 mm from a blood vessel and the cancer is too large for all of its cells to be reached by diffusing oxygen. Without oxygen, the cancer cells are at risk of dying and these cancers must develop a new mechanism for obtaining oxygen. The mechanism is angiogenesis: the growth of new blood vessels. By promoting the growth of new vessels, cancers are able to once again obtain molecules needed for cell growth and division. The point at which a cancer acquires the ability to promote blood vessel growth is known as the “angiogenic switch.” The vessels that grow during tumor angiogenesis tend to be more morphologically and functionally different from normal vessels: they are more disorganized in arrangement, and leakier (Carmeliet and Jain 2000). This latter property is exploited in a certain type of imaging which will be described in the next chapter.
Invasion and metastasis

In order to colonize other areas of the body, cancer cells must leave their original location (local invasion), enter a new vessel that can transport them to a new location (intravasation), leave the vessel (extravasation), deposit in a new location, and thrive (metastasis). Except in the case of direct seeding, this process is generally thought to be largely dependent on a dramatic transformation in the genes that cancer cells expressed. This transformation is called the “epithelial-mesenchymal transition,” or EMT (Weinberg 2007).

All cells in the body contain the same genes, but they express different genes based on their location and their role in tissue functioning. The cells that make up most breast cancers are epithelial cells of the lobules or ducts. These cells express particular proteins that are hallmark of epithelial cells, and this expression profile gives the cells certain characteristics, such as a tendency to adhere to other similar cells, and to be less motile. After EMT, the cells express proteins that are hallmark of mesenchymal cells, cells that are more mobile and less adherent, ideal for invading tissue and traveling to new locations. In normal human development and wound healing, EMT takes place and this same process is utilized by cancer cells to spread.

A protease is a type of enzymatic protein that degrades other proteins and protease expression increases as cells locally invade. As proteins surrounding the cell are degraded by proteases (local invasion) and the cancer cells themselves acquire the ability to move more freely and disassociate from their neighboring cells, the cancer becomes increasingly able to leave its environment and enter a vessel (intravasation). Vessels carry the cells to new locations (transport), and the cells then exit the vessels (extravasation),
and deposit in new tissues (micrometastasis). For reasons that are not yet fully understood, some cancer cells are able to thrive in these new locations and multiply, developing a larger metastasis. These processes of invasion and metastasis account for 90% of cancer-related deaths (Weinberg 2007).

1.4. Breast Anatomy

The process of cancer invasion and metastasis was just described, in which cancer cells leave the location in which they first developed and travel to distant locations. In breast cancer, the cancer first develops in cells that are considered to be part of the breast. The anatomy of the breast is important and impacts the growth and spread of breast cancer, as well as the imaging, physical examination, and surgical methods targeted against it. Breast anatomy is briefly reviewed and clinical correlates to breast cancer presentation and treatment are discussed.

The breast is primarily a glandular organ, made up of lobules and ducts (Figure 1-5). Lobules secrete milk during lactation and ducts move this milk to the areola at the surface of the breast. The areola is made of keratinizing squamous epithelium (Donegan and Spratt 2002).

Paget’s disease, the presence of neoplastic cells in this region, manifests as an eczema-like dermatological condition and can be associated with underlying in situ or invasive breast cancer. It is present in 0.5-5% of all breast cancer cases (Sakorafas, Blanchard et al. 2001). Due to its appearance and rare incidence, Paget’s disease can be mistaken for common dermatological conditions such as eczema or atopic dermatitis (Sakorafas, Blanchard et al. 2001).
The breast contains approximately $10^4$ lobules, each made of two cell layers: cuboidal and myoepithelial layers. Lobules are only 0.3-0.6 mm in diameter, which means that they fall below the resolution of most magnetic resonance imaging acquisitions. Each breast contains approximately 10-15 ducts, which are made of 1-2 layers of columnar epithelial cells. Ductules have 2 cell layers: epithelial and myoepithelial cells. Ducts are 2-4 mm in size (Donegan and Spratt 2002). Ducts are considerably larger than lobules and within the resolution of an MRI acquisition; however, the large size of ducts also means that measurement of anisotropy within the ducts, described in the next chapter, is difficult.

Figure 1-3. Breast Anatomy. The structure of the breast is illustrated in a figure modified from ACS, “Breast Cancer,” www.cancer.org.

The breast is anterior to the chest wall. (According to the AJCC staging criteria (2010), the chest wall includes the ribs, intercostal and serratus anterior muscles.). The pectoral muscles are posterior to the breast, with nerve supply provided by the medial and
lateral pectoral nerves (Donegan and Spratt 2002). The lymph drainage of the breast traverses the pectoral muscles and breast cancer can invade these muscles. The fact that the breast is immediately anterior to the pectoral muscles also impacts imaging because fibroglandular and pectoral muscle tissue are iso-intense on magnetic resonance imaging, creating a challenge for breast segmentation. Breast tissue can communicate with the chest wall, creating additional difficulties in the manual delineation of the breast region on images (Figure 1-4); this is discussed in a later chapter on breast segmentation.

Figure 1-4. Chest Wall. The fact that the breast is anterior to the chest wall means that all axial slices in an MR volume will require segmentation from the chest wall. The similar intensities between breast and chest wall can be seen in the axial image at left.

The location of the breast anterior to the chest wall also means that magnetic resonance imaging may be affected by air-tissue interfaces medial, lateral, and anterior to both breasts, as well as posterior to the breast due to the location of the lungs. Air-tissue interfaces may cause problems in breast MRI and this is discussed in later chapters on technical developments. In an axial MRI acquisition, the left to right distance occupied by the breasts determines the in-plane imaging field of view and the longest diameter of
the breasts along the superior-inferior axis determines the field of view required in the slice select direction. These imaging parameters are discussed in the next chapter.

Breast tissue can extend laterally into the axilla. Regional lymph nodes draining the breast include axillary nodes. Removal of lymph nodes in breast cancer staging requires access to the axilla. The medial wall of the axilla is formed by the muscle serratus anterior, with nerve supply provided by the long thoracic nerve. Injury to this nerve, which could occur during breast cancer surgery, can result in a “winged scapula” and limited abduction (Donegan and Spratt 2002). The posterior wall of the axilla is formed in part by the latissimus dorsi, with nerve supply provided by the thoracodorsal nerve. Injury of this nerve could lead to trouble adducting, medially rotating, and extending the arm. The axilla is divided into upper, middle, and lower regions by the pectoral minor muscle, supplied by the medial pectoral nerve (Donegan and Spratt 2002).

The breast is typically the first site in which breast cancer is identified; however, this is not always the case because breast cancer may spread into surrounding areas before it is detected at the primary site. Cancer can spread through the blood, through lymph, via direct extension into other organs, or by seeding. The arterial supply to the breast includes the lateral thoracic artery and internal mammary artery and venous flow includes the internal mammary and lateral thoracic vein, as well as a superficial venous plexus. Because the breast is mainly drained by axillary nodes, axillary lymph nodes are usually the first nodes to be affected in breast cancer. Internal mammary nodes have been found to provide 1-3% of lymph drainage from the breast; axillary nodes have been found to provide 97-99% (Donegan and Spratt 2002). A sentinel lymph node biopsy identifies the first node to receive lymph drainage from the breast; if cancer has not spread to this
node, it is unlikely to have spread beyond it, sparing a patient excision of additional
dnodes (Chen, Skarin et al. 2007).

1.5. Breast Cancer Diagnosis

While breast cancer may be first identified by clinical exam, self-breast exam, or
screening mammography, the diagnosis of breast cancer, and all cancers, requires
pathological confirmation of disease. Other diseases can mimic the signs and symptoms
of breast cancer. Even on imaging, features that are hallmark of cancer can also be
hallmark of benign conditions. For example, on magnetic resonance imaging, a
fibroadenoma, which is benign, can a have contrast kinetic profile mimicking that of a
malignant lesion. On mammography, calcifications may be seen in association with a
cancer, but they may also be seen following trauma or another condition.

The pathological diagnosis of breast cancer consists of determination of the
invasiveness of the cancer (in situ or invasive carcinoma), and of identification of the
breast cancer histopathological subtype (lobular carcinoma, ductal carcinoma, or other
subtypes), tumor grade, hormone receptor status and degree of HER2/neu
overexpression, and properties such as lymphovascular invasion or necrosis. Tumor grade
relates to the degree that the cancer cells resemble normal cells and the rate at which the
cancer cells are dividing. The Scarf-Bloom-Richardson system is a method of objectively
defining tumor grade and grades cancer on a 9 point scale, summing the values scored on
3-points scales for tubule formation, nuclear pleomorphism, and mitotic count. In this
system, 1-3 points corresponds to a low grade (also known as Grade 1, or well-
differentiated), 4-6 points corresponds to intermediate grade (Grade 2, or intermediate-differentiated), and 7-9 points corresponds to high grade (Grade 3, poorly differentiated).

The most common histopathological subtypes of invasive breast cancer are infiltrating ductal (76%) and infiltrating lobular (8%) carcinoma; other subtypes are mixed ductal/lobular carcinoma (7%), mucinous or colloid (2.4%), and tubular (1.5%) (Bleiweiss 2010). Histopathological subtyping categorizes the macroscopic and microscopic features of a cancer. Cancers are also described by cell of origin. Carcinomas refer to cancers originating in epithelial cells, such as those lining the ducts and lobules of the breast. Sarcomas refer to cancers originating in connective tissue cells, such as those in bone and muscle.

As described in the previous section on breast anatomy, in addition to fibroglandular breast tissue, the breast consists of other tissue types, such as lymph, blood vessels, and adipose tissue and while rare, breast cancer can also originate in cells comprising these other tissue types (lymphomas and leukemias (Ginat and Puri), leiomyosarcomas (Cobanoglu, Sezer et al. 2009), and liposarcomas (Nandipati, Nerkar et al.), respectively). Primary breast lymphomas constitute 0.04% to 1.1% of all breast cancers (Yhim, Kang et al.) and sarcomas constitute <1% of all breast cancers (Nandipati, Nerkar et al.). Most breast cancers are adenocarcinomas, referring to the fact they are derived from the glandular breast tissue.

1.6. **Breast Cancer Staging**

The properties of a cancer at the time of diagnosis, as reflected in physical evidence of disease, relate to the stage of a cancer. The hallmarks of cancer described in a
previous section are not acquired by a cancer simultaneously. Some cancers acquire only the ability to invade; others acquire the ability to invade and increase blood supply, but not the ability to invade and thrive in other organs. Whether a cancer is in situ, invasive, or metastatic (Figure 1-5) relates to the biological properties of the tumor and helps determine the clinical stage. The clinical stage is important in guiding treatment and determining prognosis.

<table>
<thead>
<tr>
<th>Normal ductal anatomy</th>
<th>Carcinoma in situ</th>
<th>Invasive carcinoma</th>
<th>Metastatic</th>
</tr>
</thead>
</table>

![Diagram showing stages of breast cancer progression](image)

**Figure 1-5. Cancer staging.** In breast cancer, the normal anatomy of the breast is disturbed and this disturbance is increased as cancer progresses. In carcinoma in situ, the cancer hallmark of unregulated cell growth is present, but the cancer cells have not spread outside of the ducts. In invasive cancer, the cells have acquired the invasive hallmark and spread beyond the ducts, and in metastatic cancer, cells have acquired the ability to spread and grow at other sites. Locally advanced breast cancer (arrow) is a clinical diagnosis referring to invasive breast cancer which has spread regionally, but not to distant sites. Figure based on (Chen, Skarin et al. 2007)

In the United States, breast cancer is staged according to the American Joint Committee on Cancer (AJCC) criteria (Appendix). In breast cancer, the AJCC criteria assign a stage to a patient’s disease based on properties related to the tumor (T), nodes (N), and metastases (M). Tumor properties include tumor size and features such as chest wall or skin involvement. Node properties include numbers and locations of lymph nodes containing cancer cells that have spread from the primary site, and metastases refer to distant nodes and organs to which the cancer has spread. The 10-year survival rate
decreases with stage (Figure 1-6). Stage 0 cancer refers to in situ disease, Stage 1 refers to early stage invasive breast cancer, Stage II and III refer to early stage and locally advanced cancers, and Stage IV refers to metastatic disease. Locally advanced breast cancer is the focus of the ensuing chapters.

![Figure 1-6. Survival and Breast Cancer Stage. Adapted from p.358 of AJCC 2010 staging criteria. Data points from p.358 AJCC 2010 staging criteria.](image)

### 1.7. Breast Cancer Treatment

Breast cancer is treated with combinations of surgery, chemotherapy, radiation, hormone therapy, and/or other therapies. Treatments are designed for: 1) local control, 2) systemic control, or 3) palliative care. Local control of disease is provided by surgery to
remove the primary tumor and local lymph nodes; radiation may also play a role in local control. Control of distant disease is provided by systemic drug treatments, including chemotherapy and hormone therapy. Systemic drugs may be given before disease has spread to distant sites in order to reduce the risk that it will spread. This is important because distant disease is not considered curable; prevention of distant disease can be life-saving. Palliative treatments are designed to relieve pain and suffering associated with incurable disease. Palliative treatments include surgery or radiation to treat spinal compression as well as pain medications.

The purpose of chemotherapy, hormone therapy, and other targeted therapies is to prevent or control distant spread of disease. This is accomplished by either arresting cell growth or causing cell death. Chemotherapy agents cause cell death, either by causing DNA damage from which the cell cannot recover, or by inhibiting proteins necessary for cell division. Some chemotherapy agents operate at specific points in the cell cycle (cell-cycle specific); others are cell-cycle nonspecific. Common chemotherapeutics used in breast cancer are listed in (Table 1-2) with their mechanisms of action.
Table 1-2. Breast Cancer Chemotherapy

<table>
<thead>
<tr>
<th>Drug (Trade Name)</th>
<th>Mechanism of action</th>
<th>Type of Drug</th>
<th>Related drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>Inhibition of DNA and RNA polymerases; intercalates in DNA; interacts with topoisomerase II; <strong>cell cycle specific</strong></td>
<td>Anthracycline antibiotic</td>
<td>Epirubicin (Ellence)</td>
</tr>
<tr>
<td>(Adriamycin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Metabolites forms crosslinks between and within DNA strands, preventing DNA synthesis and functioning; <strong>cell-cycle nonspecific</strong></td>
<td>Alkylating agent; nitrogen mustard</td>
<td>Nitrosourea alkylating agent: Carmustin</td>
</tr>
<tr>
<td>(Cytoxan)</td>
<td></td>
<td></td>
<td>Metal salts: Carboplatin, Cisplatin</td>
</tr>
<tr>
<td>Paclitaxel (Taxol)</td>
<td>Stabilizes microtubules, preventing the normal breakdown needed for the cell to divide (<strong>cell cycle specific</strong>). Inhibits anti-apoptotic protein Bcl-2 (\rightarrow) induces apoptosis</td>
<td>Anti-microtubule agent; Plant alkaloid</td>
<td>Other taxane: Docetaxel (Taxotere)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Microtubule destabilizers: Vincristine and Vinblastine</td>
</tr>
<tr>
<td>Fluourouracil, 5-FU (Adrucil)</td>
<td>Noncompetitive inhibitor of thymidylate synthase needed for DNA replication; pyrimidine analog incorporated RNA and DNA (<strong>cell cycle specific</strong>).</td>
<td>Anti-metabolite</td>
<td>Capecitabine (Xeloda, prodrug for 5-FU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methotrexate (anti-metabolite for folic acid metabolism)</td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>Slows destruction of bone cells in metastatic cancer</td>
<td>Bis-phosphonate</td>
<td></td>
</tr>
<tr>
<td>(Zometa)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In addition to chemotherapy agents targeted against cell division and DNA synthesis, other forms of drug treatment that may be prescribed in the systemic control of cancer include hormone therapy (tamoxifen), targeted agents (trastuzumab, or Herceptin, targeted against the HER2 receptor overexpressed in some breast cancers and bevacizumab, or Avastin, targeted against VEGF, which is important in the growth of
cancer blood vessels), and immunotherapy (such as immune-cell stimulating factors, tumor vaccines, and interleukins).

Chemotherapy can be administered prior to surgery (neoadjuvant), or after surgery (adjuvant); in both cases the intent is the systemic control of breast cancer. Hormone agents may also be prescribed prior to a breast cancer diagnosis in high risk patients (prophylactic). In the treatment of locally advanced breast cancer, adjuvant and neoadjuvant chemotherapy have been shown to result in the same long-term patient outcomes (Rastogi, Anderson et al. 2008); however, neoadjuvant chemotherapy may increase the chance for breast conserving surgery and allow for tumor response to be assessed while the tumor is still present. Assessment of tumor response to chemotherapy is discussed in the next section.

1.8. Chemotherapy Response

Chemotherapy response can be assessed using a variety of methods, but the relevance of particular methods is highly dependent on the timing of the chemotherapy administration. When chemotherapy is implemented following surgery (adjuvant chemotherapy), the tumor has been removed and is no longer available to be monitored during chemotherapy treatment. In the case of metastatic disease, inoperable metastatic sites can be monitored with imaging methods. Positron emission tomography (PET) can assess changes in uptake of radioactive glucose within metastases and identify any new regions of abnormal uptake. Depending on the location of metastases, chest x-ray, magnetic resonance imaging, or other imaging modalities may be used to monitor treatment response.
In the case of early stage breast cancer, for which surgery removes all primary disease and metastasis has not yet occurred, monitoring of response to adjuvant chemotherapy is difficult. In vitro assays have been explored as tools to predict treatment response to particular drugs (Cortazar and Johnson 1999; Samson, Seidenfeld et al. 2004); however, cancer cells behave differently in the human body than in vitro, and responsiveness to particular agents in vitro may not translate to responsive in vivo (Nagourney 2006). Consequently, in vitro assays are not standard practice. Monitoring of isolated tumor cells in bone marrow (de Boer, van Deurzen et al. 2009) and circulating tumor cells in peripheral blood (Riethdorf, Muller et al. 2010) for assessment of treatment response is under research. Screening for recurrence is essential.

When chemotherapy is administered primary to surgery (neoadjuvant chemotherapy), the tumor is still present and available for treatment response monitoring. A variety of methods have been used, including clinical palpation, mammography, ultrasound, and conventional magnetic resonance imaging; however, these currently available methods are poor predictors for residual pathological size (Yeh, Slanetz et al. 2005). Pathological complete response (pCR), refers to absence of all invasive disease in the breast, and it is a strong predictor for long-term outcomes. At the 9 year follow-up point for patients enrolled in NSABP-18, patients with a pCR had a 75% disease-free survival rate, compared to 58% in those not achieving pCR (Mathew, Asgeirsson et al. 2009). Unfortunately, pCR can only be assessed following completion of chemotherapy and a method able to predict pCR or long-term outcomes at an earlier timepoint would be valuable in breast cancer treatment. One such method is described in more detail in Chapter 2.
Criteria have been established for assessing treatment response in clinical trials. Response Evaluation Criteria in Solid Tumors (RECIST) determines how tumor size is measured, and what changes constitute a response (Eisenhauer, Therasse et al. 2009). RECIST was developed for assessment of treatment response using a one-dimensional measurement: longest diameter of the tumor. Functional volume measurements calculated from contrast-enhanced MRI data correlate with residual pathological size (Partridge, Gibbs et al. 2002) and patient outcomes (Partridge, Gibbs et al. 2005). While RECIST does not currently provide guidelines for response assessment using volume, the one-dimensional RECIST criteria have been extended to volumetric measurements in multiple research studies (Prasad, Jhaveri et al. 2002; Pickles, Lowry et al. 2005; Iacconi, Giannelli et al. 2009). Diffusion-weighted magnetic resonance imaging (DW-MRI) shows promise in monitoring tumor response, but does not measure a size-based parameter encompassed by RECIST. Response criteria for diffusion, or measurements from other forms of molecular imaging such as PET and magnetic resonance spectroscopy, are needed and should follow validation of these metrics in large, prospective studies. Assessment of diffusion in prediction of treatment response is the focus of this work.

1.9. **Personalized Medicine for Breast Cancer**

Difficulties in accurately, and preemptively assessing tumor response to treatment were discussed in the previous section. Personalized medicine has the potential to improve all aspects of breast cancer prevention and treatment, from high-risk screening and routine screening, to diagnosis, treatment selection, and treatment response.
monitoring, to recurrence prediction. Personalized medicine is rooted in the biological basis of cancer: mutations lead to unlimited replication and genetic instability. It is this instability that ensures that no two cancers are likely to be genetically identical. Because genes determine the proteins present in a cell and most chemotherapies are targeted at either genes or proteins, the efficacy of treatments are likely to depend on the specific mutations present in an individual’s cancer. Some individuals may have genetic predispositions to cancer, and this information can similarly guide preventative therapies.

Currently, personalized medicine for breast cancer is already in practice. In 1998, Herceptin was introduced to specifically treat HER2 positive breast cancer. In 2004, Oncotype Dx was introduced to help determine whether a patient with early stage breast cancer would benefit from chemotherapy. Testing for estrogen receptor (ER) positivity is used to guide whether or not a patient will be treated with hormone therapy (Patlak, Levit et al.).

Despite these advances, the current successes that can be achieved with personalized medicine are limited by the available biomarkers and treatments, and by the implementation of these medicines. While targeted drugs exist for ER and/or HER2 positive breast cancers, triple negative cancers have been deprived of similarly targeted therapies. It is now known that triple negative cancers often have defects in DNA repair dependent on BRCA-mediated homologous recombination and poly ADP-ribose polymerase I (PARP) inhibitors have shown promise in treating triple negative breast cancer (Jain 2009). Oncotype Dx is currently validated as a predictor for survival in women with node negative, ER+ breast cancer (Paik, Shak et al. 2004), but similar prediction assays are needed for other breast cancer groups.
The genome of cancer is not static and better methods are needed to determine if a patient is responding as predicted. Better treatments are needed to provide non-responding patients with alternatives. Personalized medicine has made significant strides in improving the treatment of breast cancer, but improvements are needed. Neoadjuvant chemotherapy for locally advanced breast cancer provides a framework for multiple points of personalized intervention, tailored treatment, and tailored treatment monitoring (Figure 1-7). Chapter 2 will introduce specific medical imaging technologies that may have potential in helping personalized medicine move towards its goal of patient-specific, tumor-biology-specific care.

Figure 1-7. Potential for Personalized Medicine in Locally Advanced Breast Cancer.

1.10. References


Chapter 2. MRI Acquisition, Processing, and Data Analysis

2.1. Introduction to Medical Imaging ................................................................. 32
   Energy in Imaging ............................................................................................ 33
   Resolution .......................................................................................................... 35
2.2. Basis of Magnetic Resonance Imaging ...................................................... 36
2.3. Steps in MRI Acquisition ............................................................................ 38
   Nuclear magnetization ....................................................................................... 38
   Excitation .......................................................................................................... 38
   Relaxation .......................................................................................................... 39
   Signal Measurement .......................................................................................... 44
   Image Reconstruction ....................................................................................... 44
2.4. MRI Pulse Sequence .................................................................................... 45
2.5. MRI Parameters ........................................................................................... 45
2.6. Contrast-enhanced MRI ............................................................................... 50
   Description ........................................................................................................ 50
   DCE-MRI Data Processing ............................................................................... 52
2.7. Diffusion-Weighted MRI .......................................................................... 57
   Introduction to DW-MRI .................................................................................. 57
   History ............................................................................................................. 57
   Diffusion in Tumors ......................................................................................... 59
   Measuring Diffusion with DW-MRI ................................................................. 62
   Processing ........................................................................................................ 65
2.8. Image Processing and Analysis ................................................................. 69
   Regions of interests .......................................................................................... 69
   Histogram Analysis ........................................................................................... 71
2.9. Statistics ...................................................................................................... 75
2.10. Imaging Studies at UCSF ....................................................................... 78
2.11. References ................................................................................................. 81
2.1. **Introduction to Medical Imaging**

Medical imaging transmits energy to human tissue and tracks it as it is emitted back to the surroundings. This is useful because when the energy is emitted back, it is emitted back at a rate that gives important information about the tissue composition. For example, in computerized tomography (CT) and mammography, energy in the form of x-rays are transmitted to the human tissue. They pass through the tissue, but they pass through depending on the ability of the tissue to transmit them. Bone does not transmit the x-rays well, but bone that is broken may better transmit x-rays, allowing a fracture to be seen. When the x-rays enter back into the surroundings, they are captured on a special type of film, allowing for a picture of their attenuation to be seen.

In positron emission tomography (PET), energy is transmitted to the patient in the form of injection with a radioactive tracer, which accumulates in areas of the body that utilize its non-radioactive analog, and thus the radioactive tracer, at a high rate. When the energy is transmitted back to the surroundings, it is transmitted back mainly from locations in which it has accumulated. These locations can be detected by the imaging apparatus.

Magnetic resonance imaging (MRI) is a medical imaging modality that utilizes these same principles of energy transmission and emission. MRI differs from the two previous imaging modalities in that it utilizes non-ionizing radiation. Like CT and PET, it is three-dimensional. The contrast in MRI results from a property that differs in soft tissues, allowing it to provide excellent soft tissue contrast. Because of these strengths, MRI is important in the clinical diagnosis of many diseases, including stroke, cancer, and musculoskeletal disease.
Energy in Imaging

As seen in these examples, imaging modalities utilize energy in different ways in order to create an image. In general, imaging methods measure differences in the ability of tissue to transmit energy, reflect energy, or emit energy (Bourne 2010). These differences between various breast imaging modalities are summarized in Figure 2-1.

<table>
<thead>
<tr>
<th>Principle</th>
<th>Differential Transmission</th>
<th>Differential “Reflection”</th>
<th>Differential Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Imaging Modality</td>
<td>Mammography, Computerized Tomography</td>
<td>MRI, Ultrasound</td>
<td>Positron Emission Tomography</td>
</tr>
</tbody>
</table>

Figure 2-1. Energy in Breast Imaging. Medical imaging modalities create images by measuring differences in energy transmission (x-ray, CT), energy “reflection” (MRI, ultrasound), or energy emission (PET and SPECT). In breast imaging, mammography is a common modality that creates images from differential transmission of x-rays; MRI creates images from the differential return of protons to equilibrium magnetization; PET creates images from the differential emission of photons resulting from positron annihilation in regions in which positron emitters have accumulated due to differences in metabolism or other functional properties of the tissue. Image adapted from p. 36, Fundamentals of Digital Imaging in Medicine (Bourne).
While imaging modalities vary greatly in the way they track energy to create an image, nearly all medical imaging modalities, with the exception of ultrasound and elastography, apply energy to the patient in the form of electromagnetic waves. The colors visible to the human eye are electromagnetic waves and the electromagnetic spectrum is the collection of all possible electromagnetic waves, ordered by increasing energy. The types of electromagnetic waves used in many breast imaging modalities can be placed along this electromagnetic spectrum (Figure 2-2).

Figure 2-2. Electromagnetic spectrum in breast imaging. The electromagnetic (EM) spectrum (Newman 2008) is a continuum of electromagnetic waves, ordered by increasing energy. Because energy, \( E \), is directly proportional to frequency, \( v \),
(\(E=nh\), where \(h=\text{Planck’s constant}\) and inversely related to wavelength, \(\lambda\) (\(E=hc/\lambda\)) (Hendee and Ritenour 2002) the spectrum is also ordered by increased frequency and decreased wavelength. Common breast imaging modalities utilize EM waves of varying energy, with breast MRI utilizing lower energy EM waves than the other imaging modalities. Breast ultrasound is a common imaging modality that does not appear on this spectrum because it utilizes pressure waves and not EM waves (Bourne 2010). MRI= Magnetic Resonance Imaging, DOS= diffuse optical spectroscopy, Mammo= Mammography, PET= Positron Emission Tomography.

Resolution

Imaging modalities vary in terms of the spatial and temporal resolution that they can provide. Spatial resolution refers to the detail of the tissue structure that the modality is able to depict, or resolve. For example, in diffusion-weighted MRI, spatial resolution is usually 2 mm or more – this means that a structure that is 1 cm in diameter will be depicted with up to 5 voxels, but a structure that is less than 1 mm, such as a lobule, will not be resolved. High spatial resolution is important in breast imaging. Calcifications, which are hallmark of injury and can be associated with cancer, are small, but within the spatial resolution constraints for mammography (spatial resolution for x-ray is .1 mm). Conversely, these structures are too small for depiction with MRI (spatial resolution, 1mm) (Bourne 2010).

Temporal resolution describes the precision of the modality in depicting changes over time. High temporal resolution is vital in dynamic-contrast enhanced MRI, in which the kinetic constants describing contrast uptake can be derived from sampling of contrast uptake and wash-out over time. The temporal resolution achieved with MRI is approximately 10 seconds; the resolution for plain x-ray is less than 1 second, and that of PET is more than 1000 seconds (Bourne 2010).
Compromises occur between spatial and temporal resolution: increased spatial resolution often results in decreased achievable temporal resolution. This is especially true in MRI. High spatial resolution requires a larger matrix size, with more frequency and phase encoding steps. In the time required to acquire an MR volume with a larger matrix, changes in contrast uptake could have been imaged with a greater number of timepoints and temporal resolution is consequently decreased. Imaging time can be spent increasing spatial or temporal resolution, or achieving intermediate increases in both spatial and temporal resolution.

Compared to other available medical imaging modalities, MRI has intermediate spatial resolution and intermediate temporal resolution. However the value of an imaging modality is not due solely to its resolution. MRI provides three-dimensional imaging data with non-ionizing radiation and high soft tissue contrast, related to both structural and functional differences in tissues. In breast imaging, MRI is used for screening of the contralateral breast in women with known cancer, screening of high-risk women, assessing extent of disease, and for monitoring tumor response to neoadjuvant chemotherapy. This last application is the focus of this research and the physical basis of MRI will be discussed in the next section.

2.2. Basis of Magnetic Resonance Imaging

Magnetic resonance imaging is possible because of the phenomenon of nuclear magnetic resonance (NMR) and the fact that one of the most common nuclei in the human body, the nucleus of hydrogen, undergoes this phenomenon. Any nucleus with an
odd number of neutrons, or an odd number of protons, can undergo NMR. Nuclei with an even number of protons and neutrons cannot undergo NMR. The hydrogen nucleus contains 1 proton and no neutrons; since it contains an odd number of protons it therefore can undergo NMR. The odd numbered protons give it a nonzero angular momentum and a nonzero spin quantum number. For hydrogen, the spin quantum number is \( \frac{1}{2} \) and the number of energy levels it occupies is equal to \( 2I+1 \), or 2. These levels are \( +\frac{1}{2} \) (spin up, parallel to the field, and low energy) and \( -\frac{1}{2} \) (spin down, anti-parallel to the field, and high energy).

In order for NMR to take place, nuclei need to be in the lower energy level so that they can be excited to the higher level with application of an RF pulse and subsequently fall back down to the lower level, at a rate that is informative for tissue contrast and construction of an MR image. The Boltzmann distribution describes the proportion of nuclei that are spin up (\( N_{\parallel} \)) vs. spin down (\( N_{\perp} \)) (Brown and Semelka 2010):

\[
\frac{N_{\perp}}{N_{\parallel}} = e^{-\frac{\Delta E}{kT}}
\]

where \( k \) is the Boltzmann constant (1.38 x 10^{-23} joule/K), \( T \) is the temperature in Kelvin, and \( \Delta E \) is the difference in energy levels between the two states, which is equal to Planck’s constant multiplied by the frequency of the photon. When exposed to the magnetic field, the frequency is equal to the gyromagnetic constant * the strength of the field. The proportion of spins parallel vs. anti-parallel can thus be calculated. At 1.5T, the difference in the number of spins aligned and anti-aligned is small (approximately 5 per million at body temperature and 1.5T (Hendrick 2008), but because there is an excess of
parallel spins relative to the anti-parallel spins, the tissue will undergo NMR and MRI can take place.

2.3. Steps in MRI Acquisition

The process of acquiring MRI data can be summarized in 5 steps: 1) nuclear magnetization, 2) excitation, 3) relaxation, 4) signal measurement, and 5) image reconstruction. These steps are described in more detail below.

Nuclear magnetization

The first step, nuclear magnetization, was introduced in the previous section and refers to the fact that the person is placed in a magnetic field, B₀, typically having a strength of 1.5-3 Tesla. Because water contains hydrogen nuclei which have a nonzero spin quantum number, and because of the high water content in the human body, and the fact that water is present throughout the body, the bulk tissue will align with the magnetic field. Not all spins will be aligned, but an excess will be aligned according to the Boltzmann distribution and nuclear magnetic resonance will be able to occur. When in the field, the spins precess at a frequency equal to their inherent gyromagnetic ratio multiplied by the field strength; at 1.5T, this frequency is approximately 64 MHz, or “resonance frequency.”

Excitation

After the person has been placed in the magnetic field and the hydrogen nuclei are precessing at resonance frequency, energy in the form of a radiofrequency (RF) wave (RF pulse), at resonance frequency, is applied perpendicular to the main magnetic field.
Application of this pulse leads to a magnetic field other than the main magnetic field of the scanner: the B1 field. Because the RF pulse is applied at the same frequency as the spins’ precession, it causes the spins to absorb energy (excitation) and move to the anti-aligned state. The spins tip into the transverse (x-y) plane and precess about the B1 field.

**Relaxation**

When the RF pulse is turned off, the spins move back to equilibrium magnetization and move back to their precession about B0. However, the rate at which they return to equilibrium magnetization depends on properties inherent to the tissue: T1 and T2. These relaxation rates, along with proton density, lead to contrast in the image and are defined in more detail below:

**T1**: This is the time constant for spin-lattice or longitudinal relaxation. The longitudinal relaxation rate (1/T1) describes the rate of return of spins to precession about the z axis, the axis of the main magnetic field B0. T1 is the time for the longitudinal magnetization vector to relax back to 63% of its equilibrium value (from 0 to 1-e^{-1}) (Elster and Burdette 2001). Regrowth of longitudinal magnetization is shown for an example tissue in which T1=600 ms (Figure 2-3).
Figure 2-3. T1 and T2. In the plot, magnetization decay (blue line) and regrowth (green line) are shown for an example case in which the T1=.6 s and T2=.06 s. On the green dashed curve, the cursor denotes the time at which the magnetization has regrown 63% (time=T1). On the blue curve, the cursor highlights the time at which the magnetization has decayed to 37% of its value after application of the 90° pulse (time=T2).

**T2**: This is the time constant for spin-spin or transverse relaxation. The transverse relaxation rate (1/T2) describes exponential decay of the spins in the x-y plane, the plane into which the spins were tipped by the RF pulse. T2 is the time for the transverse magnetization to decay to 37% of its value (to e⁻¹), as shown in the figure (Elster and Burdette 2001).

T2* relaxation, or spin-static relaxation, is the observed transverse relaxation due to external field inhomogeneities. The time constant for relaxation due to field
inhomogeneities is $T2i$. The relationship between the $T2$, $T2i$ and $T2^*$ relaxation rates ($1/T2$, $1/T2i$, and $1/T2^*$), respectively, is (Elster and Burdette 2001):

$$\frac{1}{T2^*} = \frac{1}{T2} + \frac{1}{T2_i}$$

**Proton density**: This is the density (concentration) of water protons and macromolecule protons in tissue.

$T1$, $T2$, and proton density describe properties inherent to the tissue; however, $T1$ and $T2$ are also dependent on magnetic field strength. All three properties relate to the environment of the spins, which is determined by the tissue type and function, and local pathophysiology. Different tissues have different $T1$ and $T2$ values and in MRI, this provides excellent soft tissue contrast (Figure 2-4, Figure 2-5).
Figure 2-4. Tissues with Different T1 Values. In this example, the T1 of one tissue (black dashed line) is twice as long as the T1 of the other tissue (pink line), and all other factors are equal. At a time=.6s, more of the magnetization has been recovered for the tissue represented by the pink line than for the tissue represented by the black dashed line. This difference will impact the signal intensities observed on MRI.
Figure 2-5. Tissues with Different T2 Values. In contrast to the results seen in the prior figure, when a tissue has longer T2 (black dashed line), more magnetization will be available in the x-y plane at a given time. This is because T2 relaxation describes decay and T1 relaxation describes regrowth.
Signal Measurement

As the spins de-phase in the x-y plane due to T2 relaxation and return to precession about the z axis due to T1 relaxation, the bulk magnetization vector in the x-y plane changes: it decays. A receive coil in the x-y plane is therefore exposed to a changing magnetic field, leading current to flow in the coil (induction). The MRI signal is measured as this wave varying over time that corresponds to relaxation of the magnetized tissue.

Image Reconstruction

The MRI signal is spatially encoded by varying the magnetic field strength in different directions. Spins at different locations will therefore be exposed to a different magnetic field strength, leading to a spatial variation in precessional frequency because frequency = gyromagnetic ratio times the magnetic field strength. To determine these frequency components (and thus ultimately the spatial information), the received signal which is sampled in time must be converted to the frequency domain. The signal is composed of many different frequencies due to the spatial encoding. The sampled signal is thus composed of different waves, each with a characteristic frequency, phase and amplitude. The Fourier transform is used to convert the time-varying signal to a spectrum of spatial frequencies. This information is stored in k-space. Once the information is stored in k-space, the inverse Fourier transform must be applied to move from k-space to image space.
2.4. MRI Pulse Sequence

While all MRI acquisitions follow the general process previously described, different types of images, with different information valuable to clinical medicine, can be acquired by slightly varying the general MRI acquisition. For example, the time at which the signal is acquired influences the tissue contrast because if the signal is acquired very quickly with a short TE and TR, different tissues will not have enough time to relax in the transverse direction (T2), and the image will not be T2-weighted. In addition, adding pulses before the standard sequence is acquired can allow for fat suppression and improved tissue contrast.

An MRI pulse sequence describes the pattern of pulses, the duration and strength of the gradients, and the intervals over which the signal is acquired. Most commercially available scanners are equipped with an arsenal of pulse sequences, which can be further modified depending on the clinical situation or research needs. New pulse sequences can be developed. An MRI pulse sequence can be thought of as a recipe, followed by the MRI scanner, for acquisition of the images.

2.5. MRI Parameters

Each MRI acquisition is defined by parameters related to both the pulse sequence and decisions made by the MR technologist. These parameters are briefly described.

*Flip angle:* This the angle through which the magnetization vector in the z axis rotates after application of the RF pulse. In spin-echo sequences, the flip angle is 90°. In
gradient-echo sequences it may be less than 90°. The flip angle (\(\alpha\)) is determined by
(Elster and Burdette 2001), p.69-70:

\[
\alpha = \gamma B_1 t_p
\]

Where \(B_1\) is the field strength produced by the RF coil and \(t_p\) is the time over which the RF is applied.

**Time to repeat (TR):** This is the amount of time after which the pulse sequence is repeated. In 90°-180° spin-echo sequences, it is the time from the first 90° to the 2\(^{nd}\) 90° RF pulse.

**Time to echo (TE):** TE describes the time period after application of the first RF pulse at which the signal in the x-y plane is detected. In spin-echo sequences, this is the time between the first 90° RF pulse and the spin-echo following application of the refocusing pulse(Hendrick 2008).

**Field of view (FOV):** This is the area of tissue to be imaged; this area does not need to be a square. Scanners may have maximum allowable FOVs. FOV should be chosen carefully because a FOV that is too small for the organ of interest may result in phase wrap.
**Acquisition matrix:** This is equal to the number of frequency encoding steps \((N_x)\) multiplied by the number of phase encoding steps \((N_y)\). With FOV, it describes in-plane resolution:

\[
x \text{ resolution} = \frac{\text{FOV}_x}{N_x} \quad \text{and} \quad y \text{ resolution} = \frac{\text{FOV}_y}{N_y}
\]

**Slice thickness:** This is the length of the voxel in the slice-select gradient direction. MRI sequences with very small slice thickness are generally referred to as 3D; otherwise sequences are referred to as 2D. In an axial acquisition, this would the length along the z axis. It is determined by:

\[
\text{Slice thickness} = \frac{\text{FOV}_z}{\# \text{ slices}}
\]

**Slice orientation:** This is determined by the direction of the slice-select gradient. Standard slice orientations are axial, sagittal, and coronal; however, slices can be “oblique” and occur along nonstandard angles.

**Number of excitations (NEX):** This is the number of times MRI data for a given tissue slice is acquired. Since noise is random, increasing the NEX improves signal to noise ratio.

**Fat suppression:** This is any of several techniques designed to obtain images without signal from protons in fat. At 1.5T, the resonance frequencies of fat and water are only 220 Hz apart and the RF bandwidth will typically excite both fat and water. Methods in
fat suppression prevent fat from participating in the RF excitation or otherwise reduce the contributions from fat protons to the detected MR signal.

**Sequence type:**

After the B1 field is turned off, dephasing of the spins in the transverse plane is generally mitigated using 1 of 2 methods. The aim of these methods is to allow the spin phase, and thus the magnetization vector, to be maximal at the time the signal is measured. In a spin-echo sequence, a 90° RF pulse flips the magnetization into the x-y plane and then uses a 180° refocusing pulse to re-phase the spins. Sequences applying a second RF pulse to refocus magnetization are considered to be spin-echo; however, refocusing can be accomplished using an alternative method. In a gradient-recalled echo sequence, gradient reversal is used to accomplish a similar re-phasing. In both sequences, the measured signal is dependent on the tissue properties described in an earlier section (T1, T2, proton density), and the acquisition parameters such at TR and TE.

In a spin-echo sequence, the measured signal, S, is dependent on T1, T2, and proton density according to the equation (Tofts 2003), p.160:

\[
S = k\rho \left( 1 - 2e^{-\frac{TR}{T1}} + e^{-\frac{TR + TE}{T2}} \right) e^{-\frac{TE}{T2}}
\]

Where k is a scaling factor. When TR is much greater than TE (Tofts 2003), p.161:

\[
S = k\rho \left( 1 - e^{-\frac{TR}{T1}} \right) e^{-\frac{TE}{T2}}
\]
In a gradient-recalled echo sequence, the measured signal is dependent on T1, T2, and proton density according to the equation (Kucharczyk, Moseley et al. 1994; Tofts 2003) p.418:

\[ S = k\rho \left( \frac{1 - e^{-\frac{-TR}{T1}}}{1 - \cos(\alpha) e^{-\frac{-TR}{T1}}} \right) \sin(\alpha) e^{-\frac{-TE}{T2}} \]

Spin-echo and gradient-recalled echo sequences can also be weighted so that the contrast in the image is due to particular tissue properties. In a previous section, three properties inherent to the tissue were described: T1, T2, and proton density. While T1, T2, and proton density always influence the signal during the MR acquisition, the acquisition parameters can be altered to reduce the effects of particular parameters on the signal, allowing for the acquired image to be more heavily weighted to represent differences in a particular parameter. When TR and TE are short, the image is considered to be “T1-weighted”; when TR and TE are long, the image is considered to be “T2-weighted,” (Horowitz 1995) and when TR is long (to reduce T1-weighting) and TE is short (to reduce T2-weighting), the image is “proton density weighted” (Westbrook and Roth 1998), p.26.

In addition to T1, T2, and proton density, another tissue property that is inherent to the tissue is diffusivity. Diffusivity refers to water mobility and will be discussed in more detail in a later section. Like proton density, diffusivity is both inherent to the tissue and exists outside of the scanner, at all times. T1 and T2 are inherent to the tissue but result from the fact that during the MR acquisition the protons in the tissue are forced into
alignment with an external magnet, disrupted from this alignment with an RF pulse applied at resonant frequency over a fixed time period, and then left to realign. In contrast, even when the tissue is not being imaged and is away from the magnet, proton density and diffusivity will relate the molecular components of the tissue and their arrangement in space. Application of diffusion-weighted gradients are required for an image to be “diffusion-weighted” and this will be discussed in more detail in a later section.

In summary, images can be weighted so that the image contrast is due to important properties related to the tissue and this is useful in medical imaging because tissue differences may be due to disease processes. T1- and T2- weighing can be further modulated by the use of contrast agents (contrast-enhanced MRI, or CE-MRI). CE-MRI is important in clinical breast imaging, as well as in research applications monitoring tumor response to treatment and will be discussed in the next section.

2.6. **Contrast-enhanced MRI**

**Description**

The dependency of the MR signal on T1 and T2 relaxation is exploited in contrast-enhanced MRI (CE-MRI), in which a patient is scanned with an MR sequence, and then rescanned following injection of a contrast-agent. The contrast agent impacts T1 and T2, and subsequent changes in signal intensity are therefore concluded to be due to the agent. Because the ability of the agent to reach certain areas of the body is dependent
on vascularity, vessel permeability, and blood flow, changes in the signal intensity 
provide important spatial information about tissue function and pathology.

In breast imaging, CE-MRI plays a role in tumor identification. In Chapter 1, the 
growth of new blood vessels was described as a hallmark of cancer. As cancers grow, 
their component cells become too distant from blood vessels to be reached by diffusing 
oxygen; at that point, their survival is dependent on their ability to influence blood vessel 
growth. Tumor angiogenesis refers to cancer-induced growth of vessels. The vessels that 
grow during tumor angiogenesis differ from normal vessels: they are branched, tortuous, 
and leaky. These differences are exploited by CE-MRI. The contrast agent leaks out of 
leaky tumor vessels faster than it leaks out of normal vessels (termed “wash-in”). It 
reaches, or washes into, cancer regions before it reaches tumor regions; consequently, it 
is able to modulate T1 and T2 in the cancer regions at an earlier timepoint. The contrast 
agent also leaks out (termed “wash-out”) of the leaky tumor vessels faster than it leaks 
out of normal vessels.

Gadolinium-based chelates are contrast agents used in imaging of the breast and 
other anatomic sites and these agents decrease T1, making the signal brighter on T1-
weighted images in regions with the contrast agent (Figure 2-6). A commonly used agent 
is Gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA). Because the contrast 
agent washes in and out of tumors faster, cancer will appear “bright” on MR images 
acquired soon after contrast injection and “dark” on MR images acquired later after 
injection. The brightness of the tumor can be monitored over time. MR images are 
acquired in the same regions, at multiple time-points, and these pre-contrast and post-
contrast MR images provide “snapshots” of the contrast behavior. Dynamic contrast-
enhanced MRI (DCE-MRI) tracks the contrast behavior with multiple MR volumes, separated by time.

![Figure 2-6. Varying concentration of contrast agent. An MR image of test tubes containing varying concentrations of contrast agents is shown. Higher concentrations of contrast decrease T1 and T2. The signal is dependent on both T1 and T2. On this T1-weighted image, the cross-section of the test tube labeled A is brighter than the other cross-sections. This is because A has a short T1 and a long enough T2 for sufficient signal to be measured.](image)

**DCE-MRI Data Processing:**

In DCE-MRI of the breast, contrast behavior is qualitatively described by identifying locations with increased contrast uptake and by noting the morphological, spatial, and temporal pattern of uptake. Enhancement is described as “mass-like” or “non mass-like.” For example, the shape of a mass may be described as “round”, the margins may be described as” smooth,” and the pattern of enhancement may be “rim-like” with
“plateau” kinetics. The American College of Radiology Breast Imaging Reporting and Data System governs the descriptors used in the clinical interpretation of DCE-MRI data (2003; Agrawal, Su et al. 2009). The contrast behavior can also be described quantitatively by calculating biologically meaningful parameters (pharmacokinetic modeling). In addition, the behavior can be described semi-quantitatively by quantifying changes in the MR signal (SER map). These latter two methods are described in more detail below.

**Pharmacokinetic Modeling**

Pharmacokinetics describe the time-course of a drug’s behavior in the human body. In DCE-MRI, the contrast agent is considered a drug and can therefore be described by pharmacokinetic principles. The ability to describe changes in the contrast agent’s concentration is important because it allows biologically meaningful information to be derived from DCE-MRI volumes acquired at multiple timepoints. The concentration of contrast agent in a particular region of the breast at a particular time is governed by factors including the cardiac output, permeability of the vessels, and proportion of tissue in the region into which the agent can leak. For example, even if contrast agent is able to quickly leak out of tumor vessels into the tumor, if the tumor is fibrotic, the contrast agent may not be able to penetrate the entire tumor.

The behavior of the contrast agent following injection has been related to the acquired MR signal by Paul S. Tofts and others (Tofts and Kermode 1991; Taylor, Tofts et al. 1999). Following injection, the contrast agent is assumed to move between multiple compartments in the body: the plasma, the tissue space into which it can leak, and the kidneys, from which it is cleared. Its movement between compartments is governed by
kinetic constants, which describe the rate of a process (Figure 2-7). The rate of the contrast agent’s movement in turn impacts the signal acquired in a particular region at a particular time.

In DCE-MRI, the main constants of interest are the transfer constant, $K^{\text{trans}}$ (in units min$^{-1}$, at times referred to as extraction flow product, $k$, $k^{\text{PS}}$, FE, or $\text{CL}_d/V_1$), describing the rate at which the contrast moves from the plasma into the lesion space, and the rate constant $k_{\text{ep}}$ (in units min$^{-1}$, at times referred to as $k_{21}$, $k_2$, or $1/\tau_c$), describing the rate at which it washes back out into the plasma (Tofts, Brix et al. 1999). A third kinetic parameter of interest is $v_e$ (unit-less), the volume of extracellular extravascular space per unit volume of tissue, also referred to as the interstitial space and leakage space. $K^{\text{trans}}$ and $k_{\text{ep}}$ are rate constants; $v_e$ is a volume (Tofts, Brix et al. 1999).

Typically, several assumptions are made when pharmacokinetic models are used to derive kinetic parameters from DCE-MRI data. Plasma decay constants are typically not measured for each patient; rather, the agent is assumed to decay in plasma with parameters found by Weinmann et al. (Tofts, Berkowitz et al. 1995). Hydrostatic and osmotic pressure across the capillaries is typically ignored, although the Jain and Baxter model includes these terms (Eyal and Degani 2009). Models also assume that the temporal resolution needed to measure rate constants can be achieved; however, in practice, temporal resolution may be compromised for necessary spatial resolution. For particular patients or studies, these assumptions may or may not be valid. Despite these limitations, pharmacokinetic modeling is used to quantitatively assess DCE-MRI data in the breast, as well as at other anatomic sites. Prior studies in the breast have tested the value of pharmacokinetic parameters in predicting whether a lesion is malignant or
benign (den Boer, Hoenderop et al. 1997) and in predicting response to neoadjuvant chemotherapy (Padhani, Hayes et al. 2006).

**Figure 2-7. Compartment model.** Based on pharmacokinetic principles, movement of contrast agent between the plasma and extravascular, extracellular leakage space \((v_e)\) can be described by rate constants, \(K_{\text{trans}}\) and \(k_{\text{ep}}\). The concentrations in the plasma and leakage space are a function of time, \(t\) \((C_p(t)\) and \(C_1(t)\), respectively) and the images acquired over time provide snapshots of these concentrations. The acquired MRI signal is related to the concentration of contrast agent at each voxel, as well as the \(K_{\text{trans}}, k_{\text{ep}}, v_e, \text{TR, TE, and plasma parameters} (Tofts, Berkowitz et al. 1995). Gd-DPTA=Gadolinium diethylenetriamine penta-acetic acid

**SER Map**

Based on the models described in the previous section, the signal intensity in each DCE-MRI voxel relates to the voxel’s concentration of contrast agent. As described in the previous section, pharmacokinetic modeling is not without limitations. Even when pharmacokinetic parameters are not calculated, important information can be derived from signal intensities and relative enhancement throughout the breast. The signal enhancement ratio (SER) can be used to describe the wash-in and wash-out occurring in each voxel over the time-course of the DCE-MRI exam. When this ratio is calculated at each voxel, an additional MR volume is obtained, the SER map and the calculation that produces this map is illustrated in Figure 2-8.
Functional tumor volume is a parameter derived from the SER map. This volume is calculated by summing the number of voxels with SER values greater than a particular threshold and multiplying this sum by the voxel size. Other semi-quantitative parameters related to the SER map include peak SER, the maximal SER in the tumor. Percent enhancement, PE, is the ratio of the difference between the signal acquired early post-contrast and pre-contrast to the pre-contrast signal. Unlike SER, PE does not include wash-out. In addition, the value of functional tumor volume and other semi-quantitative parameters in predicting treatment response has been assessed (Martincich, Montemurro et al. 2004; El Khoury, Servois et al. 2005; Partridge, Gibbs et al. 2005; Li, Partridge et al. 2008). Functional tumor volume has been shown to predict residual tumor size (Partridge, Gibbs et al. 2002; Belli, Costantini et al. 2006). However, these correlations
are imperfect and it is still not possible to predict response to neoadjuvant chemotherapy at the individual level.

2.7. **Diffusion-Weighted MRI**

**Introduction to DW-MRI**

As described in the previous section, DCE-MRI is an imaging modality that has shown promise over the last decade in predicting tumor response to treatment. However, DCE-MRI provides an assessment of functional tumor size, and changes in tumor size occur later than changes at the molecular and cellular level. Even before a tumor changes size, an opportunity exists to predict treatment response. Imaging modalities with contrast derived from molecular and cellular differences may be able to predict tumor response to treatment at an earlier stage than DCE-MRI. Contrast in diffusion-weighted MRI is derived from differences in properties such as cell density and cell content and its physical basis has been under study since at least the 1800s.

**History**

Diffusion in the random motion of water molecules. Milestones in the understanding of diffusion and in the development of methods to assess it in the breast are summarized in Figure 2-9. The first milestone was in 1827, when the botanist Robert Brown examined pollen grains immersed in water and noticed that the pollen grains moved and changed location (Newell 1923). This motion was attributed to the random movement not of the pollen grains themselves, but of the water molecules, termed diffusion. Later, the scientists Stokes, Fick, and Einstein related diffusion to measurable
properties such as the size of the diffusing molecule and its concentration gradient (Chang 2000). Based on these relationships, the predicted diffusion of a substance could be calculated from physical parameters. However, in human tissue, calculation of diffusion from these physical parameters would be difficult.

Nuclear magnetic resonance provides a means to noninvasively measure properties in vivo. In 1965, Stejskal and Tanner used gradients to measure diffusion with nuclear magnetic resonance (Stejskal and Tanner 1965). The pulsed spin echo gradients used by Stejskal and Tanner are still popular today and are referred to as Stejskal-Tanner diffusion-weighted gradients. In 1984, Wesbey, Moseley, and Ehman published results from measurement of diffusion DW-MRI (Wesbey, Moseley et al. 1984; Wesbey, Moseley et al. 1984). Initially, the clinical impact of DW-MRI was tied to its value in diagnosing stroke. Today, DW-MRI has been applied to many anatomic sites, including the kidney (Damasio, Tagliafico et al. 2008), colon (Oussalah, Laurent et al. 2010), pancreas (Yamashita, Namimoto et al. 1998), gallbladder (Sugita, Yamazaki et al. 2009), ovaries (Moteki and Ishizaka 2000), brain (Hamstra, Chenevert et al. 2005), head and neck (Hermans 2010), prostate (Henzler, Schmid-Bindert et al. 2010; Kim, Park et al. 2010), lungs (Henzler, Schmid-Bindert et al. 2010), and breast. It has been used in multiple human diseases, including breast cancer.

The use of DW-MRI in the human breast did not occur for over 10 years after the initial DW-MRI experiments by Wesbey, et al. Based on a search of PubMed, the first DW-MRI study of the breast in humans was published in 1997 and evaluated normal subjects (England, Ulug et al. 1997). Although abstracts reporting use of DW-MRI in patients date back to at least 1998, the first paper was published in 2002 (Sinha, Lucas-
Early studies focused on differences between benign and malignant lesions. In 2004, the first paper evaluating DW-MRI in predicting treatment response was published. In this study, response was assessed in patients with metastatic breast cancer. Studies evaluating treatment response with DW-MRI in patients with locally advanced breast cancer were published beginning in the mid-2000s. DW-MRI was included in neoadjuvant imaging studies at UCSF beginning in the late 1990s.

**Figure 2-9. DW-MRI Timeline**

**Diffusion in Tumors**

In the previous section the history of DW-MRI was reviewed and it can be seen that the journey of diffusion from the experiments of Robert Brown to breast imaging protocols was not fast. It occurred over a period of 160 years or more. Similarly, the diffusion of water molecules in slow, and it can be even slower in tumors. The distance a water molecule travels is related to its diffusivity by the equation: distance= √(2Dt), where D is the diffusivity, or ADC, t is the time over which the molecule diffuses, and distance refers the root mean square displacement (Neil 1997). At body temperature, the diffusivity of free water is approximately 3 *10^-3 mm²/s (DeVita, Lawrence et al. 2008),
In a typical DW-MRI exam, TE may be 50ms. In that time, a water molecule travels approximately 30μm.

The motion of water molecule due to diffusion is random and a water molecule’s trajectory due to this random motion is depicted in Figure 2-10.

![Figure 2-10. Random motion. The circle represents a water molecule and the arrows depict the random trajectory of this water molecule. This random motion is termed diffusion.](image)

In the figure above, the trajectory of the water molecule represents unrestricted diffusion; however, in the human body, the motion of water is restricted by cell membranes, organelles, and other structures. In human tissue, the diffusivity of water is expected to be less than 3*10^{-3} mm²/s, the diffusivity of free water. In normal fibroglandular breast tissue, the diffusivity is approximately 2*10^{-3} mm²/s (1.95±0.24*10^{-3} mm²/s, (Partridge, Murthy et al. 2010). In breast tumors, the diffusivity is typically around 1*10^{-3} mm²/s (1.09±0.27*10^{-3} mm²/s, (Kim, Cha et al. 2009). The hallmarks of cancer - - unlimited potential to replicate, gain of pro-growth signals, and loss of anti-growth signals - - lead to increased cell division in the tumor; consequently, tumors typically have a higher cell density than normal tissue. The increased cell density restricts water diffusion, leading to lower diffusivities.
In general, malignant breast tumors are associated with lower ADC than benign lesions and normal fibroglandular tissue, but this is not always the case. Some malignant tumors may have higher diffusivities in some regions, approaching that of free water (Figure 2-11). A tumor with a necrotic core may have a low diffusivity at the outer edges of the tumor and a low diffusivity in the center of the tumor. Mucinous breast cancers are also associated with increased diffusivity (Woodhams, Kakita et al. 2009).

![Figure 2-11. Diffusion in breast lesions. This schematic shows three different possible lesion patterns. At left, a lesion with high cellularity is shown and would be expected to have a low diffusivity (dark blue); this pattern would be expected for many tumors. In the middle, a tumor with a lower cellularity is shown and would be expected to have higher diffusivity (light blue); this pattern is expected for benign lesions such as fibroadenomas. At right, a tumor with a necrotic core is shown. The core of the tumor has a high diffusivity and is surrounding by regions of low diffusivity. This pattern is expected in large tumors, when the core exceeds the diffusion distance of oxygen and its cells die. In contrast, the outside of the large tumor is still close enough to the blood supply to obtain oxygen and the tumor is viable, with rapidly growing cells leading to a low diffusivity.](image)

Diffusion varies between tumors and within tumors. It is difficult to predict a tumor’s diffusivity and in prior studies, molecular and cellular characteristics of breast cancer were, in general, not correlated with the tumor’s diffusivity (Yoshikawa, Ohsumi et al. 2008; Kim, Cha et al. 2009). This highlights the need for an ability to measure
diffusion throughout the tumor, in patients. Diffusion-weighted MRI provides a fast means to do this and is described in the next section.

**Measuring Diffusion with DW-MRI**

Diffusion-weighted MRI (DW-MRI) utilizes paired gradients to indirectly measure diffusion (Figure 2-12). The first gradient spatially encodes the positions of the hydrogen nuclei, similar to other MRI gradients. A 2nd gradient, either equivalent to the first and preceded by a 180° pulse or the reverse of the first and without a 180° pulse, reverses the spatial encoding. However, since the gradient varies in space, it can only reverse the encoding for hydrogen nuclei that have not moved. If water molecules have moved due to diffusion, their spins are not exposed to the field strength that will cause re-phasing. The spins therefore are not oriented in the same direction and the associated magnetization vectors do not sum maximally, resulting in signal attenuation. In contrast, if water molecules have not moved, the spins are therefore in phase following application of the DW gradients, allowing the magnetization vectors to sum maximally and the detected signal is not attenuated (Neil 1997). DW-MRI voxels with lower signal intensities therefore correspond to regions of more water motion due to diffusion and voxels with relatively higher intensities are associated with regions of little or no diffusion.

It is important to remember that the physics of DW-MRI imply that compared to signal acquired without diffusion-weighting (b=0), the signal acquired with diffusion-weighting is decreased (high diffusion), less decreased (low diffusion) or equal (no diffusion); the signal is not increased. Diffusion-weighted images may be windowed such
that low diffusing regions appear to have an increased signal compared to $b=0$ images; however, the signal is increased compared to other regions in the image and not compared to the respective locations in the $b=0$ image. In DCE-MRI, the signal following contrast is increased due to the effect of Gd-DTPA on T1; however, in DW-MRI, diffusion-weighting does not cause increased signal. Diffusion is measured with DW-MRI indirectly, as signal attenuation.

**Figure 2-12. Spin echo DW-MRI.** In the figure, the central components of a spin-echo diffusion-weighted MRI sequence are shown. Like other spin echo sequences, the DW-MRI sequence utilizes a 90 pulse to rotate magnetization into the x-y plane. Two gradients of equal strength ($G$) and duration ($\delta$) are applied, one before a 180 pulse and one after. The gradients are separated by time, $\Delta$. $G$, $\delta$, and $\Delta$ determine the b-value, or diffusion-weighting of the sequence. Not shown are the slice select, frequency select, and phase select gradients. As in other MR sequences, these gradients are necessary for the imaging of multiple voxels over multiple slices.

The sensitivity of an acquisition to diffusion is termed the b-value. The b-value is determined by the diffusion-weighted gradient strength, $G$, the duration of the gradients, $\delta$, and the time interval between gradients, $\Delta$. Measurement of diffusivity requires at least two diffusion-weighted acquisitions: one with a low b-value, often 0, and one with a higher b-value. If two series were not acquired, regions of low signal following DW-MRI
could be interpreted as regions of high diffusivity when in fact the regions may have had an equally low signal prior to application of the gradients. In DCE-MRI, images acquired post-contrast are compared to those acquired pre-contrast; similarly, in DW-MRI, images acquired following diffusion-weighting are compared to images acquired prior to diffusion-weighting, or under a different diffusion-weighting. Diffusivity, $D$, is calculated at each voxel based on an assumption of monexponential decay of the original signal, $S_0$, acquired without diffusion-weighted, to the signal acquired with diffusion-weighting, $S_D$, and this relationship is shown below:

$$S_D = S_0e^{-bD}$$

When diffusion is measured with DW-MRI, the measured diffusivity is termed the apparent diffusion coefficient, or ADC. The “apparentness” of the diffusion is attributed to the fact that diffusion in humans is restricted diffusion (Parker 2004). In addition, the measured water motion may have contributions from water motion due to cell streaming or other types of motion, in addition to that considered to be true diffusion (Neil 1997).

The ADC is impacted by the b-values used in the acquisition. In the breast, one study found that ADC varied with the b-values used in the acquisition but that the diagnostic performance of DW-MRI was the same across sets of b-values (Peters, Vincken et al. 2010). The optimal b-value for an acquisition has been related to the diffusivity of the tissue type (Neil 1997):

$$b = 1.1/D$$

Based on this equation, regions with lower diffusivities would require higher b-values for optimal imaging. Diagnostic studies, in which the goal is to detect low diffusing regions,
may require higher b-values than treatment monitoring studies, in which the goal is to monitor a tumor as it is treated and possibly increases in diffusivity. Responding tumor regions may have diffusivities more similar to that of normal fibroglandular tissue. In addition, the b-value inversely varies with signal-to-noise (SNR), and high b-values may reduce SNR and reduce the ability to detect small lesions that remain following treatment with chemotherapy. The optimal b-value for treatment monitoring studies in the breast has not been determined. In our studies, a b-value of 600 or 800 s/mm² has typically been used.

**Processing**

Processing of DW-MRI involves application of an equation that was previously introduced, $S_D = S_0 e^{-bD}$, to each voxel of imaging data, producing an ADC map. This is illustrated in Figure 2-13. The ADC map is analogous to the SER map in that it represents a processed image volume, derived from acquired MR data. The SER map is derived from 3 acquired MR volumes but the ADC map incorporates information from 4 or more volumes. Typically, a DW-MRI scan includes 1 or more $b=0$ acquisitions, and three nonzero b-value acquisitions with diffusion-weighting gradients in orthogonal directions. In diffusion tensor imaging, diffusion-weighting is applied in 6 or more directions.

In DW-MRI, the purpose of acquiring with multiple diffusion-weighting gradients is to allow for averaging of the ADC measured in each direction, in case the motion of the water is different in different directions (Padhani, Liu et al. 2009). Directional motion is termed anisotropy and is due to the underlying directionality of the biological structure.
Motion that is the same in all directions is termed isotropy. Motion in breast tumors is expected to be isotropic due to unregulated cell growth and loss of normal structure. Even in normal tissue which is clearly structured by the orientation of branching ducts anisotropy is small. This may relate to the fact that water molecules diffuse a small distance (on the order of microns) during the DW-MRI acquisition and are unlikely to encounter the borders of ducts, which are sized on the millimeter scale.

Measurement of anisotropy requires acquisition with diffusion-weighted gradients in 6 or more directions and this type of acquisition is termed diffusion tensor imaging (DTI) (Neil 1997). While DW-MRI allows for calculation of the magnitude of diffusivity (ADC), DTI allows for calculation of both the magnitude of diffusivity and the direction of diffusivity (anisotropy). Directionality is measured using a variety of anisotropy indices (fractional anisotropy, relative anisotropy, and others). In preliminary studies, anisotropy, measured as fractional anisotropy, has been found to be 0.24 ± 0.07 (mean ± SD) in breast tumors (Partridge, Ziadloo et al. 2010), 0.29 ± 0.07 (mean ± SD) in normal tissue (Partridge, Ziadloo et al. 2010), and 0.15 (median, with interquartile range = .11-.18) in benign lesions (Baltzer, Schafer et al. 2010).
Figure 2-13. ADC Map. The ADC map is calculated by taking the logarithm of the ratio of signal intensities at each voxel in b=0 and nonzero b-value images and dividing by the b-value. While not shown in the figure, DW-MRI acquisitions typically involve obtaining three of the volumes labeled V2, each acquired with diffusion-weighted gradients oriented in a different direction. The ADC at each voxel is the average of the ADCs obtained from the three directional acquisitions.
Regions of high and low intensity on ADC maps and high and low intensity on acquired images can be qualitatively assessed using the guidelines in Table 2-1. Note that the signal intensity on DW-MRI is inversely related to the intensity on the ADC map. Areas of decreased diffusion (low ADC, dark on ADC) are associated with decreased signal attenuation on DW-MRI (high signal, bright on DW-MRI). Identification of areas that are bright on DW-MRI and dark on ADC therefore provides a means of qualitatively evaluating DW-MRI data.

**Table 2-1. Qualitative interpretation of DW-MRI Data**

<table>
<thead>
<tr>
<th>Diffusion-weighted image</th>
<th>ADC</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bright</td>
<td>Dark</td>
<td>Low diffusivity</td>
</tr>
<tr>
<td>Dark</td>
<td>Bright</td>
<td>High diffusivity</td>
</tr>
<tr>
<td>Bright</td>
<td>Bright</td>
<td>T2-shine through on DW-MRI</td>
</tr>
<tr>
<td>Dark</td>
<td>Dark</td>
<td>Low water content; fibrotic</td>
</tr>
</tbody>
</table>

In addition to being qualitatively analyzed, DW-MRI data can be quantitatively assessed. Quantitative methods allow for measurements related to the diffusivity throughout a particular region to be calculated. Typically, a mean diffusivity in the tumor is reported. The mean tumor ADC is a commonly tracked parameter in treatment monitoring studies and is also used in diagnostic studies. The tumor is identified as a region-of-interest (ROI) and the region is delineated. The mean within the ROI is then calculated. In addition, the upper or lower quartile ADC or the peak height location related to the tumor ADC histogram can be calculated. ROIs and histograms are described in more detail in the next section.
2.8. Image Processing and Analysis

Two operations involving image processing were introduced earlier: calculation of SER and ADC maps. Acquired MR images were manipulated, producing new processed images. The resulting images did not represent original MR data; however, they did contain meaningful information. Image processing has been described as a field in which images constitute both the input and output of operations, but also as a field in which the boundaries are not clearly defined (Gonzalez, Woods et al. 2004).

In the study of diffusion as a predictor of treatment response, diffusion measurements need to be extracted from imaging data. As previously described, this extraction first involves image processing and creation of an ADC map from which parameters will be averaged or otherwise derived. Image processing can also facilitate identification of the regions in the image from which parameters are extracted and these regions of interest are introduced in the next section and are discussed in later chapters. Once processing is complete, additional analysis of the processed image allows parameters to be extracted and compared to meaningful endpoints. One method in image analysis - - histogram analysis - - will be introduced in this section and discussed in later chapters.

Regions of interests

Regions of interest are locations in an image that have clinical or biological value. Further analysis of these locations is facilitated by placement of a region of interest (ROI). One or more ROIs can be placed on a single slice, or multiple slices. ROIs may be manually delineated, in which case a user places the ROI using a pre-defined shape, or by
drawing the ROI free-hand. Alternatively, ROIs may be automatically placed by developing criteria for ROI inclusion. Automatic ROI placement between imaging series requires careful image registration. Following ROI placement, summary statistics, such as the mean and standard deviation of values within the ROI, can then be calculated.

In this study, diffusion is investigated as a predictor for tumor response to treatment; the tumor is therefore the primary region of interest (ROI). However, tumor ROIs vary greatly depending on study criteria. In a study on treatment response for metastatic breast cancer or on breast cancer staging, the ROIs are likely to be at the metastatic sites and multiple ROIs at distant sites may be required (Theilmann, Borders et al. 2004; Heusner, Kuemmel et al. 2010). In the planning or assessment of local surgical treatments, placement of ROIs around the primary tumor may be helpful in investigating whether surrounding regions are cancerous (Yili, Xiaoyan et al. 2009).

In the studies in the following chapters, patients primarily had locally advanced breast cancer, and breast cancer was not yet metastatic. Therefore, assessment of treatment response was limited to the breast region. Cancers with chest wall extension or local lymph node involvement are considered locally advanced. ROIs in a treatment monitoring study could therefore be located in the chest wall or in affected nodes in addition to encompassing the disease within the breast. Exclusion of muscle from the ROI has previously been specified (Nilsen, Fangberget et al. 2010). In addition, in locally advanced breast cancer, DCE-MRI data is used to identify the primary tumor. An SER map can be derived from DCE-MRI data to automatically designate voxels with abnormal contrast-enhancement as tumor. The SER map, which provides a measure of tumor volume that has been shown to correlate with residual disease on pathology, has
not been validated for use in the chest wall or local nodes. ROIs in this study were therefore limited to disease within the breast.

Different methods for breast tumor ROI delineation on ADC maps have been used in different studies. In one study, circular ROIs of a 5-pixel diameter were placed on hypointense regions (Sharma, Danishad et al. 2008). Another study placed 5mm circular ROIs on the brightest region on DW-MRI (Tozaki, Oyama et al. 2010). In other studies, ROIs have been manually drawn on the tumor (Iacconi, Giannelli et al. 2009; Partridge, Demartini et al. 2010). Some studies do not specify method of ROI delineation and the optimal method is not known. In a later chapter, a study is presented in which different methods of ROI delineation, including a new method, are described and compared.

**Histogram Analysis**

A histogram is used for data visualization. While the concept of a histogram is old and is thought to date back to the 1660s (Rao, Wegman et al. 2005), it is integral to applications in statistics, photography, medical imaging, business, and other fields. Simply, a histogram illustrates how many points in a data set fall between particular intervals. The range of values in a data set is divided into intervals, or bins. The length of each interval is the bin width. A histogram plots bin widths on the x axis and the count of values in each bin on the y axis. The result is a display of the distribution of values in the data (Figure 2-14).

When the y axis refers not to the frequency of data points in each bin, but to the proportion of data points within in each bin, the histogram is referred to as a normalized histogram (Figure 2-14). Normalized histograms are particularly important in comparing
datasets with unequal sample numbers and are used in Chapters 11 and 12 to compare ADC distributions obtained with high and low-resolution MRI acquisitions. Resolution determines the number of voxels covering a particular area and histograms are then used to bin voxels according to ADC value. A third type of histogram is the density histogram, in which the normalized histogram is further normalized to bin width (Figure 2-14) such that the area of each bar is equal to the density within the bin and the sum of all bars is equal to 1 (Chen, Härdle et al. 2007).
Figure 2-14. Histograms. The three histograms differ only in the units of the y axis: pixels, proportion of pixels, and density of pixels for the standard, normalized, and density histograms, respectively.

Note that in the figure above, the normalized and density histograms look similar to the standard histogram and only the units of the y axis differ between the three
Histograms. The x-axis is the same for the three histograms. Histograms can change drastically depending on the x-axis increments, determined by the bin width. The choice of bin number can also impact the histogram. Formulas have been developed to guide bin width and bin number determinations (Dekking 2005):

\[
\text{bin number} = 1 + 3.3 \log_{10}(\text{sample size})
\]

\[
\text{bin width} = 3.49(\text{sample SD})(\text{sample size})^{\frac{-1}{3}}
\]

Histogram smoothing or construction of alternative density plots may be needed for optimal data visualization (Dekking 2005). However, the standard histogram is useful in understanding the distribution of values in a dataset. A histogram allows for calculation of relevant parameters such as peak height and peak height location.

Independent of the histogram, several parameters can be used to summarize the distribution of data: mean (average), median (50th percentile value), mode (most commonly occurring value, analogous to peak height but not limited by histogram binning), lower quartile (25th percentile value), upper quartile (75th percentile value), median of the lower quartile (12.5th percentile value), median of the upper quartile (87.5th percentile value), skew, and kurtosis. Because a histogram can be used to visualize these values, “histogram analysis” often refers to calculation of values derived from both the histogram, and values related to the distribution of the data, for which calculation does not require construction of a histogram.

Histograms do not provide information about the location of particular data points; however, histogram analysis can be combined with tumor ROI delineation so that
the distribution of values within a tumor can be visualized. Chapters 8, 11, and 12 describe histogram analyses of ROIs. This technique is still limited in that the locations of particular histogram frequencies cannot be derived. In diffusion-weighted MRI, the frequency count of a bin in the lower ADC range may be thought to represent the densest aspect of the tumor; however, these voxels could be located within adipose tissue or within regions affected by partial voluming with adipose tissue. Despite this shortcoming, histogram analysis of regions of interest have been useful in multiple anatomical sites, for multiple diseases. In the breast, heterogeneity of contrast uptake measured with DCE-MRI has been correlated with a poor treatment response (Venkatasubramanian, Arenas et al. 2010). In the next section, methods used to test relationships between MR variables and outcomes are discussed.

2.9. Statistics

In Chapter 1, the origins and prevalence of breast cancer were discussed. Each cancer arises from a unique set of mutations, making it unlikely that two individuals with breast cancer will have the exact same cancer. Breast cancer is the most common non skin-cancer in women, and each year, hundreds of thousands of new cases are diagnosed. The population of individuals with breast cancer is therefore large and diverse. Research studies attempt to improve the diagnosis and treatment of breast cancer, but it would be practically impossible for a researcher to include all breast cancer patients in a study. Due to this constraint, researchers recruit a sample of patients from the larger population of all breast cancer patients. The population may include only patients with a particular stage of breast cancer or with a particular molecular profile. Even when the population is
narrow, the study sample is unlikely to perfectly represent the population, but inferences can still be made. Using statistics, study of the sample is extended to the larger population from which it was drawn (Samuels and Witmer 1999).

Statistical analyses can be divided into two groups: descriptive and inferential statistical analyses. Descriptive statistics allow data to be summarized (Samuels and Witmer 1999). Variables such as the mean and standard deviation are used to describe quantitative data in a sample. For example, in a particular study, mean MR tumor volume may be 10 cm³; this mean is a descriptive statistic. Proportions may be used to describe qualitative data. For example, 20% of the patients may have a grade 2 tumor and the percentage 20% is a descriptive statistic.

Inferential statistics allows findings from the sample to be extended to the population and involves testing of hypotheses. Hypothesis testing provides a large role in the chapters that follow. In the study of treatment response, MR measurements may be made in a group of patients later determined to be responders or non-responders. Differences in the MR measurements between responders and non-responders can be assessed using the t-test, or in the case of nonparametric data, using the Wilcoxon rank-sum test. In general, a statistical test includes 4 parts: 1) a null hypothesis, $H_0$, 2) an alternative hypothesis, $H_A$, 3) a test of the hypothesis and calculation of a p-value, and 4) a decision regarding the rejection of $H_0$. The null hypothesis states that the groups are the same, or that there is no effect; the alternative hypothesis states that the groups are not the same, or that there is an effect. The hypothesis test is an appropriate statistical test used to evaluate differences between the groups or to evaluate the hypothesis. The probability that the difference found in the test could be obtained if the null were true is the p-value.
(Samuels and Witmer 1999). A small p-value is therefore associated with a small chance that the null is true and if the p-value is smaller than a pre-determined threshold, the significance level (alpha), the null hypothesis is rejected.

In this work, diffusion is evaluated as a biomarker for treatment response. MR parameters, such as tumor diffusivity, are evaluated as predictor variables for outcome variables describing treatment response. Recurrence-free survival time describes patient outcomes. Cox regression is a statistical method that can be used to evaluate if particular predictor variables are associated with a time-dependent increased risk of recurrence and is used in multiple chapters. Another statistical method used throughout this work is the Bland-Altman method for comparing measurements made with two methods. The Bland-Altman is used both in comparing measurements made with different MR acquisitions and in comparing measurements made with different processing algorithms.

Statistical analyses are central to hypothesis testing, but they do not necessarily provide “final answers” to research questions (Figure 2-15). A study may be too small to have sufficient power to observe an effect. In addition, the experimental design may have been prone to confounding variables. Even if an experiment is well-designed and large enough for sufficient power, and even if a study finding is statistically significant it may not be clinically significant. The magnitude of the difference may not be great enough to warrant change in clinical practice or to confer meaningful benefits to patients. Statistical analyses are an important component of hypothesis-driven research but the impact of the results is dependent on the experimental design, research question, and clinical significance of the effect.
Figure 2-15. Methods in Imaging Studies. A hypothesis forms the basis for methods used in imaging studies. Methods related to image acquisition (DCE-MRI, DW-MRI), image processing (SER and ADC map calculation), image analysis (ROI delineation, histogram construction), and statistics (Cox regression) were discussed in this chapter.

2.10. Imaging Studies at UCSF

Since the late 1990s, patients with locally advanced breast cancer and treated with neoadjuvant chemotherapy have participated in imaging studies at UCSF. These studies have used MRI to monitor the tumor before, during, and after treatment with chemotherapy. Five independent treatment monitoring studies have occurred at our institution: 1) a single institution pilot study, 2) the multi-center study ACRIN 6657, 3)
the multi-center study ACRIN 6657 Extension, 4) a single institution study, and 5) the multi-center study ACRIN 6693 (Appendix 2). Throughout this work, the patient groups enrolled in these studies are referred to as Groups 1-5, respectively. Due to availability of DW-MRI data, this work only includes imaging data from patients enrolled at UCSF. In addition, not all patients enrolled at UCSF were scanned with DW-MRI.

Although all five patient groups were scanned with similar imaging protocols and treated with a similar sequence of therapies (chemotherapy, surgery, adjuvant therapy), differences in chemotherapy regimens, timing of MR exams, and DW-MRI parameters prevent aggregated analyses of all data from these patients. Some of these differences are highlighted in Figure 2-16. Despite the fact that these differences prevented aggregation of all data into a larger sample, these differences also allowed additional research questions to be addressed. The neoadjuvant DW-MRI studies at UCSF span a timeframe of more than ten years, allowing for both retrospective and prospective analyses. In the following chapters, factors associated with the ability of diffusion to predict treatment response are investigated.
Figure 2-16. Patients treated with neoadjuvant chemotherapy and scanned with DW-MRI. Differences between groups of patients enrolled in neoadjuvant studies at UCSF are illustrated. Each yellow arrow denotes an MR exam. Spacing between arrows represents the time interval between exams. AC = adriamycin (doxorubicin)/cyclophosphamide. Taxol = paclitaxel and may be substituted with another taxane. In Group 5, Drugs A and B are investigational drugs. Trial arms including other investigational drugs and/or trastuzumab for HER2+ cancers are not shown.
2.11. References


Part II: Retrospective Study

Diffusion reflects underlying cellular and molecular properties of tissue, and because chemotherapy results in cell death, and subsequent decreases in cell density, diffusion is expected to change in tumor regions responding to chemotherapy. This expectation has been investigated in a variety of cancer types, including breast cancer. In the last few years, studies from around the world have both supported and refuted this hypothesis in patients with locally advanced breast cancer treated with neoadjuvant chemotherapy. In these prior studies, changes in diffusion were related to surrogate outcomes such as tumor size. At the time of this writing, the ability of diffusion to predict long-term outcomes in patients with locally advanced breast cancer is not known; this data is much needed for improved understanding of the role of diffusion in treatment response monitoring.

In this section, a study is presented in which diffusion measurements are related to long-term patient outcomes. Prior to this study, it was known that diffusion correlated with patient outcomes, but its relationship to long-term outcomes had not been reported. The additional studies described in later chapters were motivated by the relationship between diffusion and surrogate outcomes described in prior studies, as well as by the relationships presented in the following chapter.
Chapter 3. Diffusion as a Predictor of Treatment Response

3.1. Introduction

Locally advanced breast cancer (LABC) accounts for 7% of newly diagnosed breast cancers in the United States (Giordano 2003) and as many as 20% to more than 50% of cases in developing countries (Newman 2009). The stages corresponding to LABC are associated with a 5-year survival of 48-70%, (Newman 2009), compared to 89% overall for breast cancer (Jemal, Siegel et al. 2010). A combination of chemotherapy, surgery, and other adjuvant treatments are the standard of care in LABC.
Chemotherapy can be implemented prior to (neoadjuvant) or after (adjuvant) surgery. Neoadjuvant chemotherapy has been shown in randomized controlled trials to be as effective as adjuvant chemotherapy in patients with locally advanced breast cancer (van der Hage, van de Velde et al. 2001; Wolmark, Wang et al. 2001; Rastogi, Anderson et al. 2008). At the same time, neoadjuvant chemotherapy provides additional benefits of allowing for the possibility of breast-conserving surgery and for tumor response to chemotherapy to be monitored while the tumor is still present.

In anthracycline-based regimens, response rates in the form of pathological complete response (pCR), the standard surrogate endpoint, have been reported as ranging from 3-46% (Mathew, Asgeirsson et al. 2009). Pathologic response to chemotherapy has been shown to be an important predictor of survival (Herold and Marcom 2008). With 54% or more patients not responding to treatment, the ability to identify patients not responding to therapy as early and accurately as possible would have the potential to improve long-term patient outcomes. Current methods of monitoring tumor response to treatment include assessment of tumor size by clinical palpation, mammography, ultrasound, and magnetic resonance imaging (MRI). However, these methods have shown poor to moderate correlation with pathological size; in one study, correlation was 19%, 26%, 35%, and 71%, respectively (Yeh, Slanetz et al. 2005). The need for effective response monitoring has motivated research into additional methods, one of which is diffusion-weighted magnetic resonance imaging (DW-MRI). DW-MRI is a fast, non-contrast, non-ionizing method of imaging and over the past few years, interest in its use to monitor neoadjuvant chemotherapy has increased.
The value of DW-MRI is derived from its ability to provide a quantitative, in vivo, measurement of water mobility (Neil 1997). Multiple investigations have reported efficacy with chemotherapy response monitoring in patients with locally advanced breast cancer using DW-MRI (Manton, Chaturvedi et al. 2006; Pickles, Gibbs et al. 2006; Yankeeelov, Lepage et al. 2007; Kuroki and Nasu 2008; Sharma, Danishad et al. 2008; Iacconi, Giannelli et al. 2009). In human breast cancer, the apparent diffusion coefficient (ADC) of the tumor has been shown to increase after treatment with chemotherapy (Yankeeelov, Lepage et al. 2007). Tumor ADC changes have also been shown to precede DCE-MRI-derived size changes (Pickles, Gibbs et al. 2006; Sharma, Danishad et al. 2008). However, the relationship of those changes to long-term patient outcomes is not yet known and this gap motivates our current study.

It was previously shown that DCE-MRI derived tumor volume and diameter are predictors of RFS (Partridge, Gibbs et al. 2002; Partridge, Gibbs et al. 2005; Li, Partridge et al. 2008). We hypothesize that change in tumor ADC would reflect response to treatment and provide complementary information to DCE-MRI measures for predicting recurrence free survival. The purpose of this study was to investigate the ability of DWI to predict treatment response and outcome in women with locally advanced breast cancer undergoing neoadjuvant chemotherapy.
3.2. Materials and Methods

Overview

Between February 1995 and May 2002, patients with locally advanced breast cancer and undergoing neoadjuvant chemotherapy were enrolled in a pilot study at our institution with institutional review board approval. Patients were scanned with MRI prior to, during, and after treatment with neoadjuvant chemotherapy. Beginning in 1998 a DWI sequence was incorporated into the MR examination and the patients scanned with both DCE- and DW-MRI cases were retrospectively evaluated for this study.

Patients

All patients were women with locally advanced breast cancer, defined as stage II and stage III cancer that required systemic chemotherapy and had not spread beyond the breast or regional lymph nodes. Patients with inflammatory breast cancer or chest wall involvement were not excluded. Only patients aged 18 or older were included in the study. An institutional review board approved the study protocol, and each subject gave informed consent. All clinical data, including ages, menopausal status, outcomes, clinical management, and pathological size and diagnosis, was obtained from the medical records. All patients had invasive breast cancer diagnosed by core biopsy or fine needle aspiration and underwent neoadjuvant chemotherapy. Additional inclusion criteria for this retrospective analysis were the following: baseline DW-MRI and DCE-MRI, completion of chemotherapy, completion of surgery, and follow-up for recurrence.
**Imaging**

Imaging was performed on a 1.5 Tesla GE Signa scanner (GE Medical Systems, Milwaukee, WI). For patients scanned before 2000, a closed breast coil was used (GE Medical Systems); for patients scanned after 2000, two open breast coils were used (MRI Devices, Waukesha, WI). *Overview:* Patients were scanned before initiation of chemotherapy (MR1), after the first cycle and before the second cycle of chemotherapy (MR2), and at its completion but prior to surgery (MR3). Each MR examination included both DCE and DW-MRI except as otherwise noted.

**Dynamic-contrast enhanced imaging**

Dynamic-contrast enhanced MRI (DCE-MRI) was acquired using a T1-weighted three-dimensional fast gradient recalled echo (3DFGRE) sequence with the following parameters: TR = 8 ms, TE = 4.2 ms, flip angle = 20° flip angle, field of view = 18-20 cm, slice thickness = 2 mm, and acquisition matrix = 256 x 192, for an in-plane resolution of approximately 0.7 x 0.94mm. The images were fat suppressed and two repetitions were acquired for oversampling to remove phase wrap. Sixty slices were acquired in the sagittal orientation to cover the affected breast. Gadopentetate Dimeglumine (Magnevist, Schering AG, Berlin, Germany) was administered as a contrast agent at a dose of 0.1 mmol/kg body weight, followed by a saline flush of 10 mL saline. Three time points were acquired during each MR examination: 1) pre-contrast injection, 2) post-contrast centered at 2.5 minutes after injection, 3) post-contrast centered at 7.5 minutes after injection.
**Contrast-enhanced MRI**

Following DCE-MRI, a T1-weighted 2D fast gradient echo sequence was acquired axially to improve lesion identification on the axial DW-MRI. Images were acquired with the following imaging parameters: TR = 100 ms, TE = 4.2 ms, flip angle = 40°, NEX = 2, field of view = 35 cm, slice thickness = 5 mm with 0 skip, and acquisition matrix = 256 by 192, for an in-plane resolution of 1.37 x 1.82 mm.

**Diffusion-weighted imaging**

Diffusion-weighted imaging data was provided by a diffusion-weighted single shot fast spin echo (DW-SSFSE) sequence (Partridge, McKinnon et al. 2001). For breast imaging, the DW-SSFSE sequence provided an important advantage over DW-echo planar imaging (DW-EPI) sequences by minimizing distortion artifacts due to magnetic susceptibility differences at air-tissue interfaces. For patients scanned prior to 2000, diffusion data was acquired with the following parameters: TR = 8.00 s, TE = 86.2 ms, NEX = 0.5, FOV = 35 cm, slice thickness = 5 mm, acquisition matrix = 128 x 128 for an in-plane resolution of 2.73 x 2.73 mm, and \( b = 0 \) and 600 s/mm\(^2\). For patients scanned after 2000, diffusion data was acquired with TR= 6.22 s, TE = 61.5 ms. Diffusion-weighted gradients were applied independently in the x, y, and z directions, with a gradient duration \( \delta = 26.8 \) msec, interval \( \Delta = 34.8 \) msec, and gradient strength of \( G_D = 21 \) mT/m. The scan duration was 2 minutes. Exams were acquired in 10 axial slices bilaterally over the tumor volume.
Image Processing and Analysis

Apparent diffusion coefficient (ADC) maps were calculated from DW-MRI data based on an assumption of monoexponential decay of the original MR signal, \( S_{b=0} \), to the signal, \( S_{b=600} \), acquired with diffusion-weighted gradients of a weighting factor = \( b \):

\[
ADC = \frac{-\ln S_{b=600}}{S_{b=0} - S_{b=0}}
\]

Tumor was manually defined on ADC maps; enhancement on the post-contrast T1-weighted image was used as a guide for ROI placement. The post-contrast T1-weighted series matched the ADC map in slice thickness, facilitating ROI placement.

The sum of all ROIs for a given tumor formed the tumor volume of interest (VOI). The same VOIs were applied to fat-suppressed and non-fast-suppressed data for a given exam, but at each new timepoint (MR1, MR2 and MR3), new tumor ROIs were defined. A mean tumor ADC (tADC) was then calculated for all voxels within the tumor VOIs for each MRI exam. For each MRI exam, a mean ADC of normal fibroglandular tissue was calculated using ROIs manually drawn in regions of contralateral fibroglandular breast tissue. A normalized tumor ADC (nADC) was then calculated as tADC divided by the mean ADC of normal fibroglandular tissue.

Statistics

To determine the role of DW-MRI in monitoring treatment, statistical analysis was performed with MR measurements as predictor variables. The primary outcome of this study was recurrence free survival (RFS). Differences between patients recurring and
not recurring were assessed with either the t-test (parametric data) or Wilcoxon rank sum test (nonparametric data). Data was assessed for normality with the Lillifors test, at alpha=.05. Univariate Cox proportional hazards analysis was used to determine the impact of predictor variables on recurrence-free survival time, with alpha=.05. As a secondary analysis, differences between patients responding and not responding to treatment were similarly assessed. Response was defined as final percent change in tumor volume of 65% or more, compatible with the RECIST criteria for partial response (Gwyther and Schwartz 2008), and non-response was defined as all other changes. The purpose of the secondary analysis was to allow comparison to results of other studies. For all tests, statistical significance was defined as p<.05. Adjustment for multiple comparisons was not made as this analysis was exploratory. Statistical analyses were performed with Matlab (Version R2007a, The Mathworks, Inc., Natick, MA). Methods and results from an ROC analysis are in Appendix 3.

3.3. Results

Patient accrual and follow-up

During the study period, 42 patients underwent neoadjuvant chemotherapy and were scanned with DW-MRI. Twelve of the 42 patients were excluded from the study due to failure to meet inclusion criteria (1 patient had metastatic disease, 1 did not sign informed consent, 1 did not have surgery, 2 were lost to follow-up and 7 had no baseline DW-MRI), resulting in a final study cohort of 30 patients. Patients with MR exam timing differing from the planned protocol, resulting in two or more cycles of chemotherapy
before MR2, and/or lack of diffusion at MR2, were excluded from analysis at the affected timepoint but not from the study (Figure 3-1).

Patients in the study cohort ranged in age from 32-71 years (mean, 48 years). For 13 of the 30 women, the chemotherapy regimen consisted of four cycles of doxorubicin and cyclophosphamide (AC), with 21 days between cycles; AC was followed by a taxane (AC-T) in 15 of the women; and for 2 of the women, the regimen was fluorouracil, epirubicin, and a cyclophosphamide (FEC). Patient characteristics are summarized in Table 3-1.

The 30 patients in this study were followed for a mean of 235 weeks, or 4.5 years. Four patients achieved a pathological complete response (pCR) as defined by no residual invasive disease in the breast. Ten patients recurred during the follow-up period. Outcomes are summarized in Table 3-1.
Figure 3-1. Flowchart of patient accrual. This flowchart shows the patients included in the study cohort. Patients were monitored with DW-MRI throughout treatment (timepoints t1, t2, t3). *Of the 12 patients excluded from the DW-MRI analysis, 2 did not meet initial inclusion criteria: 1 had metastatic disease and 1 did not give consent. **Of the 10 patients excluded for other reasons, 2 were lost to follow-up, 1 did not have surgery, and 7 did not have baseline DW-MRI. ***Eleven patients were excluded from t2 analysis due to protocol deviations: 2 patients did not receive DW-MRI at t2, 8 patients received chemotherapy cycle 2 before t2, and 1 patient met both of these exclusion criteria.
<table>
<thead>
<tr>
<th>Table 3-1. Patient Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Period of scanning</td>
</tr>
<tr>
<td>Mean age (range)</td>
</tr>
<tr>
<td>Number pre/post/perimenopausal</td>
</tr>
<tr>
<td>Mean interval (wks) from first MRI to second MRI (range)</td>
</tr>
<tr>
<td>Mean interval (wks) from first MRI to date of surgery (range)</td>
</tr>
<tr>
<td><strong>Clinical Assessment:</strong></td>
</tr>
<tr>
<td>Mean (cm) initial clinical tumor size (range)</td>
</tr>
<tr>
<td><strong>Pathological Assessment:</strong></td>
</tr>
<tr>
<td>Invasive ductal</td>
</tr>
<tr>
<td>Invasive lobular</td>
</tr>
<tr>
<td>Mixed (invasive ductal/invasive lobular)</td>
</tr>
<tr>
<td>Mucinous</td>
</tr>
<tr>
<td>Inflammatory</td>
</tr>
<tr>
<td>Adenocarcinoma not otherwise specified</td>
</tr>
<tr>
<td>Grade 1</td>
</tr>
<tr>
<td>Grade 2</td>
</tr>
<tr>
<td>Grade 3</td>
</tr>
<tr>
<td>Unknown Grade</td>
</tr>
<tr>
<td><strong>Treatment regimens:</strong></td>
</tr>
<tr>
<td>AC (Adriamycin (doxorubicin)/ cyclophosphamide)</td>
</tr>
<tr>
<td>AC-T (Adriamycin/cyclophosphamide + taxane)</td>
</tr>
<tr>
<td>FEC (fluorouracil/epirubicin/cyclophosphamide)</td>
</tr>
<tr>
<td><strong>Surgery</strong></td>
</tr>
<tr>
<td>Lumpectomy</td>
</tr>
<tr>
<td>Mastectomy</td>
</tr>
<tr>
<td><strong>Outcomes:</strong></td>
</tr>
<tr>
<td>Number of recurrences</td>
</tr>
<tr>
<td>Local recurrence</td>
</tr>
<tr>
<td>Distant recurrence</td>
</tr>
<tr>
<td>Mean (wks) time-to-recurrence (range)</td>
</tr>
<tr>
<td>Mean (wks) disease-free interval (range)</td>
</tr>
<tr>
<td>Pathological complete responders</td>
</tr>
<tr>
<td>Following chemotherapy, mean invasive path size (range)</td>
</tr>
<tr>
<td>Following chemotherapy, number with positive lymph nodes</td>
</tr>
</tbody>
</table>
Primary outcome: Recurrence free survival

The primary aim of this study was to determine if ADC predicts recurrence free survival (RFS). While none of the variables evaluated were significantly predictive (p<.05) of whether someone would recur or not within 3 years after surgery, (Table 3-2), early percent change in normalized tumor ADC (nADC) (p=.054) showed a trend towards significance, and it was associated with the lowest p-value of any MR variable in prediction of 3-year RFS. The median early percent change in nADC in patients recurring was -1.59%, compared to a median percent change of 28.19% in patients who did not recur. Early percent change in MR longest diameter (p=.063), and initial tumor volume (p=.070) also showed trends towards significance. In patients recurring, the median early percent change in MR LD was -4.11%, less than the median of -12.45% in patients not recurring and the median MR volume at baseline was 33.1 cm³ in patients recurring, compared to 16.48 cm³ in patients not recurring.

The ability of ADC to predict recurrence free survival time (RFST) was also assessed (Table 3-3). In univariate Cox analysis, no variables at MR1 or MR2 were significant predictors at alpha=.05. The only variable measured at MR1 or MR2 to show a trend towards significance in predicting RFST was nADC at MR2 (HR=0.086, p=.062), suggesting higher nADC after 1 cycle of chemotherapy was associated with longer RFS. Final tumor volume at MR3 was significantly predictive of RFST (HR=1.14, p=.032), with increased tumor size associated with increased risk of recurrence.
Table 3-2. Predictors of 3-year RFS

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>N, total</th>
<th>N, recurring</th>
<th>N, not recurring</th>
<th>Median, recurring</th>
<th>Range, recurring</th>
<th>Median, not recurring</th>
<th>Range, not recurring</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>30</td>
<td>9</td>
<td>21</td>
<td>1192.70</td>
<td>954.39 - 1811.2</td>
<td>1156.40</td>
<td>241.9 - 1649.6</td>
<td>0.208</td>
</tr>
<tr>
<td>Post cycle-1</td>
<td>18</td>
<td>7</td>
<td>11</td>
<td>1086.40</td>
<td>984.6 - 2085.1</td>
<td>1322.90</td>
<td>772.8 - 1606.1</td>
<td>0.614</td>
</tr>
<tr>
<td>Final</td>
<td>26</td>
<td>8</td>
<td>18</td>
<td>1124.75</td>
<td>889.15 - 1588</td>
<td>1170.55</td>
<td>406.63 - 1620</td>
<td>0.893</td>
</tr>
<tr>
<td>Early % change</td>
<td>18</td>
<td>7</td>
<td>11</td>
<td>8.33</td>
<td>-8.91 - 32.14</td>
<td>9.35</td>
<td>-5.94 - 219.47</td>
<td>0.659</td>
</tr>
<tr>
<td>Final % change</td>
<td>26</td>
<td>8</td>
<td>18</td>
<td>-8.16</td>
<td>-26.54 - 21.48</td>
<td>0.62</td>
<td>-34.61 - 263.5</td>
<td>0.846</td>
</tr>
<tr>
<td>Normalized ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Initial</td>
<td>26</td>
<td>9</td>
<td>17</td>
<td>0.94</td>
<td>0.71 - 1.75</td>
<td>0.94</td>
<td>0.36 - 1.54</td>
<td>0.834</td>
</tr>
<tr>
<td>Post cycle-1</td>
<td>15</td>
<td>7</td>
<td>8</td>
<td>0.84</td>
<td>0.67 - 1.73</td>
<td>1.16</td>
<td>0.61 - 1.97</td>
<td>0.409</td>
</tr>
<tr>
<td>Final</td>
<td>23</td>
<td>7</td>
<td>16</td>
<td>1.03</td>
<td>0.53 - 2.22</td>
<td>1.05</td>
<td>0.28 - 3.28</td>
<td>0.664</td>
</tr>
<tr>
<td>Early % change</td>
<td>15</td>
<td>7</td>
<td>8</td>
<td>-1.59</td>
<td>-15 - 34.31</td>
<td>28.19</td>
<td>-0.55 - 173.75</td>
<td>0.054</td>
</tr>
<tr>
<td>Final % change</td>
<td>22</td>
<td>7</td>
<td>15</td>
<td>13.97</td>
<td>-32.8 - 51.67</td>
<td>17.46</td>
<td>-50.3 - 297.07</td>
<td>0.724</td>
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<tr>
<td>MRI Volume</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>30</td>
<td>9</td>
<td>21</td>
<td>33.10</td>
<td>5.32 - 137.47</td>
<td>16.48</td>
<td>0.52 - 97</td>
<td>0.070</td>
</tr>
<tr>
<td>Post cycle-1</td>
<td>21</td>
<td>8</td>
<td>13</td>
<td>16.98</td>
<td>0.53 - 80.93</td>
<td>4.73</td>
<td>0.54 - 40.96</td>
<td>0.491</td>
</tr>
<tr>
<td>Final</td>
<td>30</td>
<td>9</td>
<td>21</td>
<td>2.27</td>
<td>0 - 23.34</td>
<td>1.43</td>
<td>0.01 - 12.39</td>
<td>0.298</td>
</tr>
<tr>
<td>Early % change</td>
<td>21</td>
<td>8</td>
<td>13</td>
<td>-33.63</td>
<td>-95.9 - 0.24</td>
<td>-31.05</td>
<td>-84.05 - 152.79</td>
<td>0.587</td>
</tr>
<tr>
<td>Final % change</td>
<td>30</td>
<td>9</td>
<td>21</td>
<td>-82.73</td>
<td>-100 - 36.29</td>
<td>-88.64</td>
<td>-99.83 - 34.15</td>
<td>0.892</td>
</tr>
<tr>
<td>MRI LD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>30</td>
<td>9</td>
<td>21</td>
<td>6.59</td>
<td>2.5 - 12.7</td>
<td>5.80</td>
<td>2.5 - 10.76</td>
<td>0.272</td>
</tr>
<tr>
<td>Post cycle-1</td>
<td>21</td>
<td>8</td>
<td>13</td>
<td>4.71</td>
<td>2.5 - 12.9</td>
<td>3.23</td>
<td>2.38 - 9.42</td>
<td>0.158</td>
</tr>
<tr>
<td>Final</td>
<td>29</td>
<td>9</td>
<td>20</td>
<td>2.25</td>
<td>0.5 - 8.1</td>
<td>3.66</td>
<td>0 - 8.98</td>
<td>0.989</td>
</tr>
<tr>
<td>Early % change</td>
<td>21</td>
<td>8</td>
<td>13</td>
<td>-4.11</td>
<td>-21.28 - 1.57</td>
<td>-12.45</td>
<td>-33.05 - 3.62</td>
<td>0.063</td>
</tr>
<tr>
<td>Final % change</td>
<td>30</td>
<td>9</td>
<td>21</td>
<td>-29.80</td>
<td>-92.41 - 13.26</td>
<td>-32.50</td>
<td>-100 - 16</td>
<td>0.761</td>
</tr>
</tbody>
</table>

Table 3-2. Recurrence-free survival was censored at 3 years and the group of patients that did not recur was compared to the group of patients that did recur. Differences between variables were tested using the t-test or Wilcoxon.
Table 3-3. Predictors of RFS Time

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>N</th>
<th>Median</th>
<th>Range</th>
<th>HR*</th>
<th>95% CI for HR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>30</td>
<td>1158.60</td>
<td>242-1810</td>
<td>0.9998</td>
<td>0.9981-1.0015</td>
<td>0.855</td>
</tr>
<tr>
<td>Post-cycle 1</td>
<td>18</td>
<td>1174.85</td>
<td>773-2090</td>
<td>0.9999</td>
<td>0.9977-1.0022</td>
<td>0.957</td>
</tr>
<tr>
<td>Final</td>
<td>26</td>
<td>1170.55</td>
<td>4077-1620</td>
<td>1.0003</td>
<td>0.9977-1.0029</td>
<td>0.827</td>
</tr>
<tr>
<td>Early % change</td>
<td>18</td>
<td>8.84</td>
<td>-8.91-219</td>
<td>1.0015</td>
<td>0.9913-1.0117</td>
<td>0.775</td>
</tr>
<tr>
<td>Final % change</td>
<td>26</td>
<td>-4.45</td>
<td>-34.6-263</td>
<td>1.0047</td>
<td>0.9973-1.0121</td>
<td>0.217</td>
</tr>
<tr>
<td>Normalized ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>26</td>
<td>0.94</td>
<td>0.361-1.75</td>
<td>0.1804</td>
<td>0.0175-1.8557</td>
<td>0.150</td>
</tr>
<tr>
<td>Post-cycle 1</td>
<td>15</td>
<td>1.00</td>
<td>0.605-1.97</td>
<td>0.0862</td>
<td>0.0066-1.1307</td>
<td><strong>0.062</strong></td>
</tr>
<tr>
<td>Final</td>
<td>23</td>
<td>1.03</td>
<td>0.280-3.28</td>
<td>0.4143</td>
<td>0.0676-2.5393</td>
<td>0.341</td>
</tr>
<tr>
<td>Early % change</td>
<td>15</td>
<td>6.41</td>
<td>-15.0-174</td>
<td>0.9974</td>
<td>0.9814-1.0136</td>
<td>0.751</td>
</tr>
<tr>
<td>Final % change</td>
<td>22</td>
<td>15.91</td>
<td>-50.3-297</td>
<td>1.0019</td>
<td>0.9943-1.0095</td>
<td>0.625</td>
</tr>
<tr>
<td>MRI Volume</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>30</td>
<td>17.46</td>
<td>0.521-137</td>
<td>1.0132</td>
<td>0.9973-1.0293</td>
<td>0.103</td>
</tr>
<tr>
<td>Post-cycle 1</td>
<td>21</td>
<td>6.33</td>
<td>0.530-80.9</td>
<td>1.0077</td>
<td>0.9752-1.0412</td>
<td>0.648</td>
</tr>
<tr>
<td>Final</td>
<td>30</td>
<td>1.87</td>
<td>0.0024-23.3</td>
<td>1.1411</td>
<td>1.0117-1.2871</td>
<td><strong>0.032</strong></td>
</tr>
<tr>
<td>Early % change</td>
<td>21</td>
<td>-31.05</td>
<td>-95.9-153</td>
<td>0.9900</td>
<td>0.9716-1.0087</td>
<td>0.293</td>
</tr>
<tr>
<td>Final % change</td>
<td>30</td>
<td>-87.93</td>
<td>-100-34.2</td>
<td>1.0097</td>
<td>0.9796-1.0407</td>
<td>0.533</td>
</tr>
<tr>
<td>MRI LD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>30</td>
<td>5.90</td>
<td>2.5-12.7</td>
<td>1.0557</td>
<td>0.8220-1.356</td>
<td>0.671</td>
</tr>
<tr>
<td>Post-cycle 1</td>
<td>21</td>
<td>3.50</td>
<td>2.38-12.9</td>
<td>1.0632</td>
<td>0.8394-1.3468</td>
<td>0.611</td>
</tr>
<tr>
<td>Final</td>
<td>29</td>
<td>2.91</td>
<td>0-8.98</td>
<td>1.0002</td>
<td>0.7886-1.2716</td>
<td>0.999</td>
</tr>
<tr>
<td>Early % change</td>
<td>21</td>
<td>-11.07</td>
<td>-33.0-1.57`</td>
<td>1.0472</td>
<td>0.9690-1.1317</td>
<td>0.244</td>
</tr>
<tr>
<td>Final % change</td>
<td>30</td>
<td>-31.15</td>
<td>-100-16</td>
<td>1.0002</td>
<td>0.9817-1.019</td>
<td>0.987</td>
</tr>
</tbody>
</table>

Tumor variables were assessed in univariate Cox regression for prediction of recurrence-free survival time. *Hazard Ratio (HR) describes instantaneous relative risk of event for individual with increase of 1 in the value of the continuous variable compared with an individual who does not have an increase of 1, given that all other variables are equal.
Secondary outcome: Volumetric tumor response

For the purposes of comparing to prior studies, which used volumetric tumor response as an outcome variable, ADC was assessed for its ability to predict response as defined as final change in MR volume. Response was defined as ≥65% final reduction in MR tumor volume. On this basis, six patients were classified as responders and 24 as nonresponders. ADC was not predictive at alpha = .05; however, the final percent change in tADC showed a trend towards significance (p=.051).

Selected patient cases

Two patient cases illustrate relationships between tumor volume, tumor diffusivity, and RFS seen on the individual patient level. In general, decreased MR volume, decreased MR LD, and increased ADC indicate a good response to chemotherapy.

In Case 1 (Figure 3-2), DCE- and DW-MRI findings were concordant for a good response, and the patient did not recur. DCE-MRI showed a final decrease in volume of -83%, a partial response by RECIST, and DW-MRI showed an increase in tADC of +18.6%. Notably, the greater changes in volume occurred after more than one cycle of chemotherapy, while the greater changes in diffusion occurred after once cycle of chemotherapy.

In Case 2 (Figure 3-3), DCE-MRI and DW-MRI findings were discordant for a good response (decreased MR volume and LD and decreased ADC) and the patient recurred. Based on DCE-MRI, the patient's tumor had a final decrease in volume of -
96%, a partial responder by RECIST, while DW-MRI showed a decrease in tADC of -14.7%.

Figure 3-2. Case 1: Decreased Volume and Increased Diffusion. This patient was diagnosed with intermediate-grade invasive ductal carcinoma of the right breast. Images shown correspond to A) Baseline DCE-MRI (tumor identified with arrow); longest diameter on imaging (MR LD) was found to be 8 cm; MR volume was 41.9 cm³, B) DCE-MRI following chemotherapy with neoadjuvant AC; the early decreases in tumor LD and volume were 6.3 and 2.1%, respectively, and final decreases were 32.5 and 83%. C) Baseline diffusion; mean tumor ADC (tADC) was 1.16 x 10⁻³ mm²/s, and D) Diffusion following chemotherapy; tADC increased to 1.36 x 10⁻³ mm²/s following once cycle, and remained elevated at the final MR, for a final percent change of 18.6%. Following mastectomy, the residual pathological size was found to be 1.5 cm and tumor grade was high; no nodes were positive. The patient did not recur during the study period and recurrence-free survival (censored) was 107.2 weeks.
3.4. Discussion

The results of this study suggest that measurements of tumor diffusivity after one cycle of chemotherapy may predict recurrence-free survival. Our most significant finding was the early percent change in normalized tumor ADC may predict whether or not a patient recurs; the importance of normalizing to benign fibroglandular tissue is consistent with the fact that tumor ADC is expected to increase with treatment, but that the increases in the tumor may need to be evaluated relative to the ADC in normal tissue. Our results suggest that diffusion information may be important in predicting long-term outcomes.

Previous studies have shown that diffusion changes with treatment occur before volume changes, that diffusion increases with effective treatment, and that diffusion changes in non-responders as determined by volume are different from those in responders, as determined by volume. Intermediate outcomes such as pathological
complete response (pCR) and tumor volume relate to long-term outcomes, but the
correlation is imperfect. The possibility that some drugs may improve RFS and OS but
not pCR (Padhani, Liu et al. 2009) and the fact that intermediate outcomes are surrogate
outcomes and not measures of long-term response highlight the need for knowledge
regarding long-term outcomes. Our findings suggest that diffusion changes may relate to
the long-term outcome of RFS.

Due to the variability in techniques and evaluation of methods of prior DW-MRI
neoadjuvant studies, direct comparisons between studies are difficult. Despite differences
in study design, our study findings generally agree with results from other studies
indicating that measuring ADC may be valuable in response monitoring (Pickles, Gibbs
et al. 2006; Yankeelov, Lepage et al. 2007; Sharma, Danishad et al. 2008; Iacconi,
Giannelli et al. 2009). Alternatively, another recent study (Nilsen, Fangberget et al. 2010)
found that tumor ADC did not predict tumor response as defined by changes in tumor
volume; however, the earliest timepoint at which ADC was measured following
chemotherapy initiation was after 4 cycles. In discussion of their results, the authors
suggest an earlier MR timepoint may be more predictive. In another study in which
measurement of ADC occurred after two cycles, ADC did not predict response (Manton,
Chaturvedi et al. 2006). Our study evaluated an earlier timepoint with ADC measured
after only one cycle of chemotherapy and our results suggest an association between this
timepoint and RFS. Variations in the timing of MRIs may contribute to differing results
in different studies. The increase in extracellular water mobility following chemotherapy-
induced cell death may be followed by fibrosis and decreased water mobility, suggesting
that an optimal window for DW-MRI does exist and this timing has yet to be determined.
Sharma et al. (Sharma, Danishad et al. 2008) found that changes in ADC after one cycle were predictive of response defined by change in tumor size. Our findings due not directly agree with those of Sharma et al. because in our study, early ADC measurement predicted long-term outcomes, but not volumetric response. In the study by Sharma et al, response was defined as a 50% or more decrease in tumor volume, measured clinically (Sharma, Danishad et al. 2008). In our study, response was defined as a 65% or more decrease in tumor volume, measured by DCE-MRI. This difference in outcome measure may have contributed to the differing results.

Technical differences in image acquisition and analysis provide further sources of variation between DW-MRI neoadjuvant studies. Our study encompassed the tumor with multiple ROIs. The study by Nilsen et al utilized a single ROI to measure ADC at each timepoint. Sub-sampling of the tumor likely occurred in both ROI-based methods of analysis. One study (Pickles, Gibbs et al. 2006) attempted to characterize heterogeneity in tumor ADC by monitoring changes in the numbers of voxels in particular ranges of ADCs throughout treatment; significant differences were found in particular ranges. Another group monitored changes in individual tumor voxels (Ma, Meyer et al. 2009). In our study, as well as most studies to date, ADC is averaged across tumor voxels and this whole-tumor mean is tracked during chemotherapy. The optimal means of measuring ADC in tumor is not yet established and further studies are needed.

There are limitations to our study. This study was retrospective and limited by the small sample size as well as the protocols in imaging and clinical medicine that were standard of care at the time of patient recruitment. Not all patients were treated with the same chemotherapy regimen; this was a consequence of the time period in which the
study took place and the fact that neoadjuvant taxanes were not yet established standards of care. RFS times may be impacted differently in the taxane and non-taxane treatment cohorts. This study was limited in its sample size and larger studies are needed to provide sufficient statistical power to validate our findings and determine if ADC predicts RFS.

Furthermore, the manual nature of the tumor ROI delineation on the ADC maps could have contributed to both intra and inter-user variation in analysis. In addition, the diffusion-weighted images were 5mm thick, and partial voluming between tumor and normal tissue could have produced less accurate tADC values. Improved DW-MRI resolution, consistent image acquisition, and more automated tumor delineation on ADC maps would help reduce these sources of variability in future studies. Our analysis did not utilize fat suppressed MR data. Care was taken to define ROIs within the tumor and fibroglandular regions and avoid fatty areas, however, subvoxel contributions from fat may have contributed to the ADC measures for tumor and normal tissue. Future work using fat-suppression methods could improve the accuracy of diffusivity information obtained with DW-MRI.

### 3.5. Conclusions

In this study, tumor ADC showed a trend towards significance as a predictor of recurrence-free survival and time to recurrence, suggesting ADC may be a promising marker of response. Larger studies are needed to confirm and prospectively validate the ability of ADC to predict patient outcome. Combined knowledge of tumor size from DCE-MRI and of tumor ADC from DW-MRI may provide more sensitive assessment of
tumor response to chemotherapy, facilitating tailoring of treatment regimens, and potentially improving overall outcomes for patients with locally advanced breast cancer.

3.6. References


106


Diffusion-weighted magnetic resonance imaging (DW-MRI) in the breast is technically challenging. Due to its anatomic location, the breast interfaces with air, creating regions of differing magnetic susceptibility and subsequent MRI artifacts. In addition, adipose and fibroglandular tissue are variably present and variably distributed throughout the breast. Due to the need to identify small lesions, breast imaging also demands high spatial resolution and meeting this demand can be difficult.

The full potential of diffusion as biomarker for breast cancer treatment response may not be fully realized unless the technical obstacles to diffusion measurement are identified and circumvented. In this section, methods of acquiring DW-MRI data in the breast are investigated and a new method is optimized for use in the breast. In Chapter 4, the impact of fat suppression on tumor diffusivity measurements is studied. In Chapter 5, the method of image acquisition used in Chapters 3-4 is compared to method used more commonly in current studies, diffusion-weighted echo planar imaging (DW-EPI). And in Chapter 6, a new DW-EPI–based sequence is optimized for use in the breast and preliminary case studies illustrating its potential value in imaging breast cancer are explored.
Chapter 4. Fat Suppression: Quantitative and Qualitative Analysis of Fat Suppression in DW-MRI

4.1. Introduction

Tissue contrast is important in identifying lesions in breast imaging. Fat suppression enhances tissue contrast by reducing the signal from adipose tissue. Fat suppression can be implemented through a variety of techniques such as a Chemical Shift Selective (CHESS) pulse, the Short Tau-Inversion Recovery (STIR) technique, water
excitation, or techniques based on the Dixon method, in which separate water and fat images are acquired (Ma 2008).

Due to the intermixing of fibroglandular and adipose breast tissues, fat and water often occupy the same voxels in breast MR, contributing to partial voluming. Fat and water diffuse at different rates, and partial voluming in diffusion-weighted MRI (DW-MRI) could lead to inaccurate diffusion measurements for fibroglandular tissue. DW-MRI shows promise in diagnostic and treatment monitoring applications related to breast cancer (Hamstra, Rehemtulla et al. 2007; Abdel Razek, Gaballa et al. 2010; Iacconi 2010). Success of these applications depends on the ability of DW-MRI to accurately and reproducibly quantify the apparent diffusion coefficient (ADC). This study aimed to characterize the impact of fat suppression on measured ADC in patients with locally advanced breast cancer.

Variable adipose content in the breast can lead to misassignment of fat and water shifts and lack of fat suppression (Harvey, Hendrick et al. 2007). The shifts of water and fat may also vary due to field inhomogeneities caused by use of the magnet off isocenter, air-fat interfaces, skin folds, and biopsy clips or other hardware (Kazama, Nasu et al. 2009). These challenges can make adequate fat suppression difficult.

The purpose of this study was to determine if fat suppression impacted measured tumor ADCs and to assess the quality of fat suppression. It was hypothesized that due to partial voluming, measured diffusion in fibroglandular tissue would differ in fat and non fat suppressed exams and that due to field inhomogeneities, fat suppression would qualitatively vary from exam to exam.
4.2. Materials and Methods

Patients: Group 1 and Group 2 patients were included in this study. Patients were enrolled in IRB approved studies and scanned with dynamic contrast enhanced MRI (DCE-MRI) and diffusion-weighted MRI (DW-MRI) before, during, and after treatment with neoadjuvant chemotherapy between 1998 and 2006. All patients signed informed consent. All patients had locally advanced breast cancer and were treated with anthracycline-based neoadjuvant chemotherapy. Only Group 1 and 2 patients were included in this study; these were the only patients enrolled in neoadjuvant imaging studies at our institution and scanned with both fat-suppressed and non fat-suppressed DW-MRI.

MR Imaging: Patients were scanned with DCE-MRI and DW-MRI. Imaging parameters varied depending on whether patients were in Group 1 or Group 2 (reported elsewhere in this work), but in both groups DW-MRI was acquired axially with and without fat suppression using a single shot fast spin echo (SSFSE) sequence (Partridge, McKinnon et al. 2001). DCE-MRI was acquired in the sagittal orientation, but patients were also scanned with a post-contrast T1-weighted imaging series matching DW-MRI in slice thickness. The purpose of this series was to assist in tumor localization on DW-MRI.

Image Processing: ADC maps were constructed from DW-MRI data using previously published methods (Partridge, McKinnon et al. 2001) based on an assumption
of monoexponential decay of the original signal ($S_0$) to the signal measured when diffusion-weighted gradients were applied ($S_{b600}$) (Equation 1):

\[
S_{b600} = S_0 e^{-bD}
\]

Signal enhancement ratio (SER) maps were constructed from the pre-contrast ($S_0$), early post-contrast ($S_1$), and late post-contrast ($S_2$), signal measured with DCE-MRI using the relationship in Equation 2 and previously published methods (Partridge, Gibbs et al. 2002; Li, Partridge et al. 2008).

\[
SER = \frac{S_1 - S_0}{S_2 - S_0}
\]

**Image Analysis**

**Quantitative Image Analysis**: In Group 2, regions with SER>1 were converted to regions of interest and automatically registered with ADC maps. In Group 1, this registration was not possible to differences in the image header between DCE and DW-MRI data. In Group 1, regions of interest (ROIs) containing tumor and excluding normal tissue and fat were manually placed on ADC maps. Information from post-contrast T1-weighted imaging assisted with tumor identification. The same ROIs were registered to both fat and non-fat suppressed ADC maps for a given exam. For both Groups 1 and 2, all ROIs were summed to define the tumor volume of interest (VOI). The mean ADC within the VOI was then calculated.
In Group 1, differences between fat suppressed and non fat suppressed data in the mean ADC of normal fibroglandular tissue were also assessed. Normal fibroglandular tissue was sampled with manually placed ROIs on the contralateral breast.

**Qualitative Image Analysis:** Quality of fat suppression was assessed retrospectively in Group 2. Only DW-MRI exams at baseline (visit 1) and after 1 cycle of chemotherapy (visit 2) were assessed. Patients were required to have baseline fat-suppressed DW-MRI for inclusion in this study.

Quality of fat-suppression was assessed on the combined b=600 image volumes from DW-MRI data. When available, the non fat-suppressed b=600 combined volume was used as reference for localization of adipose and fibroglandular tissue. Images were viewed using in-house software developed in Interactive Data Language (IDL, ITT Visual Information Solutions, Boulder, Colorado). Bright areas on the non-fat suppressed combined image that were dark on the fat-suppressed image were interpreted as regions of adequate fat suppression. Bright areas on the non-fat suppressed images that were also bright on the fat-suppressed indicated areas of incomplete fat suppression. Bright areas were noted and completeness of fat suppression was judged qualitatively relative to the affected breast.

Fat suppression quality was judged on a scale from 1 to 3. A rating of 1 (poor quality) was assigned to cases judged as having poor fat suppression - - adipose was mainly bright on the fat-suppressed b=600 images. A rating of 2 (intermediate, with some problems) was assigned to cases judged as having overall adequate fat suppression but areas of bright adipose tissue, such as in the axilla or anterior breast. A rating of 3 (good, with no problems) was assigned to cases judged as having overall good fat suppression.
with no problems noted. Ratings were tracked at visit 1 and visit 2, separately and longitudinally, using Microsoft Excel.

**Statistics:** Mean ADCs from fat-suppressed exams were compared pair-wise to mean ADCs from the corresponding non-fat suppressed exams. All available timepoints were considered: it was assumed that the impact of fat suppression would remain constant throughout treatment. The normality of fat and non fat suppressed ADCs was assessed and when both groups of ADCs were normally distributed, a two-sided paired t-test was used to determine if ADCs were the same, with alpha=.05. When both groups were not normally distributed, a two-sided paired Wilcoxon sign rank test was used to determine if ADCs were the same, with alpha =.05. Correlation between paired fat suppressed and non fat suppressed measurements was also tested at alpha =.05; Spearman’s rho was calculated. A linear model was fit to the data to describe the pair-wise relationship between fat suppressed and non-fat suppressed mean ADCs. The norm of the residuals was calculated to evaluate appropriateness of a linear model for the data. All statistical analyses and data fitting were done in MATLAB (R2007a, The Mathworks, Natick, MA).
4.3. Results

Quantitative Analysis

The relationship between tumor ADC measurements in fat suppressed in non fat suppressed DW-MRI was assessed separately in Group 1 and Group 2 and then in a combined study of both Group 1 and Group 2 patients (Figure 4-1; Figure 4-2). Normal tissue ADC measurements were compared in Group 1 only and fat suppressed ADCs were found to be higher than non-fat suppressed (Figure 4-3).

Figure 4-1. Bar graph comparing mean tumor ADC in fat-suppressed and non-fat suppressed exams. In a combined study of Groups 1 and 2, fat suppressed ADCs (blue) were higher than non-fat suppressed (red). In one case in Group 1, non-fat suppressed ADC was recorded as higher, but this was found to be an error.
Figure 4-2. Box plots of ADC distributions. In all groups (A and B: Group 1, fat suppressed and non fat suppressed ADCs, respectively; C and D: Group 2; E and F: Group 1 + 2), the group mean fat suppressed ADC was higher than the non-fat suppressed group mean.

Figure 4-3. Bar graph comparing mean normal fibroglandular tissue ADC in fat-suppressed and non-fat suppressed exams. In Group 1, in all cases, mean fibroglandular tissue ADC measured from fat-suppressed DW-MRI was found to be higher than the mean measured from non fat-suppressed DW-MRI.

In all cases, ADC measurements differed between fat suppressed and non fat suppressed acquisitions; for all comparisons except the normal tissue comparison, a linear correlation was identified between fat suppressed and non fat suppressed ADCs (Table 4-1).
<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ADC, fat suppressed (SD)</th>
<th>Mean ADC, non fat suppressed (SD)</th>
<th>Test for paired comparison of fs and nonfs ADC</th>
<th>p-value for paired comparison of fs and nonfs</th>
<th>Linear correlation p-value</th>
<th>Linear correlation coefficient, r (r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1, tumor</strong></td>
<td>1538 (410.4)</td>
<td>1144 (313.3)</td>
<td>T-test</td>
<td>.000005778</td>
<td>.0210</td>
<td>.3965 (1.1572)</td>
</tr>
<tr>
<td><strong>Group 2, tumor</strong></td>
<td>1367 (268.1)</td>
<td>814.9 (314.1)</td>
<td>Sign rank</td>
<td>1.468*10^{-12}</td>
<td>5.443*10^{-7}</td>
<td>.5677 (3.223)</td>
</tr>
<tr>
<td><strong>Group 1+2, tumor</strong></td>
<td>1425 (331.1)</td>
<td>925.8 (349.2)</td>
<td>T-test</td>
<td>6.626*10^{-27}</td>
<td>7.515 *10^{-8}</td>
<td>.5044 (2.544)</td>
</tr>
<tr>
<td><strong>Group 1, normal tissue</strong></td>
<td>1980 (225.3)</td>
<td>1213 (306.6)</td>
<td>Sign rank</td>
<td>.000003789</td>
<td>.5324</td>
<td>-.1232 (0.0152)</td>
</tr>
</tbody>
</table>

Based on the Spearman’s R^2 and p-value, a linear relationship was not expected to exist between fat suppressed and non fat suppressed normal tissue ADCs. However, the relationship between fat suppressed and non fat suppressed tumor ADCs was thought to be linear and a linear regression line was fit to the data sets. Linear models for the relationship between fat suppressed and non fat suppressed tumor ADC showed that non fat suppressed mean ADCs were approximately ½ non fat-suppressed mean ADC (range for slope = .25 - .67 in 3 groups) (Figure 4-4).
Figure 4-4. Linear models of relationship between fat- and nonfat-suppressed ADCs in patient groups. In A, linear model for Group 1 data is shown; in B, a model for Group 2 is shown; in C, a model for the combined Group 1+2 is shown. In A, the norm of residuals was 1698; in B it was 2100; in C it was 3015. These large norms describe the distance of the data points from the fit line. Regression lines and corresponding linear equations are shown. nonFS = non-fat suppressed ADC; FS=fat-suppressed ADC.
Qualitative Analysis

In 44 fat-suppressed Group 2 DW-MRI acquisitions, no rating of 3 (good) was assigned. A rating of 1 (Figure 4-5) was assigned 6 times and a rating of 2 (Figure 4-6) was assigned in 38 acquisitions. Only 1 case had a rating of 1 on both visit 1 (baseline) and visit 2 (post cycle-1).

Figure 4-5. Case of poor fat suppression. A) Image from fat suppressed exam. As seen in A, adipose tissue is bright and not fat-suppressed (green arrow) in the right breast. In the left breast, fat suppression was improved (orange arrow). This exam was rated 1 (poor) for fat suppression. The cancer was in the right breast for this patient. B) Image from non fat-suppressed exam, shown for comparison. As seen in B, that adipose tissue is bright bilaterally (green arrow); it was intentionally not suppressed in this acquisition.
At visit 1, most exams were given a rating of 2 and had some problems identified (Figure 4-7). At visit 2, most exams were also given a rating of 2 (Figure 4-8). And longitudinally, patients with a 1 at baseline were equally likely to be assigned a 1 or 2 on visit 2. Patients with a 2 at baseline were more likely to be assigned a 2 than on 1 on visit 2 (Figure 4-9).
Figure 4-7. Bar graph of Group 2 baseline fat-suppression ratings. Most (24/27) patients were assigned a rating of 2 on baseline fat-suppressed DW-MRI.

Figure 4-8. Bar graph of Group 2 MR2 fat-suppression ratings. Most (14/17) patients were assigned a rating of 2 on fat-suppressed DW-MRI at visit 2.
Figure 4-9. Bar graph of Group 2 longitudinal fat-suppression ratings. Categories corresponding to combinations of visit 1 and 2 ratings are displayed on the x axis; frequency of each combination is displayed on the y axis. Patients with a rating of 1 at baseline were equally likely to have a rating of 1 or 2 on visit 2; patients with a 2 at baseline were most likely to have a 2 at visit 2.

4.4. Discussion

In retrospective analysis of two independent studies, mean tumor ADC measured from fat suppressed DW-MRI was found to significantly differ from mean tumor ADC measured from non fat suppressed DW-MRI. The differences occurred despite the use of the same regions-of-interest to define the tumor. Mean tumor ADC measurements from non fat-suppressed DW-MRI were lower than those from fat-suppressed DW-MRI.

This finding is compatible with the effect that partial voluming would have on the tumor ADC measurements. Due to the resolution of DW-MRI, the presence of both normal adipose tissue and tumor in the same voxel is probable. Without fat suppression,
DW-MRI measurements are thus likely to incorporate the diffusivities of both fat and water (partial volume effects).

Even with fat suppression, low diffusing water content in adipose tissue may, however, impact tumor ADC measurements. Adipose tissue has a lower water content than fibroglandular tissue (Graham, Ness et al. 1997). In addition, a greater proportion of the water in adipose tissue may be trapped and therefore have decreased diffusivity compared to the water in fibroglandular tissue (Baron, Dorrius et al.) Reduction in tumor and fibroglandular tissue ADC measurements may therefore be due to two partial voluming contributions from adipose tissue: 1) low diffusing fat in adipose tissue, and 2) low diffusing trapped water in adipose tissue. Fat suppression would be expected to reduce contributions from fat to the tumor and fibroglandular ADC measurements but complete suppression of water adipose contributions may not be possible. Nevertheless, differences in tumor ADC from fat suppressed and non fat suppressed exams shows that the impact of fat suppression on tumor ADC measurements is substantial.

Based on a qualitative assessment of fat suppression in a subset of cases (Group 2), most fat suppressed exams had adequate fat suppression, but some exams had poor fat suppression. Given the impact of fat suppression on ADC measurements, poor fat suppression could impair longitudinal tracking of ADC changes. In a study of normal breast tissue, different ADCs were obtained when different fat suppression methods were used (Baron, Dorrius et al.). Partial fat suppression may result in mean tumor ADCs similar to those seen in non fat-suppressed data due to partial volume effects; however, decreased ADC could be erroneously interpreted as lack of tumor response to treatment,
rather than lack of fat suppression. This underscores the important of 1) fat suppression in DW-MRI and 2) complete fat suppression.

In Group 2 exams, the lack of good ratings (3) for fat-suppressed DW-MRI does not necessarily invalidate the technique; other sequences may have similar challenges in achieving very high quality of fat suppression. To better put the DWSSFSE quality ratings in perspective, quality in a subset of DCE-MRI exams could be judged, as well as fat-suppression quality in a subset of EPI-based DW-MRI, which is more prevalent on today’s commercially available scanners.

This study was limited in that it relied on a qualitative assessment of fat suppression completeness. Given the importance of fat suppression in DW-MRI, quantitative methods should be identified for a more exact assessment of fat suppression. A quantitative assessment may also be amenable to automation, expediting analysis. The quality ratings used in this study may also exhibit inter-rater variability and if used in other studies, the rating scale should be defined with example cases. Illustrated examples are used to better explain the BI-RADS standards and ratings (D'Orsi, American College of Radiology. et al. 1998).

In addition to developing quantitative methods of fat suppression evaluation, future studies could also focus on identifying relationships between fat suppression quality and breast composition. Identification of patterns could be used to predict fat suppression quality prior to acquisition with a DW-MRI sequence, allowing for possible use of additional or alternative methods of fat suppression. Breast volume, breast density, and breast tissue patterns may impact the completeness of fat suppression.
The preponderance of intermediate fat suppression ratings assigned in this study suggests that fat-suppression was overall adequate, with some problems in most cases. These problems do not necessarily invalidate ADC measurements. The prevalence of fat suppression incompleteness in the axilla supports this conclusion; even if disease were present in the axilla, it would not have been assessed on DW-MRI and included in the tumor average because the enhancement in the breast and not the axilla was used to localize the tumor on ADC.

Despite the shortcomings of a qualitative analysis, fat suppression ratings may help identify cases that should or should not be included in an analysis. Given the impact of fat suppression on the ADC, it may be problematic to pool poorly fat suppressed ADC data with fat suppressed data; such pooling could impair study analyses.

4.5. Conclusion

This study showed that measured tumor ADC is higher with fat suppression than without, and that fat suppressed exams often contain areas of inadequate fat suppression. Due to the fact that ADC is decreased without fat suppression, fat suppressed DW-MRI data should be analyzed in treatment monitoring studies when possible, with the fat suppression ratings used to provide some context for unexpected results and possible exclusion of subsets of cases from study-wide analyses.
4.6. Acknowledgements:

This work was made possible through a collaboration with Savannah Partridge at University of Washington in Seattle.

4.7. References

Chapter 5. Other Parameters: Sequence Type and Treatment Protocol

5.1. Introduction

Developments in imaging acquisition and cancer treatments have resulted in shifts in clinical care. Advances in diffusion-weighted echo planar imaging (DW-EPI) sequences resulted in the ability to scan quickly with multiple diffusion-weighted acquisitions, with reduced affects from bulk noise. This allowed for measurement of diffusion in acute situations such as stroke (Mukherjee, Chung et al. 2008). DWEPI sequences became the type of sequences commercially available to clinical and research
centers. Advances in pharmaceutical research led to the development of taxane chemotherapies. In NSABP-27, the addition of taxanes to anthracycline-based regimens was found to improve pathological completer response rates for patients with locally advanced breast cancer (Liu, Melstrom et al.). A meta-analysis of several randomized control trials found that the addition of taxanes to neoadjuvant chemotherapy improved the rates of both pCR and breast-conserving surgery (Cuppone, Bria et al. 2008). While not all studies have shown support for taxanes and use in early stage breast cancer is not without controversy, taxanes are thought to provide patient benefits (Bedard, Di Leo et al. 2010). In the United States, taxanes are now used in standard breast cancer chemotherapy protocols (Chen, Skarin et al. 2007).

These shifts in the standard of care make it difficult to prospectively study the impact of these parameters on the ADC in patients. Variability in chemotherapy regimen and sequence type must be clinically justified. At UCSF, neoadjuvant chemotherapy and imaging studies in patients with locally advanced breast cancer have been occurring since the 1990s. As standards in imaging and treatment have changed over time, these changes were integrated into the neoadjuvant studies at our institution and retrospective assessment of the impact of these variables on the ADC is possible. Between- and within-study comparisons allow for some insight into the impact of difficult to study parameters on the ADC.

The goal of this study was to retrospectively evaluate DW-MRI data acquired in patients with locally advanced breast cancer and treated with neoadjuvant chemotherapy. ADC measurements were studied in relation to 2 parameters: sequence type and chemotherapy type. Due to well-known problems with EPI distortion (Jezzard and
Balaban 1995; Andersson, Skare et al. 2003), it was hypothesized that EPI acquisitions would contain more severe artifacts than non-EPI sequences. Due to the fact that the taxanes have a distinctive mechanism of action (Horowitz 1995; Altmann 2001), it was hypothesized that ADC changes would differ in patients treated with taxane versus non-taxane regimens.

5.2. Materials and Methods

Overview of research design: DW-MRI and DCE-MRI exams were acquired in patients with locally advanced breast cancer, treated with neoadjuvant chemotherapy, and enrolled in studies at UCSF between 1995 and 2010 (Groups 1-5). All patients signed informed consent and all studies were approved by the institutional review board. MRI exams were acquired prior to, during, and following chemotherapy treatment. ADC maps were constructed from DW-MRI data and SER maps were constructed from DCE-MRI data. Exams were retrospectively reviewed in 2 investigations into other parameters that may impact ADC: sequence type and treatment protocol. Patients and methods for each investigation are described below.

Sequence type

Patients: Patients in Groups 1 and 2 were scanned with a diffusion-weighted single shot fast spine echo (SSFSE) sequence; patients in Groups 3, 4, and 5 were scanned with a diffusion-weighted echo planar imaging sequence. Due to sequence availability and experimental constraints, no patient group was scanned with both...
DWSSFSE and DWEPI, making direct comparisons between sequences difficult. However, since dynamic-contrast enhanced MRI (DCE-MRI) provides a measure of tumor volume and all patient groups were scanned with DCE-MRI, misregistration between DCE-derived tumor volumes and ADC maps can provide some insight into the impact of sequence type on diffusion measurements. Assessment of other characteristics related to image quality, such as signal to noise (SNR) and tissue contrast can also provide information about differences between these two sequences.

**Method:** For this comparison, baseline DW-MRI exams from randomized subsets of patients enrolled in Group 2 and Group 3 studies were assessed. Patient IDs from exams with diffusion analyzed at baseline and after 1 cycle chemotherapy (15 in Group 2 and 18 in Group 3) were randomly sampled without replacement in MATLAB (R2007a, The Mathworks, Natick, MA); the first 6 IDs from each randomly reordered group were included in this analysis.

1. **Motion and distortion:** Misregistration between DCE and DW-MRI exams was assessed by measuring the linear shift in a tumor region of interest (ROI) derived from the SER map and automatically registered with the diffusion-weighted imaging.

2. **SNR:** SNR was calculated for 1 diffusion-weighted acquisition for each baseline exam. A 2.2mm circular ROI was placed over tumor (hyperintense on DWI) on the first b=600 acquisition and mean intensity was calculated. A large (30.2-78mm, depending on amount of background included in image) square ROI was placed in the background noise and SD was calculated. A second 2.2mm circular ROI was placed in tissue adjacent to the tumor (Figure 5-1).
Figure 5-1. SNR Calculation. A 2.2mm ROI is placed on hyperintense tumor on diffusion-weighted images (green arrow); a 2.2mm ROI is placed on adjacent normal tissue (orange arrow); a large square-shaped ROI defines background noise.

SNR was calculated as the mean signal in the circular ROI divided by the SD in the square ROI. Tissue contrast was calculated by comparing SNR for tumor to SNR to normal-appearing surrounding tissue.

3. **Fat suppression**: Fat suppression on each exam was rated on a scale from 1-3: 1= poor and majority of breast not fat suppressed; 2= intermediate with some areas of inadequate fat suppression; 3=good. Fat suppression was rated on the first b=600 acquisition.

4. **Artifacts and SAR**: Artifacts were described for each exam and the presence of artifacts was noted. Differences in specific absorption rate (SAR) between imaging series was quantitatively compared.
**Statistics:** Differences between the two sequences in parameters measured quantitatively (shift, SAR, SNR, tissue contrast) were compared in Matlab (R2007A, The Mathworks, Natick, MA) with a two-sided Mann-Whitney U-test, at alpha=.05. Differences in fat suppression, measured on a qualitative scale, were assessed in STATA (IC 11.1, StataCorp LP, College Station, TX) with the Fisher exact test.

**Chemotherapy protocol**

**Patients:** The only patient group in which it was possible to study chemotherapy affect was in Group 1. Group 2 and Group 3 patients were enrolled in clinical trials and the treatment regimen was standardized, preventing significant variation in treatment and within-group comparisons, especially in the smaller diffusion subset. In Group 4, protocols varied, but too few patients were enrolled to allow for comparisons. In Group 5, patients are treated with different regimens, but at the time of this writing, not enough patients have been enrolled to allow for study of associations between therapies and diffusion changes.

**Method:** For assessment of chemotherapy protocol on diffusion change, patients treated with regimens including a taxane (AC+T, in all cases) were considered to be part of the taxane group. Patients treated with either of two regimens (FEC or AC only) that did not include a taxane were considered to be part of the non-taxane group. Due to the fact that the study size was 30 and only a subset of patients were scanned with a fat-suppressed DW-MRI protocol, only non fat-suppressed DW-MRI measurements were considered in this analysis. While traditionally diffusion changes from baseline to the
first MR and from baseline to the final MR are considered, late change, from the 2nd MR
to the final MR were considered because in this study, taxanes, when given, were given at
the end of the chemotherapy protocol and the late change period was most likely to
capture differences in diffusion between taxane and non-taxane groups. Measurements of
mean tumor ADC (tADC) and mean tumor ADC normalized to the mean of ADC of
normal contralateral fibroglandular tissue (nADC) were considered. ADC was measured
as previously described in Chapter 3.

Statistics: The distribution of each variable in taxane and non-taxane treatment
groups was first assessed for normality with the Lilliefors test at alpha=.05. Normally
distributed variables were compared with the t-test; variables not normally distributed
were compared with the Wilcoxon rank sum (Mann-Whitney U-test); for both tests,
p<.05 was considered statistically significant and p<.10 was considered a trend towards
significance. Differences in MR diffusion (mean tumor ADC and normalized mean tumor
ADC), MR LD, MR volume, residual pathological size, and DFS time were assessed.
Statistical analysis was conducted in Matlab (R2007A, The Mathworks, Natick, MA).
5.3. Results

Sequence type

Registration of DCE-derived tumor volume with ADC maps was improved in Group 2 (SSFSE) exams as compared to Group 3 (EPI) exams (p<.05). SNR, tissue contrast, and fat suppression were better with EPI (Table 5-1), but the only statistically significant improvement was in SNR. All SSFE suffered from problems with signal homogeneity (Figure 5-2); all EPI exams suffered from artifacts due parallel imaging, and a subset of EPI exams also had ghosting artifacts and misregistration (Figure 5-3). Mean SAR was increased almost 11-fold in SSFSE as compared to EPI (p<.05).

Table 5-1. Differences between SSFSE and EPI

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Mean Linear Shift (Range)</th>
<th>Mean SNR (range)</th>
<th>Mean Tissue Contrast (range)</th>
<th>Mean FS Rating (Range)</th>
<th>Most Common Artifact/ Problem</th>
<th>Mean SAR (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW-SSFSE</td>
<td>1.667 (1.3-2.2)</td>
<td>45.8 (29.5-64.0)</td>
<td>3.57 (2.11-6.13)</td>
<td>1.667 (1-2)</td>
<td>Signal drop-off</td>
<td>1.807 (1.677-1.922)</td>
</tr>
<tr>
<td>DW-EPI</td>
<td>6.383 (2.8-9.8)</td>
<td>133.5 (58.3-262.1)</td>
<td>4.52 (0.52-6.8)</td>
<td>2.167 (2-3)</td>
<td>Parallel imaging artifact; distortion</td>
<td>0.166 (0.101-0.211)</td>
</tr>
</tbody>
</table>

p-value | .0022 | .0043 | .3095 | .455 | NA | .0022 |
Figure 5-2. SSFSE Technical Problems. In the top image, signal drop-off from the affected to unaffected breast is seen. Fat suppression is also inadequate in the anterior affected breast. In the bottom image, fat suppression is adequate in the anterior breast, but hyperintensity is seen in the axilla (green arrow) and across the contralateral breast (orange arrow).

Figure 5-3. EPI Artifacts. The DCE-derived tumor ROI appears misregistered on the DW image (right breast). A layer of noise surrounds the breasts due to parallel imaging artifact. The left breast appears distorted.
Chemotherapy protocol

In patients treated with taxane regimens, median DFS was 293 weeks (range = 30-356 weeks). In patients not treated with taxanes, median DFS was 241 weeks (range = 39-429 weeks) and this difference was not significant (p=.21). Differences in baseline MR volume (cm³), MR longest diameter (cm), MR diffusion (10⁻⁶ mm²/s), and residual pathological size (cm) were not statistically significant at alpha=.05 (Table 5-2). Differences in late percent change in nADC, and late and final percent change in MR longest diameter were statistically significant (p=.009, .038, .028, respectively).
Table 5-2. Differences Between Treatment Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>N, taxane group</th>
<th>N, non-taxane group</th>
<th>N, total</th>
<th>Median, taxane group</th>
<th>Range, taxane group</th>
<th>Median, non-taxane</th>
<th>Range, non-taxane group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>1276.5</td>
<td>705.27 - 1811.2</td>
<td>1074.4</td>
<td>241.9 - 1535</td>
<td>0.062</td>
</tr>
<tr>
<td>Post cycle-1</td>
<td>8</td>
<td>10</td>
<td>18</td>
<td>1204.65</td>
<td>950 - 2085.1</td>
<td>1174.85</td>
<td>772.8 - 1606.1</td>
<td>0.468</td>
</tr>
<tr>
<td>Final</td>
<td>14</td>
<td>12</td>
<td>26</td>
<td>1093.2</td>
<td>575 - 1620</td>
<td>1187.25</td>
<td>406.63 - 1490.3</td>
<td>0.848</td>
</tr>
<tr>
<td>Early % change</td>
<td>8</td>
<td>10</td>
<td>18</td>
<td>6.39</td>
<td>-8.91 - 32.14</td>
<td>10.22</td>
<td>-5.94 - 219.47</td>
<td>0.408</td>
</tr>
<tr>
<td>Late % change</td>
<td>7</td>
<td>8</td>
<td>15</td>
<td>-9.06</td>
<td>-16.75 - 9.72</td>
<td>-3.61</td>
<td>-18.84 - 15.92</td>
<td>0.552</td>
</tr>
<tr>
<td>Final % change</td>
<td>14</td>
<td>12</td>
<td>26</td>
<td>-9.06</td>
<td>-33.98 - 30.41</td>
<td>1.64</td>
<td>-34.61 - 263.5</td>
<td>0.341</td>
</tr>
<tr>
<td>nADC</td>
<td></td>
<td></td>
<td></td>
<td>0.98</td>
<td>0.75 - 1.53</td>
<td>0.89</td>
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<td>0.28 - 2.22</td>
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<tr>
<td>Final</td>
<td>13</td>
<td>10</td>
<td>23</td>
<td>1.13</td>
<td>0.53 - 3.28</td>
<td>1.13</td>
<td>0.28 - 2.22</td>
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<td>7</td>
<td>15</td>
<td>14.65</td>
<td>-15 - 47.89</td>
<td>6.41</td>
<td>-1.59 - 173.75</td>
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<td>6</td>
<td>13</td>
<td>-16.75</td>
<td>-52.61 - 24.8</td>
<td>28.43</td>
<td>1.41 - 45.05</td>
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<tr>
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<td>10</td>
<td>22</td>
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<tr>
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<td>15</td>
<td>15</td>
<td>30</td>
<td>22.03</td>
<td>2.33 - 137.47</td>
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<td>0.52 - 97</td>
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<td>0 - 23.34</td>
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<tr>
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<tr>
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<td>21</td>
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<td>-16.1</td>
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5.4. Discussion

Sequence type

This investigation assessed qualitative and technical differences in MRI data acquired with two types of diffusion-weighted MRI sequences. In this study, the DW-EPI acquisition was associated with improved SNR, improved tissue contrast, improved SAR, and improved fat suppression relative to the DWSSFE acquisition, but worse distortion and worse misalignment between diffusion and DCE images. Even in this small study, image quality and technical parameters differed between images acquired with the DWEPI versus the DWSSFSE sequence. These findings are important because they illustrate potential advantages of each sequence and different diffusion applications may benefit from particular sequence types.

Studies that rely on registration between DCE and DW-MRI may benefit from SSFSE sequences; studies that require multiple diffusion-weighted acquisitions and series, such as in DTI or that require tissue contrast such as in the diagnostic setting may benefit from EPI. Neoadjuvant treatment monitoring studies fall somewhere between both requirements: the tumor location is known, but treatment effect reduces tumor size; later in the course of treatment a less conspicuous tumor may need to be distinguished from normal tissue for accurate ROI placement and diffusion measurement in the tumor. DTI does not have a clear role in neoadjuvant treatment monitoring as the fractional anisotropy of normal breast tissue has been shown to be small (Partridge, Murthy et al. 2010), and cancers would be expected to have even smaller anisotropy; therefore, the ability to scan with more than 3 diffusion-weighted gradients may not necessarily need to guide sequence choice in the clinical setting. However, high signal to noise is essential in
DW-MRI and fat suppression has been shown to be important due to partial voluming and effects on the ADC. Therefore, EPI may be best suited for DW-MRI of the breast, despite the accompanying artifacts due to distortion.

The findings in this study are compatible with the features of the two sequences and scanning protocols at the time. The EPI sequence had greatly improved SNR compared to the SSFSE but the EPI also utilized parallel imaging and had a much greater number of excitations as compared to the SSFSE sequence. The increased misregistration in tumor ROIs with EPI is compatible with the fact that EPI sequences are prone to artifacts due to distortion. This can be traced to the fact that EPI sequences rapidly alternate gradients, creating eddy currents. Increased SAR in SSFSE is expected due to the use of multiple 180 pulses to invert the magnetization vector, rather than alternating gradients as in EPI. Finally, the left to right variation in signal intensity and fat suppression quality in the SSFSE data would not be unexpected if shimming had only occurred over the affected breast. At the time of the acquisitions, DCE-MRI was acquired unilaterally and the shim settings and center frequency determination from the DCE-MRI acquisition may have been used for the DWSSFSE. The DWSSFSE was however bilateral, so unilateral shims would not have provided as optimal of field homogeneity. In contrast, DCE-MRI was acquired bilaterally when the EPI sequence was in use.

Despite the fact that the results of this study lead to a recommendation to scan with DW-EPI over DW-SSFSE, there were several limitations to this study. First, this study compared modern-day EPI to SSFSE data acquired four to eight years ago. Improvements in SSFSE may mean that SNR, fat suppression, and tissue contrast have since improved, making it a better candidate for routine DW-MRI. However, the nature
of SSFSE and the use of multiple 180° pulses means that the SAR is less likely to improve. Studies with larger numbers of diffusion-weighted gradients per acquisition (DTI, multi b value imaging) may be better suited to EPI. Another limitation of this study is that the measure of linear shift did not fully capture EPI distortion; distortion was likely to be nonlinear. In EPI studies, the ROI shift may have been underestimated and the true shift may be unacceptable for some processing algorithms.

Despite this shortcoming, the difference in linear shift was still statistically significant between EPI and SSFSE. While differences in multiple variables were observed between the two sequences, only differences in SNR, SAR, and linear shift were statistically significant. However, this study was small. Furthermore, the scale used to assess fat suppression quality may not have fully captured differences between the two sequences; many exams were given a score of 2, the intermediate rating. A scale with additional increments may have led to a more statistically significant finding.

Prior to this study, it was thought that EPI image quality was inferior to SSFSE image quality. The distortion in EPI is apparent, which may lead to an unfavorable view of EPI image quality. However, even though SSFSE data was found not to have artifacts due to distortion, all SSFSE images assessed in this study had other technical problems. In addition, in SSFSE images, fat suppression, SNR, tissue contrast, and SAR were poorer, and these are all qualities that are important in clinical and research applications of DW-MRI in the breast. In conclusion, despite the apparent distortion and misalignment between DWEPI and other imaging series, the DWEPI sequence commercially available today is preferred to the SSFSE sequence used at our institution between 1998 and 2006.
DWEPI provides higher SNR and higher tissue contrast, qualities that are crucial to breast imaging.

**Chemotherapy protocol**

This investigation assessed differences in tumor diffusivity measurements between patients treated with taxane and non-taxane chemotherapy regimens. Significantly, during the timeframe when treatment regimens differed, taxane-treated patients were found to have larger decreases in MR longest diameter, but smaller nADC changes. The larger decrease in tumor size in taxane-treated patients is compatible with findings from clinical trials showing that taxanes are beneficial to patient outcomes. The smaller change in diffusion may be explained by the fact that the mechanism of action of the taxanes differs from that of the other drugs used simultaneously in the non-taxane group (Gewirtz 1999; Wang, Wang et al. 2000; Symmans 2001; de Jonge, Huitema et al. 2005). While this study was small, this finding has important implications for treatment monitoring studies that rely on early changes in diffusion to predict treatment response. It is possible that not all drugs will result in diffusion changes.

Diffusion differences between the two groups cannot be fully explained by biological differences in the tumor. Up until MR2, diffusion differences were not statistically significant between the treatment groups and up until MR2, both groups were treated with similar drug classes. The decreased change in the taxane group also cannot be explained by a hypothesis that these patients were truly not responding well: their tumors decreased in size during this time period to a greater extent than did the tumors in patients not treated with a taxane. Disease-free survival did not differ between the two
groups. The only apparent difference during the time of taxane treatment was the fact that while patients in the taxane group were treated with a taxane, patients in the other group were treated with either doxorubicin and cyclophosphamide or fluorouracil, epirubicin, and cyclophosphamide. The mechanisms of these drugs differ from the mechanism of taxanes.

It is however unclear why late changes in tumor ADC (tADC) did not differ between treatment groups while late changes in nADC did differ. Notably, in Chapter 3 differences in nADC were more predictive of response than changes in tADC and the difference in nADC between treatment groups warrants further investigation. If nADC is used as a biomarker for treatment response, expected changes in nADC may vary by treatment type. The nADC incorporates a measurement of normal fibroglandular tissue ADC and changes in normal fibroglandular tissue differed between groups (p<.05), with larger increases in the taxane group relative to the non-taxane group. The decreased late change in nADC in the taxane group may therefore be due to the fact that the normal tissue ADC increased during this time; however, if the normal tissue ADC increased it is surprising that the tumor ADC did not increase.

Chemotherapy-induced cell death is accomplished by variable combinations of apoptosis, necrosis, and mitotic catastrophe (Mansilla, Bataller et al. 2006; Portugal, Bataller et al. 2009). Based on pre-clinical studies, ADC increase is typically thought to be a generalized response to chemotherapy-induced cell death (Morse, Galons et al. 2007). However, in this study, nADC change varied by treatment type. Cell swelling preceding apoptosis could decrease extracellular water and decrease overall water mobility (Mardor, Roth et al. 2001; Koh and Collins 2007), dampening increases in
tADC relative to normal tissue ADC. In contrast, cell death due to necrosis and apoptosis is associated with decreased cell density and increased extracellular water mobility (Koh and Collins 2007). Conversely, fibrosis following necrosis is associated with decreased diffusivity. The proportion of cells in particular phases of cell death at particular timepoints may differ when taxanes versus anthracyclines are used.

The findings in this study were limited by the small study size. Due to protocol deviations (explained in Chapter 2), multiple patients were excluded from the MR2 analysis, and thus change from MR2 to the final MR (late change) could not be assessed in these patients. In addition, normal tissue ADC could not be assessed in all patients. 15 patients were in each of the taxane and non-taxane treatment groups, but only 7 and 6 patients, respectively, could be included in the analysis of late change in nADC. The ability to verify these findings in independent populations is also limited by the fact that taxanes are now frequently incorporated into treatment regimens, so a comparison between taxane and non-taxane groups would be difficult. In neoadjuvant breast cancer trials at our institution, taxanes have been given at the beginning of the treatment regimen since 2007; therefore, if any changes between treatment groups were to be assessed, early and not necessarily late change would be important to assess.

Despite these limitations, the findings of this study are important for integrating information from multiple trials. The type of chemotherapy used, and the sequence of treatments, should be considered when interpreting diffusion changes. This information is not always reported in the literature. In addition, early in the drug development process, a drug’s impact on diffusion should be reported to ensure that diffusion is in fact the most appropriate biomarker for treatment response (Padhani, Liu et al. 2009). When drugs are
combined, the impact on diffusion may differ from the impact on diffusion when the drug is used alone. Currently, a trial at our institution and other institutions is evaluating changes in MR tumor measurements during treatment with a combined regimen involving administration of taxanes with other investigation (Barker, Sigman et al. 2009) and changes in the ADC should be closely monitored and related to hypotheses based on the drugs’ mechanisms of action.

In conclusion, in this small study treatment with a taxane versus non-taxane regimen was associated with greater decreases in tumor size but simultaneously smaller changes in tumor diffusivity. This suggests that the drug mechanism of taxanes may impact the timing or the ability of this class of drugs to change tumor diffusivity. Larger studies assessing diffusion changes with taxanes are needed. Continued work relating drug mechanisms to ADC changes is needed for treatment monitoring with DW-MRI to be more valuable to patient care.

5.5. Conclusions

In this study, sequence type and chemotherapy regimen were associated with measurable differences in DW-MRI parameters. The newer DW-EPI sequence was associated with improvements in parameters related to image quality compared to the older DW-SSFSE sequence, but problems related to misregistration were increased with DW-EPI. As new sequences are developed, both advantages and disadvantages may be introduced and these issues will be examined in the next chapter, when a new DW-EPI sequence is optimized for use in the breast.
In the second investigation on chemotherapy type, taxane-based regimens were associated with increased tumor size reductions but decreased diffusion changes compared to non-taxane based regimens and these differences were apparent during the time period when treatment regimens differed. New treatments may lead to both survival benefits and the requirement for new biomarkers, or the modification of existing biomarker tests. Diffusion has been shown to detect response to a variety of cytotoxic drugs, but its value as a response biomarker may vary by treatment type and the time-course of detection. In some studies, it may not be feasible to select MR timepoints based on the timeframe of expected diffusion changes. The optimal timing of DW-MRI is not yet known and the particular contexts in which diffusion may be most valuable are still being identified.

As new sequences are developed, improvements in measuring the ADC in treatment response studies should be assessed and as new drugs are developed, the value of the ADC in detecting response should be identified early in the drug development process and validated in large, prospective clinical studies.

5.6. References


6.1. Introduction

Diffusion-weighted MRI (DW-MRI) provides information about cell density and cell content that has been shown to correlate with tumor response to chemotherapy in vitro, and in vivo, in multiple organs (Chenevert, Meyer et al. 2002; Colagrande, Carbone et al. 2006; Hamstra, Rehemtulla et al. 2007; Padhani, Liu et al. 2009). At most research and clinical centers, the ability to measure diffusion in patients is provided by the echo
planar imaging (EPI)-based DW-MRI sequences available on commercially available scanners. Despite its strength, DW-EPI is susceptible to artifacts due to distortion (Andersson, Skare et al. 2003), potentially impairing image quality and quantitative analysis of diffusivity. In the breast, artifacts due to distortion are exacerbated by the presence of multiple air-tissue interfaces and differences in magnetic susceptibility. DW-MRI sequences are also limited in spatial resolution, and in breast imaging high spatial resolution is needed for accurate diagnoses.

Efforts to improve DW-MRI acquisition and processing have included the use of parallel imaging (Kuroki, Nasu et al. 2004), development of methods to correct EPI distortion (Jezzard and Balaban 1995), and implementation of image registration to account for distortion (Partridge, Murthy et al. 2010). Recently, diffusion-weighted images have been acquired in other organs with a reduced field of view (rFOV) to increase resolution for a given acquisition matrix size (Wilm, Svensson et al. 2007).

A sequence for rFOV DW-MRI was developed at Stanford University for use in the spine, utilizing a 2D RF pulse for restriction of the in-plane FOV to a specified region (Saritas, Cunningham et al. 2008). In collaboration with Stanford University and GE Medical Systems, we hypothesized that restriction of the FOV with this new sequence would reduce artifacts from differing magnetic susceptibility at air-tissue interface while simultaneously improving resolution, both of which would improve image quality in DW-MRI of breast tumors. We further hypothesized that these improvements in image quality would improve the ability to characterize ADC heterogeneity and to predict treatment response to chemotherapy.
Our initial experiences with rFOV DW-MRI involved optimizing this sequence for breast imaging. We tested and modified a variety of parameters with the aim of improving rFOV DW-MRI image quality and acquisitions so that we could ultimately test our hypotheses related to tumor characterization and treatment response monitoring. In this study, results from sequence optimization and early testing are described.

6.2. Materials and Methods

Phantoms: Two types of phantoms were used for testing: one utilizing saline spheres suspended in oil to simulate breast tissue and the second utilizing a dual cavity design with doped water surrounded by fat.

Spheres: The spherical phantom was a quality control phantom developed by Patrick Bolan at University of Minnesota for ACRIN 6657. This phantom consisted of two bottles, Bottle A and Bottle B. The contents of Bottle A (20°C, 68°F) were: 2 Liters canola oil 40mM OD sphere containing 1mM phosphocholine chloride, 10mM TSP, 0.2mM Gd-DTPA, and 0.1% NaN3 in saline .9%. The contents of Bottle B (20°C, 68°F) were 2 Liters canola oil 40mM OD sphere containing 10mM TSP (NMR frequency reference), 0.2mM Gd-DTPA, and 0.1% wt NaN3 (sodium azide) in saline .9% wt. Bottle A was placed in the left breast coil and Bottle B was placed in the right coil, with each bottle surrounded by bags of water for stabilization (Figure 6-1).
Dual Cavity: The second phantom was developed by Sentinelle Medical and consisted of two breast-shaped structures, each filled with an inner layer of doped water, surrounded by an outer layer of fat. Each structure contained an inner plate allowing for resolution to be ascertained.

Human Subjects: Patients signing informed consent and meeting inclusion criteria were enrolled in studies approved by the institutional review board and were scanned with research sequences, including rFOV DW-MRI.

MR Acquisition: T1-weighted images were acquired with a fat-suppressed 3D fast gradient recalled-echo (3DFGRE) sequence with the following parameters: TR=8.95 ms, TE=4.40 ms, TI = 8ms, FOV=300mm, matrix = 512 x 320, slice thickness = 2mm, number of slices = 124, and number of averages = .75. Standard diffusion was acquired with a fat-suppressed, diffusion-weighted sequence with the following parameters: TR = 6000 ms, TE = 108.5 ms, FOV = 400mm, matrix = 128 x 128, slice thickness =3mm,
number of slices = 20, number of excitations = 6, b=0, 600, and number of diffusion-weighted acquisitions = 6. rFOV DW-MRI was acquired with a fat-suppressed, diffusion-weighted sequence with the following parameters: TR = 4000 ms, TE = 64.8 ms, FOV = 140 x 70 mm, matrix = 128 x 64, slice thickness = 4mm, number of slices = 8, number of excitations = 16, b=0, 600, and number of diffusion-weighted acquisitions =3. During testing, parameters were varied as follows: FOV= 9-25cm, b = 0, 600 or 800, TR = 3000 or 4000, number of slices = 8 or 16.

**Image processing:** For standard diffusion, ADC maps were calculated offline using in-house software. For rFOV diffusion, diffusion-weighted images were first automatically complex-averaged; ADC was then automatically calculated on-line.

**Image analysis:** Images were qualitatively and/or quantitatively assessed for optimization of imaging parameters and evaluation of protocol modifications.

**Image Quality:** For assessment of image quality, images from patient scans were qualitatively assessed and rated on scale from 1-3: 1= poor quality and not able to be quantitatively analyzed, 2= grainy or otherwise noisy, and 3= good. Ratings were based on images resulting from complex reconstruction; b600 combined, b0 and ADC maps were rated separately. For each acquisition, as many slices as possible were assessed. For an overall assessment of image quality in the study cohort, frequencies of each rating were calculated. Ratings for acquisitions with particular slice orientation and slice numbers were compared.
**FOV:** For testing of the relationship between ADC and FOV, ROIs were manually placed on images acquired in the dual cavity and mean ADC was calculated within each ROI. Mean ADCs were compared across acquisitions.

**Shimming method and number of slices:** Multiple parameters were tested and images were qualitatively compared. Mean ADCs were calculated and compared for selected acquisitions.

**Image Artifacts:** Artifacts were identified and tracked for frequency in occurrence.

**Case Studies:** The potential clinical utility of the sequence was assessed in two case studies. In the first case, a patient with invasive breast cancer was scanned with rFOV diffusion, standard diffusion, and DCE-MRI. In the second case a patient with in situ breast cancer was scanned with rFOV diffusion, standard diffusion, and DCE-MRI.

### 6.3. Results

**Image Quality**

**Overall image quality:** In patients, 33 axial exams were assessed for image quality. A rating of 3 (good) was more common on b=0 images than on b=600 images, and a rating of 3 was even less common on ADC maps (Figure 6-2).
Patients initially scanned with rFOV DW-MRI were scanned in both the axial and sagittal orientation, when possible, to assess the effects of orientation on image quality. Image quality was better on sagittal exams (Figure 6-3).

**Image quality on axial versus sagittal exams:** Patients initially scanned with rFOV DW-MRI were scanned in both the axial and sagittal orientation, when possible, to assess the effects of orientation on image quality. Image quality was better on sagittal exams (Figure 6-3).

Figure 6-2. Image Quality on Axial Exams. A rating of 3 was more common on T2-weighted (b=0) image and a rating of 1 was more common on diffusion-weighted images. Most ADC maps were noisy and rated 2. Some ADC maps were not available (NA=9%) due to inability to information handling errors and inability to obtain complex images off-line during the timeframe of the study.

Figure 6-3. Axial vs. Sagittal Image Quality. On T2-weighted (b=0) images, all sagittal exams were rated 3 (good), but fewer axial exams were rated good; on diffusion-weighted imaging, no sagittal exams were rated poor, but axial exams occupied all rating categories.
FOV

Four different fields of view were tested in the rFOV DW-MRI acquisition: 9, 14, 20, and 25 cm. Mean ADC was found to vary minimally (Figure 6-4) from the mean calculated from images obtained with the standard FOV of 14 cm (-5% change in 9cm FOV to +4.3% change in 20cm FOV)

Figure 6-4. Change in ADC with FOV. Mean ADC tended to increase with imaging FOV.
Shimming method

Due to the low SNR in reduced FOV images, shimming over a larger volume was determined to be optimal and a new shimming method was introduced in which a shim box was placed and expanded so as to cover the dimensions of slab to be imaged. Improvements were seen after the shimming method was introduced (Figure 6-5).

Number of slices

A 16 slice acquisition was developed for use in patients in large tumors that would require more than 8 slices for adequate coverage. To avoid doubling scan time as compared to the 8 slice acquisition, TR was reduced by 25% to 3000 ms; all other parameters were kept the same. On select slices, image quality did not appear to suffer as compared to the 8 slice acquisition (Figure 6-6); however, more slices at the end of the image volume suffered in quality and this 16 slice acquisition was discontinued in patient scans.
Figure 6-6. Comparison of Images from 8 and 16 slice rFOV DW-MRI Acquisitions. Images are shown for 8 and 16-slice b=0 and b=600 acquisitions, and computed ADC maps. Qualitatively, 8 and 16 slice rFOV images looked similarly. Mean ADC was increased in the 16 slice acquisition, but within the expected limits for test/retest variability.
Artifacts

Three artifacts were primarily identified: 1) a noise band at the level of the tumor (Figure 6-7), 2) a wrap-like artifact in the complex recon images but not in the acquired images (Figure 6-8), and 3) a wrap-like artifact in recon and acquired images (Figure 6-9). Frequencies of these artifacts are in Table 6-1.

Table 6-1. Artifacts in rFOV

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<tr>
<td>Noise band</td>
<td>2/38 exams</td>
</tr>
<tr>
<td>Wrap in recon only</td>
<td>4/38 exams</td>
</tr>
<tr>
<td>Wrap in recon and acquired</td>
<td>24/38 exams</td>
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</table>

Figure 6-7. Noise band. A band of noise is seen at the level of the tumor, in both axial and sagittal acquisitions.
Figure 6-8. Images with a wrap-like artifact in complex reconstructed but not acquired rFOV DW-MRI data. A wrap-like artifact was seen in reconstructed images but not acquired images. This artifact is considered to be wrap-like and not phase wrap because the excited breast tissue was not larger than the FOV and phase wrap would not be expected.
Figure 6-9. Images with a wrap-like artifact in complex reconstructed and acquired rFOV DW-MRI data. A second wrap-like artifact was seen in both acquired and recon images.

Both cases of the noise band were in the same patient (different visits). The noise band was replicated in a phantom with phase-encoding direction set to the anterior/posterior direction, as in the patient. When the phase-encoding direction was switched to the optimal right/left direction, the noise band was no longer apparent (Figure 6-10).
Figure 6-10. Images from phantom studies exploring relationship between noise band and rFOV DW-MRI phase-encoding direction. The noise band was not apparent when the phase-encoding direction was changed to the non-anterior/posterior direction, the optimal phase-encoding direction for breast imaging due to the location of the breast anterior to the lungs. Note that the noise band is apparent in both sagittal (top row) and axial acquisitions (bottom row) when phase-encoding direction is anterior/posterior. PE=phase-encoding direction, R/L=right/left, S/I=superior/inferior.

A problem with fat suppression and water excitation occurred in one patient (Figure 6-11), and was attributed to incorrect identification of the center frequency of water.

Figure 6-11. Images acquired in a patient with invasive breast cancer illustrating water suppression in rFOV DW-MRI b=0 and b=600 acquisitions. In the images from the rFOV acquisition, tissue in the tumor appears hypointense on both b=0
and b=600 rFOV images (orange arrow). Areas of fibroglandular tissue (green arrow) also appear hypointense (green arrow) and fat appears bright. The tumor is hyperintense on post-contrast DCE-MRI (orange arrow).

Artifacts thought to occur due to incorrect identification of water center frequency were replicated in the spherical phantom (Figure 6-12). Misidentification of center frequency by more than 200 Hz resulted in water suppression, paralleling the effect seen in Figure 6-11. Misidentification of center frequency by 100 Hz resulted in wrap in the acquired images. Misidentification by 150 Hz resulted in partial water suppression. Misidentification by 50 Hz resulted in noisier images, a problem which had been noted in select patient cases. Due to the importance of center frequency in the rFOV acquisition, a method of manual pre-scan and manual adjustment of the center frequency was implemented.

Figure 6-12. Images from phantom studies exploring relationship between center frequency and artifacts in rFOV DW-MRI. The rFOV sequence utilizes water excitation and fat suppression. In the far right image, a large shift in center frequency resulted in suppression of water and excitation of fat; a similar situation occurred in a patient (Figure 6-11). As seen in the images, misidentification of the center frequency of water resulted in different artifacts depending on the magnitude of the misidentification.
Case studies

Case A. rFOV DW-MRI in Invasive Breast Cancer:

In Case A, the patient is a pre-menopausal woman with cancer of the right breast. The patient was scanned with magnetic resonance imaging sequences on a 1.5 T Signa scanner (GE Medical Systems, Milwaukee, Wisconsin) prior to the start of treatment (Figure 6-13). On dynamic-contrast enhanced MRI (DCE-MRI), the cancer is seen as a lobulated, enhancing mass, 6.9*6.7*5.8 cm, with central necrosis. Right axillary lymphadenopathy and right internal mammary lymphadenopathy were also seen. The patient was scanned post-contrast with a standard FOV DW-MRI protocol (40cm FOV, diffusion tensor imaging, (DTI)) and rFOV DW-MRI. The ADC map shows a large hypointense mass, with central areas of higher intensity; possibly correlating with the areas of central necrosis seen on DCE-MRI. The ADC map shows that tumor regions vary in intensity and this variation may be more apparent with rFOV DW-MRI than on with standard FOV DW-MRI.

Figure 6-13. Invasive breast cancer. A patient with invasive breast cancer was scanned with standard diffusion (DTI, ADC map above at left), rFOV diffusion (ADC map above, at center), and DCE-MRI (post-contrast image above, at right).
Pathology obtained at core biopsy determined the cancer to be invasive ductal carcinoma; the modified Bloom-Richardson grade to be 8/9, or high grade. No lymphovascular invasion, microcalcifications, lobular carcinoma in situ, or ductal carcinoma in situ were identified. The patient was found on imaging to have metastatic disease; pathology was concordant with this finding.

**Case B. rFOV DW-MRI in In Situ Breast Cancer:**

A patient with biopsy-proven ductal carcinoma in situ (DCIS) of the left breast was scanned with MRI. On DCE-MRI, mass-like enhancement was seen in the left upper outer breast with areas of washout kinetics; additional enhancing lesions were seen throughout the upper left breast. The exam was given a BIRADS 4, indicating suspicion of invasive disease and the need for a biopsy to examine the tissue. Post-contrast, the patient was scanned with rFOV DW-MRI and standard diffusion (Figure 6-14). Diffusion findings were not part of the clinical report (at the time of this writing, DW-MRI is generally considered investigational in breast imaging), but areas of hyperintensity can be visualized on rFOV diffusion. These regions may or may not correspond to in situ disease.

Two weeks after MR imaging, ultrasound-guided core biopsy was performed 8 cm from the nipple of the left breast. Solid, cribiform, intermediate to high grade DCIS was identified with microcalcifications in benign ducts. No lobular carcinoma in situ or invasive disease was identified. (Absence of invasive disease was confirmed by immunostaining for p63 and SMM). DCIS was measured to be 0.7cm in 0.4cm thick cores of length 1.8, 1.6, 1.6, 1.5cm. Pathology was determined to be concordant with imaging findings.
Figure 6-14. In situ breast cancer. A patient with in situ breast cancer was scanned with rFOV, standard diffusion, and DCE-MRI. Regions of hyperintensity are seen on both diffusion acquisitions, but lesions are more well-defined and conspicuous on rFOV diffusion (b=600). These regions may or may not correspond to in situ disease, but the hyperintensity on diffusion is compatible with the increased cell density that would be seen in in situ cancer.

6.4. Discussion

In early studies, a reduced field-of-view diffusion-weighted magnetic resonance imaging (rFOV DW-MRI) sequence was optimized for use in patients with breast cancer. Initial experiences with rFOV DW-MRI were not without challenges. Image quality of rFOV diffusion varied considerably, with several exams being judged to be of too poor quality for quantitative analysis. The cause of this poor quality was investigated and
adjustments were made to improve quality. With these acquisition improvements, image quality improved.

One gap in quality centered on image orientation. Images obtained with sagittal acquisition appeared to be of higher image quality than images from axial acquisitions in the same patients. Later studies showed that good placement of the shim box and accuracy in capturing the center frequency (CF) of water within the image volume were essential. This finding may explain why sagittal acquisitions resulted in improved image quality. In sagittal acquisitions, more of the imaging FOV was occupied by breast tissue, perhaps allowing for a more accurate CF of water to be calculated; in contrast, in axial acquisitions, more of the FOV was occupied by background noise. To address the problem of poor axial quality, we first implemented a new shimming method which reduced inclusion of air in the shim volume. While image quality appeared to improve, we continued to see artifacts that were thought to be due to center frequency problems. We then implemented a protocol of manually inputting the center frequency of water; image quality dramatically improved.

Improvements in rFOV diffusion image quality are exciting, particularly because this sequence appears promising for multiple clinical applications. In in situ breast cancer, regions of hyperintensity on DW-MRI were seen in the rFOV acquisition and not seen in the standard acquisition. These regions would need to be biopsied to determine if they were regions of cancer, regions of another pathological process such as fibrosis (which would also result in low diffusion), or non-pathological variations in tissue. Improved identification of small in situ or invasive lesions with a high resolution sequence could improve the diagnostic imaging of breast cancer. In invasive disease,
tumor heterogeneity was better captured on rFOV diffusion than on standard diffusion. Studies in multiple organs have found that histogram analysis is beneficial in monitoring treatment response. The ability of rFOV diffusion to better capture tumor heterogeneity could improve treatment response monitoring.

6.5. Conclusions

In conclusion, early studies optimizing and testing rFOV diffusion were successful in 1) improving image quality, and 2) demonstrating potential clinical utility in the imaging of both in situ and invasive disease. Further work assessing differences in tumor depiction with rFOV versus standard FOV DW-MRI is needed and this is addressed in Chapter 11. The ability of the ADCs derived from each sequence to predict treatment response should be prospectively assessed and Chapter 12 presents a related investigation. The rFOV DW-MRI sequence evaluated in this chapter provides high resolution imaging of the breast and has the potential to improve the diagnosis and treatment response assessment of breast cancer.

6.6. Acknowledgements

This work was made possible through a collaboration with GE Healthcare and Stanford University.

6.7. References


In this section, image processing methods aimed at improving the extraction of tumor diffusivity information from DW-MRI data are explored. Use of DW-MRI in monitoring tumor response to treatment requires: 1) delineation of tissue regions of interest, and 2) measurement of diffusivity in these regions. Measurement of diffusivity in normal fibroglandular tissue is needed for calculation of normalized tumor ADC, a parameter that was tested in Chapter 3 for prediction of recurrence-free survival. Measurement of this parameter first requires delineation of normal fibroglandular tissue on diffusion data and a method for breast segmentation is explored in Chapter 7. Whether normalized to the diffusivity in normal tissue or not, treatment monitoring primarily tracks diffusivity changes in the tumor; however, tumors can exhibit varying degrees of heterogeneity that may not be adequately captured by measurement of a mean ADC. Alternative measurements derived from the ADC distribution are explored in Chapter 8. Tumor response to treatment may also be heterogeneous, with some regions responding and other regions not responding and a method to capture this heterogeneity is described in Chapter 9. Methods for defining tumor regions in which to subsequently measure diffusivity are explored in Chapter 10. In order for treatment monitoring to help guide chemotherapy, measurement of tumor diffusivity must be fast enough for timely feedback to clinicians.
Chapter 7. Breast Segmentation

Chapter 7. Breast Segmentation .......................................................................................... 169
7.1. Introduction ............................................................................................................. 169
7.2. Materials and Methods ......................................................................................... 172
    Image Processing ...................................................................................................... 173
    Comparison of our new technique with a manual method ...................................... 177
7.3. Results .................................................................................................................... 179
    Comparison of new vs. manual technique .............................................................. 181
7.4. Discussion .............................................................................................................. 183
7.5. Conclusion ............................................................................................................. 189
7.6. Acknowledgements ............................................................................................. 190
7.7. References ........................................................................................................... 190

7.1. Introduction

Breast cancer is the most common cancer in women, the second most common cause of cancer death in women (Kopans 2007), and the leading cause of overall death in women age 45-55 (O'Leary, Tabuenca et al. 2008). Breast density, as measured by mammography, is an independent risk factor for breast cancer (Boyd, Martin et al. 2009). The importance of breast density assessment in breast cancer screening has led to breast density reporting standards (American College of Radiology Breast Imaging Reporting and Data System lexicon) for mammography exams (D'Orsi, American College of Radiology. et al. 1998; Burnside, Sickles et al. 2009).

Mammography is the current imaging standard in breast cancer screening and thus the most common method of measuring breast density and other measures of breast
composition; however, mammography utilizes ionizing radiation which could pose risks in serial studies of serial breast density and breast composition monitoring. Sensitivity of mammographic screening is also impaired by high breast density (Kolb, Lichy et al. 2002), a density category that is seen more often in younger women. In contrast, magnetic resonance imaging (MRI) is a three-dimensional, non-ionizing imaging method with high soft tissue contrast that provides information on breast composition and functional activity, which could potentially lead to new markers of risk. The sensitivity of MRI is not impaired by high breast density and MRI does not utilize ionizing radiation, making it a valuable alternative for populations of women who cannot undergo mammographic exams due to their young age or high breast density. Recently, studies have used MRI to measure breast density and breast composition (Khazen, Warren et al. 2008; Nie, Chen et al. 2008; Boyd, Martin et al. 2009; Thompson, Leach et al. 2009). MRI breast density has been found to correlate with mammographic breast density(Graham, Bronskill et al. 1996; Klifa, Carballido-Gamio et al. 2010). Breast composition as measured by MRI may provide important information about breast cancer risk (Thompson, Leach et al. 2009; Dorgan, Liu et al. 2010), treatment response, and prognosis; however, these relationships are still being elucidated.

Despite its strengths, use of MRI in quantifying breast tissue composition is limited by the fact that current computerized analysis methods are time and user-intensive. User interaction results from the need to delineate the breast from the chest wall in order to analyze the tissue within the breast; on MRI volumes made of multiple slices (20-100 or more slices), breast volume delineation can be a repetitive, laborious task. Faster methods of volumetric breast tissue quantification could improve the clinical
adoption of MRI breast composition measurements as metrics for breast cancer risk and treatment response assessment.

Previous solutions for quantification of breast composition from MRI data have targeted MR image acquisition or image processing. A method targeting image acquisition is the use of the Dixon method to acquire separate water and fat images and calculation of breast density as the proportion of water content (representing fibroglandular tissue) to total content (representing adipose and fibroglandular tissue) (Graham, Stanchev et al. 1995). This method has been shown to correlate with mammographic density (Boyd, Martin et al. 2009); however it requires the use of a non-standard sequence, which could limit its use and add cost to the overall clinical MRI exam.

Other methods utilize image processing to improve breast density measurement. One method involves the implementation of a “V-shape” across each axial slice to better standardize the axillary borders of the breast included in the breast density measurement (Ke, Jeon-Hor et al. 2008); however, the problem of delineating the pectoral muscles from posterior fibroglandular tissue remains.

To date, a fully-automated, accessible method has not been developed using MRI data. A fast, user-free technique is much needed for clinical use of these breast tissue composition measurements. The need for such a method motivated us to explore computer automation of measurements of breast composition. In this study, we aimed to develop a faster, three-dimensional method for quantifying breast tissue composition and volumetric breast density from MRI data while reducing user interaction. Our goal is to provide clinicians with a fast and robust technique to measure breast tissue composition.
7.2. Materials and Methods

The goals of our algorithm were to 1) quantify fibroglandular breast tissue volume and total breast volume with improved speed in order to measure volumetric breast density as defined in Figure 7-1, and 2) to spatially map fibroglandular breast tissue.

Figure 7-1. Calculation of Volumetric Breast Density. Hyperintense (bright) areas of the T1-weighted, non-contrast magnetic resonance image correspond to fibroglandular tissue. Hypointense (dark) areas correspond to adipose tissue. Volumetric breast density is calculated as the total volume of fibroglandular tissue divided by the total volume of breast tissue.

Volumetric Breast Density = \frac{\text{Fibroglandular tissue volume}}{\text{Total breast Volume}}
We applied our technique to breast MRI data obtained under a standard clinical imaging protocol. All MR data presented in this project were from healthy volunteers who signed a consent for projects approved by our institution review board. Fat-suppressed T1-weighed images were acquired using the following parameters: TR/E = 27/4.76 ms, FOV=320 x 320 mm, acquisition matrix = 512 x 256, slice thickness = 1mm, number of slices = 144-160, number of averages = 1, orientation= axial.

**Image Processing**

Due to the visibility of the chest wall throughout the axial MRI volume (the chest wall is visible as the near-horizontal separation between breast tissue and muscle), we chose the axial orientation as the input for our algorithm. Standard breast MRI data acquired in other orientations could be reformatted to the axial orientation for compatibility with this algorithm. In order to quantify breast tissue composition and volumetric breast density we first quantified fibroglandular and total breast volumes. We did this by 1) segmenting the breast volume from the chest wall, 2) suppressing the background noise and removing skin on the breast volume, and 3) extracting fibroglandular tissue from adipose tissue in the selected volume. These steps are described below and illustrated in Figure 7-2.
Figure 7-2. Overview of Processing Algorithm. Non-contrast axial MRI data is first acquired in the axial orientation. The axial MRI data is utilized to define the chest wall on a central slice and then reformatted to a coronal orientation. The coronal data is then processed using the following steps: 1) clean background and remove skin, 2) identify total breast volume, and 3) extract fibroglandular tissue. The output of the algorithm is the quantification and locational mapping of fibroglandular breast tissue and total breast volumes, quantification of MRI breast density, and indirect quantification and locational mapping of adipose tissue volume (total breast volume minus fibroglandular tissue volume).
1. Segmentation of Chest Wall: In each axial MRI volume, the slice containing approximately the largest anterior-posterior chest diameter (roughly the middle slice) was selected for chest wall delineation. Using in-house software developed in Interactive Data Language (IDL Version 7.0, ITT Visual Information Solutions, Boulder, Colorado), the chest wall was manually defined by creating an irregular region-of-interest (ROI) that separated the pectoral muscle from the posterior breast and then loosely enclosed the anterior breast (Figure 7-3). This single ROI was projected throughout the entire axial MRI volume; the algorithm only analyzed the content of the three-dimensional region defined by the ROIs.

Figure 7-3. Chest Wall Delineation. The chest wall is delineated on one slice, the slice visually determined to have the greatest anterior-posterior diameter, which is roughly the middle slice of the volume.
2. Suppression of background noise and skin: For this step, we utilized the coronal orientation due to the regularity of the breast shape (circular). In addition, the coronal orientation also provided a more regular skin thickness on each slice, facilitating skin removal. Utilization of both axial and coronal orientations gave us flexibility in our processing algorithm and was feasible due to the ability to automatically reformat 3D MR volumes.

Speckled noise from the background of each MRI slice was removed by median filtering in the coronal plane. A threshold was then used to suppress the remaining background in order to extract the breast volume from the background and minimize modification to its content. The default threshold value was empirically defined as twice the mean background pixel intensity on a selected slice, or a pre-specified value when that value was 0 or when the user desired a different threshold. After this step, the extracted breast volume included the total breast and skin volume.

The hyper-intense skin pixels were removed by eroding the resulting breast volume using a structuring element size empirically defined as a matrix of 15x15 pixels (Serra 1982). The resulting processed data included the total breast volume without skin. This total breast volume was then quantified. Since voxels in this volume were processed (median filtering) for background correction, we used the total breast volume mask obtained after skin removal to select the corresponding region from the original MRI data, in order to proceed to the breast tissue segmentation step. The next steps in the algorithm were therefore applied to the extracted total breast volume containing original unprocessed data.
3. Fibroglandular tissue segmentation: The extracted total volume of the breast obtained from the pre-processing algorithm was segmented into adipose and fibroglandular tissue by first selecting the volume under study. To do this, control points were interactively placed by the user on one slice of the coronal data, in a loosely circular shape around the breast. B-splines were then automatically fitted to the control points using interpolation and copied to all slices in the MRI volume. The volume selected by the splines was then segmented into three groups (fibroglandular tissue, adipose tissue, and background) using a fuzzy-c-means based technique (Bezdek, Hall et al. 1993) developed in our group (Klifa, Carballido-Gamio et al. 2004). The group corresponding to fibroglandular tissue was then selected and its volume was quantified. Volumetric breast density was calculated as a volumetric ratio of fibroglandular tissue volume to total breast volume.

Comparison of our new technique with a manual method: The efficiency of our new technique was tested by recording the number of slices requiring manual delineation of breast contours and comparing that number to the number required with manual methods (Klifa, Carballido-Gamio et al. 2004). Accuracy of the algorithm was tested by comparing measurements of total breast volume, total fibroglandular tissue volume, and breast density with measurements obtained using manual methods. For each measure of breast composition, the percent change relative to the measurement made with the manual method was calculated:
\[ \text{percent change (\%)} = \frac{\text{new measurement} - \text{manual measurement}}{\text{manual measurement}} \times 100 \]

Volumetric breast density measurements made with the new and manual methods were compared with Bland-Altman (Bland and Altman 1986) plots and the 95% confidence interval for the agreement between the two methods was calculated.
7.3. Results

The result of our new algorithm is presented for an example case in Figure 7-4. The MRI data was acquired in an axial orientation and the result of the reformat to coronal is shown in Figure 7-4 (far left image). The total breast volume was segmented from the background and the skin was removed, as seen in Figure 7-4 (center image). The breast was then segmented into fibroglandular and adipose tissues and the resulting map of fibroglandular tissue is seen in Figure 7-4 (far right image). The hyperintense areas in the processed image correspond to the hyperintense areas in the original MRI data, showing that the algorithm did in fact select the fibroglandular tissue.

![Figure 7-4 Tissue Segmentation. At left, the original axial MRI data, reformatted to coronal and cropped to the chest wall is shown for one slice. At center, the result of background suppression and skin removal is shown for the same slice. At right, the resulting fibroglandular tissue map is shown.](image)

Spatial line profiles drawn through identical central horizontal planes of the original MRI volume and processed total breast volume (Figure 7-5) illustrate the changes produced by the algorithm. The background of the original data is noisy, as
illustrated by the heterogeneous peaks at the far left and far right sides (right arrow) of the profile at left in Figure 7-5. Reduction of background noise, skin removal, and selection of total breast volume reduced the background noise, as seen in the empty left and right-hand sides (right arrow) of the line profile at right in Figure 7-5.

Finally, the result of the algorithm is evaluated against the result for manual processing in Figure 7-6. At left in Figure 7-6, a slice from the original non-contrast MRI data acquired in the axial orientation is shown. The result of our new technique (reformatted back to the axial orientation and original resolution) is shown at center in Figure 7-6 and the result from the manual technique at right in Figure 7-6. (Klifa, Carballido-Gamio et al. 2004).

Figure 7-5. Comparison of Background Intensities. At left, a horizontal line profile for the original MR data is shown. The background in the original MRI data is noisy, with pixels fluctuating in intensity (arrow). In addition, the outer contours of the breast have high intensity pixels, with intensity greater than 100 (corresponding to skin pixels). At right, a horizontal line profile for the processed MR data is
shown. After processing and selection of the total breast volume, the intensity spikes at the outer contours are reduced and the background noise is reduced (arrow).

Figure 7-6. Comparison of New versus Manual Method. At left, original axial MRI data is shown. At center, the fibroglandular tissue map is shown for the same slice, obtained after processing with a new method utilizing axial and coronal images. At right, a fibroglandular tissue map is shown, obtained after manual processing and segmentation utilizing axial images. The center and far right images are comparable, illustrating the efficacy of the new method.

Comparison of new vs. manual technique

*Algorithm efficiency:* Our new algorithm reduced the number of regions-of-interests (ROIs) manually drawn from 20-50 to two (90-96% reduction in ROI number). Our new algorithm also reduced variability in this number, as the number of ROIs previously depended on the number of slices in the MRI volume and on the user technique (Klifa, Carballido-Gamio et al. 2004). Our novel algorithm fixes this number to two ROIs per MRI volume: one ROI to define the chest wall on the central axial slice and
one ROI to envelope the entire breast volume prior to segmentation with fuzzy-c-means. Our new algorithm also eliminated the need to precisely define the full breast contours, allowing a more general and free flowing selection around the breast on one slice. This reduces the time required for breast volume delineation by a factor of 10 or more: from 20 minutes to less than 2 minutes.

**Algorithm accuracy:** Measurements of breast composition from MRI data made using the new and manual methods were compared in 6 different cases. On average, the new method resulted in the following percent changes in measurement as compared to the manual method: -5.3%, 5.3%, and -9.9% for fibroglandular volume, total breast volume, and density, respectively. Measurements for breast density using the new method compared well measurements made using the manual method (Figure 7-7), with differences falling within the 95% confidence interval for the limits of agreement between the two methods (Figure 7-7).
Figure 7-7. Accuracy of New Method. At left, volumetric breast density measurements made with new and standard methods are shown for each case. As seen in the barplot, differences in measurements are minimal. At right, a Bland-Altman plot is used to evaluate agreement between the methods. Measurements (mm3) are within 1.96 SD (dotted lines) of the mean difference (dashed lines).

7.4. Discussion

Our new method for quantification of breast composition and breast density reduces user interaction to the selection of only two regions-of-interest (ROIs) per MRI volume. It excludes isointense contributions from background noise, skin, and pectoral muscle. While differences were observed in the quantification of breast composition as compared to a manual method, differences ranged from 5-9% and are similar to differences due to intra- and inter-user variation reported with the manual technique(Klifa, Carballido-Gamio et al. 2004). In addition, these differences may be clinically tolerable and do not invalidate the locational mapping of these parameters.
Currently, calculation of MRI total breast volume with commercially available software takes an average of ten minutes (Herold, Reichelt et al. 2010). Our method provides a much faster result for breast volume and tissue delineation (less than 2 minutes), thus facilitating the quantification of volumetric breast density and measurements of breast tissue composition in the clinic. This could help make possible future larger studies evaluating MRI density for assessment of breast cancer risk or treatment response assessment.

Our previous work focused on measuring volumetric breast density from MRI data acquired in the coronal orientation, with exclusion of image slices posterior to the chest wall region from the calculation (Singer, Hylton et al. 2008). This helped avoid manual segmentation of the breast fibroglandular tissue from the chest wall region, but risked the loss of important posterior regions of fibroglandular breast tissue. Tumors and other lesions may occur in these posterior regions of the breast. To minimize this issue, we changed our method to incorporate user-defined information about chest wall location from axial data and limited the chest wall delineation to only one slice, as described in the methods section. Our new technique includes posterior breast tissue and presents an improvement over algorithms that limit analysis to anterior breast tissue.

A main source of variability in our current method is individual variability in chest wall shape. Individual variation makes automation of chest wall delineation on the central axial slice difficult. In our method, we rely on a user to delineate this contour with a manually drawn region-of-interest (ROI). Delineation of the chest wall on the central slice of the MR volume is a conservative approach in that if the anterior chest wall boundary on another slice is posterior to this delineation, breast tissue may be lost but
chest wall will not be included. This helps avoid the risk of measuring chest wall as fibroglandular tissue. Chest walls with greater superior-inferior differences in anterior-posterior (AP) diameter (greater slopes), or chest walls with peak diameters far away from the central axial slice could result in exclusion of breast tissue from the analysis. The fact that the new method overestimates total volume by 5% suggests that some pectoral muscle may remain in the breast volume after segmentation. Delineating the chest wall on an axial maximum intensity projection image may help exclude pectoral muscle and include breast tissue otherwise lost due to individual variation in chest wall morphology.

Currently, our method also involves a second ROI: delineation of the breast volume prior to segmentation with fuzzy-c-means. The need for this ROI was due to the constraints of our in-house software programmed in Matlab and this ROI could be eliminated by a commercial system. Since all voxels in the MRI volume can be included in a fuzzy-c-mean segmentation based on three tissue groups (background, fibroglandular tissue, adipose tissue), an ROI should not be necessary for breast segmentation. Integration of the programming platforms used in our new method could further increase efficiency and reduce variability in measurement of breast composition.

By providing a fast method of total breast segmentation and breast density measurement, this new method of breast segmentation could facilitate use of breast composition measures in the clinic. Routine measurement of breast composition on screening breast MRIs could be feasible since they are performed on pre-contrast MRI data, common to clinical breast MRI protocols. This could provide quantitative information from large cohorts of women undergoing breast MRI for screening or
diagnosis and participating in imaging studies. Our algorithm has applications to breast cancer risk assessment, diagnosis, and treatment monitoring through its measurement of markers of breast composition: breast density, total breast volume, and total fibroglandular volume.

Breast density as measured by mammography has been correlated with breast cancer risk (Threatt, Norbeck et al. 1980; Brisson, Merletti et al. 1982) and more recently, in a meta-analysis of 42 studies, individuals in the densest category were found to have a nearly five-times increased risk of breast cancer compared to those in the least dense category (McCormack and dos Santos Silva 2006). The relationship between breast cancer risk and MRI volumetric density could be further explored with a more efficient method of MRI density measurement.

The correlation between total fibroglandular volume and breast cancer risk has also been studied. In one study, MRI fibroglandular tissue volume was found to be associated with increased breast cancer risk in BRCA 1 and BRCA 2 carriers (Thompson, Leach et al. 2009). Fibroglandular tissue volume may also be important in assessing response to methods aimed at decreasing risk. Fibroglandular tissue volume has been shown to decrease in high-risk pre-menopausal women given raloxifene; in these women, mammographic density did not change significantly (Eng-Wong, Orzano-Birgani et al. 2008). Measurement of fibroglandular tissue volume may be important in risk assessment and in monitoring response to treatments aimed at reducing risk.

Our algorithm also quantifies total breast volume. Measures of total breast volume have applications in post-operative assessment of breast reconstruction and may also be helpful in surgical planning (Herold, Reichelt et al. 2010). MRI total breast volume has
been shown to correlate with obesity measures (Janiszewski, Saunders et al. 2009). By providing direct measures of multiple breast tissue quantities, efficient breast segmentation has the potential to advance research and clinical applications related to breast composition.

The ability of this method to provide a spatial map of fibroglandular tissue in a variety of orientations allows for additional important applications. Calculation of total fibroglandular tissue volume involves creation of a tissue map and this map can be transferred onto other MRI functional images (derived from other MRI sequences), allowing for extraction of diverse fibroglandular tissue parameters. Overlay of the fibroglandular tissue map with dynamic-contrast enhanced MRI (DCE-MRI) data could allow for quantification of background enhancement (Teifke, Hlawatsch et al. 2002); (Morris 2007). Overlay of the tissue map with the apparent diffusion coefficient (ADC) map derived from diffusion-weighted MRI (DW-MRI) could also be useful. The ADC of normal tissue has been studied as a comparison to the ADC in tumor for diagnostic and prognostic purposes (Kim, Cha et al. 2009; Partridge, Ziadloo et al. 2010). For background enhancement and normal tissue ADC calculations, regions-of-interest (ROIs) are drawn on selected regions of breast tissue, but utilization of a tissue map could allow for these parameters to be studied throughout the entire fibroglandular tissues and decrease inter-user variability as manual outlining of regions-of-interest are often dependent on user technique.

Our new method provides a means to segment and quantify breast tissue types and these parameters could be important across breast cancer imaging, from screening to the prognostic setting. In treatment monitoring, changes in normal fibrogladular tissue
may be important and use of the fibroglandular tissue map produced in this algorithm could enable tracking of those changes in a variety of parameters (volume, density, enhancement, ADC). In the prognostic setting, changes in the tissue surrounding the tumor could be important in predicting overall survival, and those changes could be measured by utilizing the tissue map. And in diagnosis and screening, if breast tissue composition is reliably quantified in large enough cohorts of patients, it could be envisioned that thresholds in normal or diseased tissue could be determined to help guide care.

Our method is still limited by the overall quality of the breast MRI data. Noisy images could be problematic if the noise reached intensities found in fibroglandular breast tissue. Techniques applied for noise suppression must also be selected with care as they could inadvertently suppress regions of similar intensity levels, such as regions of fibroglandular tissue. In addition, parameters used in the removal of skin around the breast total volume have been defined empirically. For patients who may exhibit thicker breast skin these parameters could be modified. A method to calculate skin volume from total breast volume has been proposed (Nie, Chang et al. 2010), and this information could help automate adjustment of structuring element size used in skin removal.

Our fast quantification technique is dependent on image quality but it is robust in that it standardizes measurement of breast composition, reduces user-interaction, and works on non-contrast data. It also can provide results in a variety of image orientations for measurement of quantities derived from MRI data acquired in other orientations.
7.5. Conclusion

Our new technique reduced the user-interaction in the quantification of breast composition and breast density from MRI data. The final result of our algorithm provides quantitative measurements of total breast volume, fibroglandular breast tissue volume, and volumetric breast density, as well as spatial maps of fibroglandular tissue and total breast tissue. This quantification and segmentation of breast tissue allows for tracking of relevant parameters in clinical settings pertaining to the screening, diagnosis and prognosis, and treatment of breast cancer. Future work should focus on implementation of this method on clinical computer aided diagnostic systems, allowing for efficient, routine measurement of breast composition in the clinical and research settings, and facilitation of studies evaluating the relationships between breast composition, risk and breast cancer outcomes.
7.6. Acknowledgements

This study was made possible through current and previous funding from California Breast Cancer Research Program (CBCRP) IDEA 131B-0171, NIH R01 CA069587, NIH R01 CA116182, Medical Scientist Training Program and Department of Bioengineering at UCSF, and the California Breast Cancer Research Program Dissertation Award.

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8.1. Introduction

Diffusion-weighted magnetic resonance imaging provides a noninvasive measurement of water mobility in-vivo (Neil 1997). Because water mobility in a tumor relates to important properties such as cell density and cell content, and because these
properties may change with chemotherapy administration, DW-MRI is a promising technique for monitoring tumor response to chemotherapy. Value in treatment monitoring with DW-MRI has been shown for a variety of anatomic sites, including the brain, head and neck, and the breast (Ross, Moffat et al. 2003; Colagrande, Carbone et al. 2006; Abdel Razek, Gaballa et al. 2010).

Traditionally, monitoring treatment response with DW-MRI has involved averaging diffusivity over the whole tumor or tumor regions of interests; however, averaging this cellular property over the tumor results in loss of information about regional differences in the tumor. Even in regards to size-based tumor measurement, it is known that the tumor does not shrink homogeneously (Belli, Costantini et al. 2006). Similarly, the molecular and cellular response of the tumor to chemotherapy, in the form of apoptosis or necrosis and decreased cell density, may not be homogeneous throughout the tumor. In the breast, heterogeneity of contrast uptake measured with DCE-MRI has been correlated with a poor treatment response (Venkatasubramanian, Arenas et al. 2010). Similarly, heterogeneity in breast tumor diffusivity may provide important information about treatment response. Histogram analysis provides a method of visualizing heterogeneity. Diffusion histogram analysis has been found to be valuable in clinical applications for other diseases (Mori, Miki et al. 2008; Tessa, Giannelli et al. 2008; Pope, Kim et al. 2009).

The full distribution of ADC values, and changes in this distribution with chemotherapy, can be visualized with an ADC histogram, and particular parameters related to the histogram can be tracked, in addition to the mean. In the breast, tracking changes within particular ADC intervals has been shown to be beneficial (Yankeelov,
Lepage et al. 2007). At the time of this writing, it is not known whether tracking properties related to the tumor distribution, such as kurtosis, skew, or upper and lower quartiles, may be beneficial in predicting long-term response to neoadjuvant chemotherapy in patients with breast cancer.

The purpose of this study was to assess the ability of parameters derived from tumor ADC histogram analysis to predict long-term patient outcomes. Due to the fact that 1) morphological changes in response to chemotherapy are known to be heterogeneous, and 2) proportions of voxels in particular ranges of ADC values have been shown to change in response to chemotherapy, it was hypothesized that histogram analysis of the tumor ADC distribution would provide a better means of predicting response to chemotherapy than an average tumor ADC. This hypothesis was tested in a group of patients with locally advanced breast cancer undergoing serial DW-MRI exams before, during, and after treatment with neoadjuvant chemotherapy.

8.2. Materials and Methods

Patients

All patients were in Group 2 as part of an IRB-approved study and gave informed consent. Patients had invasive breast cancer, confirmed by pathology and were considered to have locally advanced disease. All patients underwent treatment with neoadjuvant chemotherapy (4 cycles of doxorubin/cyclophosphamide, followed by 4 cycles of a taxane). Only patients scanned with both dynamic-contrast enhanced MRI and
diffusion-weighted MRI at baseline and after the first cycle of chemotherapy were included in this analysis.

**Imaging acquisition**

*Dynamic-contrast enhanced MRI*: Unilateral, sagittal DCE-MRI data was acquired in accordance with the required study protocol for ACRIN 6657.

*Diffusion-weighted MRI*: Bilateral, axial diffusion-weighted MRI (DW-MRI) was acquired with a fat-suppressed, 2D, single shot fast-spin echo (SSFSE) sequence. Imaging parameters were: TR/TE= 6257/61 ms, FOV= 350mm, acquisition matrix= 128 x 128, for an in-plane resolution of 2.73x2.73mm, slice thickness= 5mm, number of slices= 10, number of averages= 0.5. Diffusion-weighted gradients were applied sequentially in 3 orthogonal directions with b=600. Images were also acquired with b=0. Non fat-suppressed data was also acquired, but only fat-suppressed data was used for this analysis.

**Image processing and analysis**

*ADC Maps*: Maps of the measured apparent diffusion coefficient (ADC, or D) were created offline for DW-MRI data. Maps were created under the assumption of monoexponential signal decay of the original signal ($S_{b0}$) to $S_{b600}$ with application of the diffusion gradients using the relationship below and previously published methods (Partridge, McKinnon et al. 2001).

$$S_{b600} = S_{b0}e^{-bD}$$
**Region of interest delineation:** Tumor on ADC maps was limited to corresponding regions of abnormal enhancement on DCE-MRI due to the established relationship between signal enhancement ratio (SER) tumor volume derived from DCE-MRI data and residual disease on pathology (Partridge, Gibbs et al. 2002). Signal enhancement ratio (SER) maps (Partridge, Gibbs et al. 2002; Li, Partridge et al. 2008) were constructed from the signals measured with DCE-MRI at the pre-contrast (S₀), early post-contrast (S₁), and late post-contrast (S₂) timepoints using in-house software programmed in Interactive Data Language (IDL Version 7.0, ITT Visual Information Solutions, Boulder, Colorado) and the relationship below.

\[
SER = \frac{S_1 - S_0}{S_2 - S_0}
\]

Regions of abnormal enhancement were then identified as values on SER maps > 1 and reformatted to the axial orientation for registration with axial DW-MRI data. Regions-of-interest (ROIs) were automatically placed on reformatted regions. Tumor was then delineated on DW-MRI data by automatically registering axial ROIs derived from DCE-MRI data with ADC maps using in-house software programmed in Interactive Data Language (IDL Version 7.0, ITT Visual Information Solutions, Boulder, Colorado). For each slice, voxels outside of tumor ROIs were reassigned to an intensity of 0 and all other voxels retained their gray-level value, creating a tumor mask; tumor masks were saved in DICOM image format.

**Quantitative Histogram Analysis:** Tumor masks were analyzed in Matlab (R2007a, The Mathworks, Natick, MA). To facilitate analysis of the ADC histogram, for each exam, ADC values from tumor mask image slices were first combined into a one-
dimensional array. Parameters related to the ADC distribution (skew, kurtosis, mean, median, minimum ADC, maximum ADC, 12.5th, 25th, 75th, 87th percentile ADCs) were then calculated from this array. Histograms were constructed with bin-width 100*10^-6 mm^2/s. Histogram peak height, normalized peak height, and peak height location were determined.

**Qualitative Histogram Analysis:** A qualitative histogram analysis was conducted to determine if histograms could be used to give an overall impression about change in the tumor ADC distribution. The tumor ADC distribution at visit 2 was qualitatively compared to the distribution at visit 1. Tumor ADC histograms were normalized and the y axes for visit 1 and visit 2 histograms were matched for each patient, allowing for assessment of change in ADC distribution. For each patient, the tumor ADC distribution at visit 2 was compared to that at visit 1 using a rating system: 1= decreased ADC distribution, 2= no change, 3= increased ADC distribution. Patients were then assigned to one of groups based on 3-year recurrence free survival: “Recurrence” and “No Recurrence,” and the number of exams in each rating category was compared between groups.

**Statistics**

Quantitative parameters related to the ADC distribution were tested for normality using the Lilliefors test; parameters were then tested as predictors for 3-year recurrence free survival using the t-test (for normally distributed parameters) or Wilcoxon rank-sum test (not normally distributed parameters). Parameters were tested as predictors for recurrence free survival time using univariate Cox regression. All tests were performed in
Matlab (R2007a, The Mathworks, Natick, MA) with alpha=0.05. Parameters with p<0.1 were considered as showing trends towards significance.

8.3. Results

Patient Accrual

At our institution, 66 patients in Group 2 were enrolled in this study and underwent follow-up. In 16 of these 66 patients, diffusion was measured with DW-MRI at baseline and following 1 cycle of chemotherapy. 1 patient was excluded from further analysis due to lack of surgery, leaving a final cohort of 15 patients for this analysis. In this group of 15 patients, 5 patients recurred during the follow-up period; 10 patients did not recur. In recurring patients, mean time to recurrence was 83.4 weeks (SD=88.8 weeks). In patients not recurring during the study period, mean recurrence-free interval was 232.5 weeks (SD=37.1 weeks). Mean MR tumor volume at baseline was 29.2 cm$^3$ (SD=24.8) and mean MR tumor longest diameter (LD) at baseline was 6.5 cm (SD=2.2, range=2.9-10.8 cm).

Quantitative Analysis

MR Variables as Predictors for 3-year RFS

MR measurements of tumor volume, longest diameter, and diffusivity were tested for prediction of the binary outcome of 3-year RFS. The variable with the lowest p-value was early percent change in MR tumor volume (p=.005, Table 8-1). No MR LD variables were significant or showed a trend towards significance (p>.1 for all variables). No mean tumor ADC variables were significant at alpha=.05, but mean tumor ADC at visit 1 and
visit 2 showed trends towards significance (p=.058 and .077, respectively). The only MR variable measured at visit 1 that was significant at alpha=.05 was maximum tumor ADC (p=.026, with the median value higher in the recurring group). Selected measurements are shown in Table 8-1; results for all other variables can be found in Appendix 8.1.
Table 8-1. Predictors of 3-year RFS

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>N recur</th>
<th>N non-recur</th>
<th>Median, recur</th>
<th>Range, recur</th>
<th>Median, nonrecur</th>
<th>Range, nonrecur</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>38.8</td>
<td>5.31 - 54.61</td>
<td>13.7</td>
<td>8.54 - 89.39</td>
<td>0.753</td>
</tr>
<tr>
<td>Visit 2</td>
<td>3</td>
<td>11</td>
<td>37.0</td>
<td>5.14 - 51.05</td>
<td>6.5</td>
<td>1.11 - 67.94</td>
<td>0.225</td>
</tr>
<tr>
<td>Early change</td>
<td>3</td>
<td>11</td>
<td>-0.2</td>
<td>-3.55 - 7.25</td>
<td>-8.6</td>
<td>-42.88 - 2.05</td>
<td>0.022</td>
</tr>
<tr>
<td>Early % change</td>
<td>3</td>
<td>11</td>
<td>-3.2</td>
<td>-6.51 - 24.32</td>
<td>-55.0</td>
<td>-88.13 - 13.4</td>
<td>0.005</td>
</tr>
<tr>
<td>MR LD</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>70.0</td>
<td>29 - 108</td>
<td>60.0</td>
<td>31 - 90</td>
<td>0.666</td>
</tr>
<tr>
<td>Visit 2</td>
<td>4</td>
<td>11</td>
<td>70.0</td>
<td>26 - 108</td>
<td>50.0</td>
<td>26 - 88</td>
<td>0.432</td>
</tr>
<tr>
<td>Early change</td>
<td>4</td>
<td>11</td>
<td>-1.5</td>
<td>-8 - 8</td>
<td>-5.0</td>
<td>-29 - 6</td>
<td>0.237</td>
</tr>
<tr>
<td>Early % change</td>
<td>4</td>
<td>11</td>
<td>-5.2</td>
<td>-12.7 - 10.39</td>
<td>-11.6</td>
<td>-50 - 7.69</td>
<td>0.224</td>
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<tr>
<td>Mean Tumor ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>1679.2</td>
<td>1435.1 - 1777.6</td>
<td>1410.8</td>
<td>1052.7 - 1787.5</td>
<td>0.058</td>
</tr>
<tr>
<td>Visit 2</td>
<td>4</td>
<td>11</td>
<td>1732.2</td>
<td>1672.4 - 1836.1</td>
<td>1338.6</td>
<td>909.52 - 2312.7</td>
<td>0.077</td>
</tr>
<tr>
<td>Early change</td>
<td>4</td>
<td>11</td>
<td>84.3</td>
<td>-4.2 - 237.3</td>
<td>-37.7</td>
<td>-877.98 - 901.9</td>
<td>0.615</td>
</tr>
<tr>
<td>Early % change</td>
<td>4</td>
<td>11</td>
<td>5.1</td>
<td>-0.24 - 16.54</td>
<td>-2.7</td>
<td>-49.12 - 63.93</td>
<td>0.743</td>
</tr>
<tr>
<td>ADC Distribution, Kurtosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>4.6</td>
<td>4.45 - 5.24</td>
<td>4.3</td>
<td>3.33 - 7.32</td>
<td>0.995</td>
</tr>
<tr>
<td>Visit 2</td>
<td>4</td>
<td>11</td>
<td>6.3</td>
<td>5.25 - 6.73</td>
<td>4.0</td>
<td>2.02 - 19.96</td>
<td>0.026</td>
</tr>
<tr>
<td>Early change</td>
<td>4</td>
<td>11</td>
<td>1.7</td>
<td>0.01 - 2.14</td>
<td>-1.0</td>
<td>-4.41 - 12.64</td>
<td>0.177</td>
</tr>
<tr>
<td>Early % change</td>
<td>4</td>
<td>11</td>
<td>38.0</td>
<td>0.16 - 46.6</td>
<td>-23.4</td>
<td>-68.58 - 172.73</td>
<td>0.280</td>
</tr>
<tr>
<td>Minimum Tumor ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>50.0</td>
<td>27 - 131</td>
<td>95.0</td>
<td>36 - 679</td>
<td>0.177</td>
</tr>
<tr>
<td>Visit 2</td>
<td>4</td>
<td>11</td>
<td>42.0</td>
<td>13 - 59</td>
<td>147.0</td>
<td>34 - 823</td>
<td>0.021</td>
</tr>
<tr>
<td>Early change</td>
<td>4</td>
<td>11</td>
<td>-7.0</td>
<td>-95 - 7</td>
<td>3.0</td>
<td>-451 - 212</td>
<td>0.661</td>
</tr>
<tr>
<td>Early % change</td>
<td>4</td>
<td>11</td>
<td>-25.9</td>
<td>-72.52 - 17.07</td>
<td>2.9</td>
<td>-92.99 - 481.82</td>
<td>0.343</td>
</tr>
<tr>
<td>Median Tumor ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>1648.0</td>
<td>1399 - 1837</td>
<td>1383.0</td>
<td>1067 - 1859</td>
<td>0.106</td>
</tr>
<tr>
<td>Visit 2</td>
<td>4</td>
<td>11</td>
<td>1730.3</td>
<td>1612 - 1901</td>
<td>1322.0</td>
<td>916 - 2353</td>
<td>0.018</td>
</tr>
<tr>
<td>Early change</td>
<td>4</td>
<td>11</td>
<td>70.0</td>
<td>41 - 260.5</td>
<td>11.5</td>
<td>-943 - 929</td>
<td>0.226</td>
</tr>
<tr>
<td>Early % change</td>
<td>4</td>
<td>11</td>
<td>-25.9</td>
<td>-72.52 - 17.07</td>
<td>2.9</td>
<td>-92.99 - 481.82</td>
<td>0.343</td>
</tr>
<tr>
<td>75th Percentile Tumor ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>1907.0</td>
<td>1601 - 2032</td>
<td>1555.0</td>
<td>1247 - 2159.8</td>
<td>0.095</td>
</tr>
<tr>
<td>Visit 2</td>
<td>4</td>
<td>11</td>
<td>1954.5</td>
<td>1855 - 2053</td>
<td>1543.0</td>
<td>1090 - 2476.5</td>
<td>0.018</td>
</tr>
<tr>
<td>Early change</td>
<td>4</td>
<td>11</td>
<td>47.5</td>
<td>21 - 254</td>
<td>-48.0</td>
<td>-1069.8 - 922.5</td>
<td>0.642</td>
</tr>
<tr>
<td>Early % change</td>
<td>4</td>
<td>11</td>
<td>2.5</td>
<td>1.03 - 15.87</td>
<td>-2.7</td>
<td>-49.53 - 59.36</td>
<td>0.412</td>
</tr>
<tr>
<td>Maximum Tumor ADC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>3132.0</td>
<td>2736 - 3367</td>
<td>2661.0</td>
<td>2131 - 3086</td>
<td>0.026</td>
</tr>
<tr>
<td>Visit 2</td>
<td>4</td>
<td>11</td>
<td>3174.0</td>
<td>2603 - 3523</td>
<td>2410.0</td>
<td>1387 - 3083</td>
<td>0.023</td>
</tr>
<tr>
<td>Early change</td>
<td>4</td>
<td>11</td>
<td>42.0</td>
<td>-133 - 156</td>
<td>-173.0</td>
<td>-1380 - 559</td>
<td>0.355</td>
</tr>
<tr>
<td>Early % change</td>
<td>4</td>
<td>11</td>
<td>1.3</td>
<td>-4.86 - 4.63</td>
<td>-6.7</td>
<td>-47.88 - 26.23</td>
<td>0.424</td>
</tr>
</tbody>
</table>
**MR Variables as Predictors for RFS Time**

MR measurements of tumor volume, longest diameter, and diffusivity were tested for prediction of RFS time. The variable with the lowest p-value was the maximum tumor ADC at visit 2 ($p=.025$, $HR=1.003$). MR volume at visit 2 was also significant ($p=.039$, $HR=1.040$); early percent change in MR volume was significant ($p=.029$, $HR=1.071$). No variables related to longest diameter were significant or showed trends towards significance. No variables measured at visit 1 were significant at alpha=.05, but normalized peak height showed a trend towards significance ($p=.076$, $HR<<.01$). (The low HR is related to the fact that the HR represents the increased hazard for an incremental increase of 1; normalized peak height ranges from 0-1 and could only increase by an amount less than 1.) Selected measurements are listed in Table 8-2; results for the complete list of variables can be found in Appendix 8.2.
Table 8-2. Univariate Cox Regression

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>N</th>
<th>Min-Max</th>
<th>Median</th>
<th>HR</th>
<th>95% CI of HR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MR Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>15</td>
<td>5.3-89.4</td>
<td>18.2</td>
<td>1.021</td>
<td>0.993-1.051</td>
<td>0.146</td>
</tr>
<tr>
<td>Visit 2</td>
<td>14</td>
<td>1.1-67.9</td>
<td>9.1</td>
<td>1.040</td>
<td>1.002-1.079</td>
<td><strong>0.039</strong></td>
</tr>
<tr>
<td>Early change</td>
<td>14</td>
<td>-42.8-7.2</td>
<td>-7.4</td>
<td>1.096</td>
<td>0.936-1.285</td>
<td>0.255</td>
</tr>
<tr>
<td>Early % change</td>
<td>14</td>
<td>-88.1-24.3</td>
<td>-39.8</td>
<td>1.071</td>
<td>1.007-1.138</td>
<td><strong>0.029</strong></td>
</tr>
<tr>
<td><strong>MR LD</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>15</td>
<td>29.0-108.0</td>
<td>63.0</td>
<td>1.031</td>
<td>0.981-1.084</td>
<td>0.233</td>
</tr>
<tr>
<td>Visit 2</td>
<td>15</td>
<td>26.0-108.0</td>
<td>51.0</td>
<td>1.033</td>
<td>0.992-1.077</td>
<td>0.117</td>
</tr>
<tr>
<td>Early change</td>
<td>15</td>
<td>-29.0-8.0</td>
<td>-5.0</td>
<td>1.087</td>
<td>0.954-1.239</td>
<td>0.212</td>
</tr>
<tr>
<td>Early % change</td>
<td>15</td>
<td>-50.0-10.4</td>
<td>-10.7</td>
<td>1.060</td>
<td>0.974-1.153</td>
<td>0.255</td>
</tr>
<tr>
<td><strong>Mean Tumor ADC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>15</td>
<td>1052.7-1787.5</td>
<td>1429.5</td>
<td>1.002</td>
<td>0.998-1.007</td>
<td>0.350</td>
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<tr>
<td>Visit 2</td>
<td>15</td>
<td>909.5-2312.7</td>
<td>1474.6</td>
<td>1.002</td>
<td>1.000-1.004</td>
<td>0.112</td>
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<td>-4.2</td>
<td>1.001</td>
<td>0.999-1.003</td>
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<tr>
<td>Early % change</td>
<td>15</td>
<td>-49.1-63.9</td>
<td>-0.2</td>
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<td>0.985-1.041</td>
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<td><strong>Normalized Peak Height</strong></td>
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<tr>
<td>Visit 1</td>
<td>15</td>
<td>0.10-0.24</td>
<td>0.2</td>
<td>2.44E-13</td>
<td>2.95e-27-20.156</td>
<td><strong>0.076</strong></td>
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<tr>
<td>Visit 2</td>
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<td>0.13-0.27</td>
<td>0.2</td>
<td>1.77E-08</td>
<td>4.36e-22-7.21e5</td>
<td>0.264</td>
</tr>
<tr>
<td>Early change</td>
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<td>-0.07-0.12</td>
<td>0.0</td>
<td>58.117</td>
<td>4.04e-7-8.36e9</td>
<td>0.672</td>
</tr>
<tr>
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<td>15</td>
<td>-32.7-79.9</td>
<td>17.2</td>
<td>1.007</td>
<td>0.978-1.038</td>
<td>0.641</td>
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<tr>
<td><strong>Maximum Tumor ADC</strong></td>
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<td></td>
<td></td>
<td></td>
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<td>15</td>
<td>2131.0-3367.0</td>
<td>2736.0</td>
<td>1.004</td>
<td>1.000-1.008</td>
<td><strong>0.056</strong></td>
</tr>
<tr>
<td>Visit 2</td>
<td>15</td>
<td>1387.0-3523.0</td>
<td>2603.0</td>
<td>1.003</td>
<td>1.000-1.007</td>
<td><strong>0.025</strong></td>
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<td>15</td>
<td>-1380.0-559.0</td>
<td>-60.0</td>
<td>1.001</td>
<td>0.999-1.003</td>
<td>0.211</td>
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<td>-47.9-26.2</td>
<td>-2.7</td>
<td>1.030</td>
<td>0.980-1.082</td>
<td>0.248</td>
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<td>1404.2-2273.4</td>
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<td>0.305</td>
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<td>1815.3</td>
<td>1.002</td>
<td>1.000-1.004</td>
<td><strong>0.098</strong></td>
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<td>0.999-1.003</td>
<td>0.458</td>
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<td>0.978-1.048</td>
<td>0.490</td>
</tr>
</tbody>
</table>

**Qualitative Analysis**

Tumor ADC distributions for 5 of 15 patients were assigned a rating of 1 (“decreased”); distributions for 4 of 15 patients were assigned a rating of 2 (“no change”); distributions for 6 of 15 patients were assigned a rating of 3 (“increased”). The difference in rating distribution between patients recurring and not recurring is illustrated
in Figure 8-1. Example histograms are shown for each rating category in Figure 8-2, Figure 8-3, and Figure 8-4.

Figure 8-1. Results of qualitative analysis. The number of times a rating was assigned for each recurrence group is displayed. Most cases of no change in tumor ADC distribution were in patients who recurred. All cases of decreased tumor ADC were in patients who did not recur.

Figure 8-2. Decreased ADC distribution. Histograms of the tumor ADC distribution are shown for the same patient at visits 1 and 2. The proportion of voxels in the low ADC range (<1000) is increased at visit 2 compared to visit 1 (thick orange arrow).
The peak height is decreased at visit 2. The qualitative rating was 1, decreased ADC distribution.

Figure 8-3. No change in ADC distribution. Histograms of the tumor ADC distribution are shown for the same patient at visits 1 and 2. A similar proportion of voxels are in the low ADC range at visit 1 and visit 2 and the peak height and peak height location appears to be similar between visits. Minimum and maximum ADC appears to be similar. The qualitative rating was 2, no change.
Figure 8-4. Increased ADC distribution. Histograms of the tumor ADC distribution are shown for the same patient at visits 1 and 2. The proportion of voxels in the low ADC range is decreased (thin blue arrow) at visit 2 compared to visit 1. The peak height is increased (large arrow) at visit 2. The qualitative rating was 3, increased ADC distribution.

8.4. Discussion

This study investigated the ability of tumor ADC histogram parameters to predict response to neoadjuvant chemotherapy in patients with locally advanced breast cancer. The results of this study indicate that quantitative parameters derived from the tumor ADC distribution may be better predictors of long-term outcomes than the mean ADC, and earlier predictors than MR tumor volume measurements. Notably, the only statistically significant predictor of 3-year recurrence free survival (RFS) at baseline was maximum tumor ADC (p=.026). The only baseline variable to show a trend (p<.1) towards prediction of time to recurrence was normalized tumor ADC histogram peak.
height (p=.076). Other variables, including tumor ADC distribution kurtosis, minimum tumor ADC, and 75th percentile tumor ADC, but not the mean tumor ADC, were statistically significant predictors of RFS.

The results of this study are important because accurate prediction of patient response to neoadjuvant chemotherapy is not yet possible and new predictive markers are needed. Quantitative DW-MRI analysis of breast tumors is often limited to calculation of the mean tumor ADC and the results of this study suggest that analysis of heterogeneity in tumor ADC and calculation of ADC distribution parameters could benefit prediction of RFS. Quantitative histogram analysis was needed to detect these differences between patients recurring and not recurring.

At visit 1 and visit 2, recurring patients had statistically significantly higher maximum tumor ADC than non recurring patients. While this may at first seem surprising because higher ADCs are associated with decreased cellularity, and presumably better response, the median maximum tumor ADC in recurring patients was close to the diffusivity of free water at body temperature and such diffusivities would not be expected in the restricted environment of human tissue. However, such diffusivities would not be unexpected in necrotic tumor regions. Due to the small study size, only four patients recurred and retrospective review of the tumors for these patients was possible. The tumors tended to remain large at visit 2 and this large size may have been associated with necrosis. Similarly, the 75th percentile tumor ADC at visit 2 was higher in recurring patients (p=.018) and this percentile would represent the upper range of tumor ADCs. Somewhat paradoxically, recurring patients also had a lower minimum tumor ADC than non recurring patients at visit 2 (p=.021). Tumors successfully able to grow - - leading to
increased cell density, decreased diffusivity, and lower minimum tumor ADC might also be expected to outgrow the reaches of diffusing oxygen, resulting in a necrotic core and high maximum tumor ADC. This scenario would result in both a lower minimum and higher maximum tumor ADC. More extreme ADCs would be expected to increase the kurtosis and kurtosis at visit 2 was higher in recurring patients (p=.026).

The relationship between increased post-treatment tumor ADC distribution kurtosis and 3-year recurrence is compatible with the idea that a responding tumor would normalize in tumor ADC and perhaps have less extreme ADC value (decreased kurtosis). Along this line, the normalized peak height was associated with a decreased hazard ratio. The presence of a greater percent of total tumor voxels in the most populated bin suggests a normalization of the ADC histogram. However, the median kurtosis in both recurring and non-recurring patient groups was greater than 3 (considered to be outlier-prone) and this suggests that outliers were in fact present for both recurring and non recurring patients. The fact that the maximum and minimum tumor ADC were significant predictors of recurrence while the mean tumor ADC was not underscores the importance of analysis of the full tumor ADC distribution. A mean tumor ADC would average the significant outlier ADCs that may in fact predict patient outcomes.

Similarly, the qualitative histogram analysis clustered all combinations of quantitative changes into 1 of 3 categories: increased, decreased, or no change in distribution, perhaps allowing for important predictors of outcome to be overlooked. The qualitative analysis did show that most recurring patients had “no change” in the histogram, while most non recurring patients had an “increase” or a “decrease” in distribution, but it is unclear why responding patients would have a decrease in
distribution at MR2 (timed long enough after treatment to likely avoid early cell swelling-induced decreases in the ADC and soon enough to reduce the change of fibrosis-induced decreases). Based on the results of this study, the significance of the qualitative histogram analysis cannot be easily assessed. The qualitative analysis was motivated by the need for an analysis fast enough for routine clinical use; however, if a tumor ADC histogram has already been constructed, as would be needed in the qualitative histogram analysis, quantitative analysis may not be too unreasonable for routine use.

The low number of published studies assessing quantitative histogram parameters in prediction of locally advanced breast cancer response to neoadjuvant chemotherapy makes comparison of these results to prior results difficult. Nevertheless, the finding that the 75th percentile ADC predicted 3-year RFS highlights the importance of the upper ADC range, and this echoes a finding by other investigators that ADCs in the range of $2.15 \times 10^{-3} - 2.45 \times 10^{-3}$ mm$^2$/s significantly increased with treatment (Yankeelov, Lepage et al. 2007). While the earlier study did not compare changes between responders and nonresponders, this upper ADC range showed change with treatment.

In addition, the finding that the mean tumor ADC was not a statistically significant predictor of RFS or RFST is supported by findings in prior studies that mean tumor ADC is not valuable in treatment response monitoring (Manton, Chaturvedi et al. 2006; Nilsen, Fangberget et al. 2010; Tozaki, Oyama et al. 2010). Some studies did find that ADC increased with treatment (Pickles, Gibbs et al. 2006; Yankeelov, Lepage et al. 2007), but a global increase in a patient cohort is not enough to predict individual patient responses. In this study, long-term patient outcomes, RFS and RFST, was utilized as the
outcome variable and it is difficult to find similar studies in the literature. In prior studies, different ADC changes were seen in responders versus nonresponders (Sharma, Danishad et al. 2008; Iacconi, Giannelli et al. 2009), but long-term measures of response were not assessed. In Chapter 3, ADC showed a trend towards prediction of RFST at MR2, earlier than MR size measurements. Similarly, this study found that ADC variables showed a trend towards prediction of RFST at MR1, earlier than MR size measurements; however, in Chapter 3, mean normalized tumor ADC (nADC) showed a trend towards prediction of long-term patient outcomes. In contrast, the mean did not show a trend in this study; however, the nADC measurement in Chapter 3 was derived from non-fat-suppressed DW-MRI data and this study utilized fat suppressed data. This study also did not normalize to the diffusivity of contralateral fibroglandular breast tissue. Based on the findings in Chapter 4, fat suppression does impact the mean tumor ADCs.

In addition to use of fat-suppressed DW-MRI data, a strength of this study was assessment of changes in ADC percentiles instead of ADC intervals. In a tumor with a shifted distribution, the 75th percentile may be located in a different ADC histogram bin (interval). The 75th percentile is dependent on the ADC distribution present in an individual tumor, whereas monitoring an ADC interval fixes this range irrespective of tumor type. For example, mucinous carcinomas have higher ADCs than the typical range for tumors and the 12.5th or 25th percentile may better capture the low ADCs than a fixed interval, which may or may not be present in the mucinous tumor type.

This study was however limited. The study size was small (N=15), although the size is comparable to that of many other DW-MRI studies in patients with locally advanced breast cancer. An additional limitation is the fact that the acquisition utilized a
DW-MRI sequence that is not common on commercial scanners. While this sequence provided images less prone to distortion and more amenable to analysis, if this study were repeated in additional cohorts, the acquisition would likely use a DW-EPI sequence, prone to artifacts due to distortion and other problems. (The advantages of DW-EPI versus DW-SSFSE were discussed in Chapter 5). Additionally, recurrence free survival was censored at 3 years in the analysis of predictors of recurrence (binary outcome).

Patients with hormone receptor positive tumors have been found to have longer disease-free intervals than patients with other tumor subtypes (Rakha, El-Sayed et al. 2007); therefore, parameters found to predict 3-year RFS in this study may no longer predict RFS when patient follow-up is increased.

The method of tumor ADC monitoring is limited in that as the tumor responds to treatment, its size is likely to decrease, to a point at which the tumor may be too small for quantitative histogram analysis. This effect was reduced in this study because only visit 1 and 2 exams were included in the histogram analysis. However, since tumor was defined on ADC maps by SER, small ROIs were included in this study. Small regions of interest may incorporate surrounding normal fibroglandular or adipose tissue, risking partial voluming and inaccurate ADC measurements. Minimum ROI sizes could be explored in future studies.

The tumor ADC distributions assessed in this study varied in distribution type. The distribution appeared bimodal for some cases and unimodal for others. In cases of bimodal distributions, tracking of upper and lower peaks may provide valuable information about tumor response, beyond the information tracked in this study (Pope, Kim et al. 2009). In addition, a limitation of histogram analysis is that it does not provide
locational information about particular tumor ADCs. The locations of tumor regions increasing or decreasing in ADC may provide important information about tumor response. Heterogeneity in tumor response may also provide valuable information and this is explored in the next chapter. Despite these shortcomings, this study explored the utility of multiple histogram parameters in predicting long-term patient outcomes.

8.5. Conclusions

In conclusion, multiple parameters derived from the tumor ADC distribution were statistically significant predictors of recurrence free survival and recurrence free survival time. Quantitative histogram parameters predicted long term patient outcomes earlier than MR tumor volume, suggesting that histogram parameters may be promising predictors of patient response to neoadjuvant chemotherapy. While histogram parameters predicted patient response, measures of mean tumor ADC were not statistically significant predictors of response, highlighting the fact that a mean tumor ADC does not fully capture the range of tumor ADC changes. Quantitative histogram analysis may be needed to detect heterogeneity in tumor diffusivity and may be beneficial for prediction of response to chemotherapy. The ability of quantitative histogram parameters to predict response should be tested in large, prospective studies.

8.6. Acknowledgements

This analysis relied on data acquired through the ACRIN 6657 study. Funding for this analysis was provided by the California Breast Cancer Research Program.
8.7. References


Chapter 9.  Heterogeneity in Tumor Response to Treatment

9.1.  Introduction

Chemotherapy is a mainstay of treatment for locally advanced breast cancer; however, not all patients respond to chemotherapy. Identifying response earlier in the course of treatment could allow for ineffective therapies to be discontinued and potentially more effective therapies to be introduced. For this reason, accurate methods of evaluating patient response are needed.
Dynamic contrast-enhanced MRI (DCE-MRI) has been shown to be valuable in evaluating response. Based on contrast kinetics and spatial localization of contrast agent, a functional MR tumor volume can be measured from DCE-MRI data. Changes in this functional volume correlate with patient outcomes; however, the correlation is imperfect. When anti-angiogenic therapies are used, DCE-MRI may underestimate tumor volume (Chen, Feig et al. 2008). In some cases, DCE-MRI may also overestimate tumor volume as compared to pathological tumor size (Kwong, Chung et al. 2006). In addition, even when DCE-MRI is accurate in capturing functional tumor volume, tumor response to chemotherapy may be heterogeneous: reductions in cancerous cells in one region of the breast may be accompanied by constant or persistent growth in another region. Identification of these differences may be important in evaluating tumor response to chemotherapy.

Diffusion-weighted MRI (DW-MRI) may be valuable in capturing local differences in tumor response due to its ability to measure water motion resulting from processes occurring at the molecular and cellular level. Traditionally, changes in diffusion in breast tumors have been identified by placing a region of interest (ROI) on one or more tumor slices and averaging the measured water motion, or apparent diffusion coefficient (ADC) in this region (Manton, Chaturvedi et al. 2006; Nilsen, Fangberget et al. 2010). However, an average ADC does not allow for local variation in ADC to be measured. In DW-MRI of the brain, histogram analysis has been used to test the association between ADC distribution parameters and clinical variables. This technique has been applied to the breast to identify particular histogram bins, or ranges, of ADCs that may change in response to chemotherapy (Pickles, Gibbs et al. 2006). In Chapter 8,
parameters related to the tumor ADC distribution, including kurtosis and normalized peak height, were shown to correlate with long-term patient outcomes.

Histogram analysis, and the tracking of histogram bins, has been extended to the voxel-level. As it may be useful to know if voxel memberships in particular bins are changing, it may also be useful to know if particular voxels are changing, the frequencies of those changing voxels, and their locations. A method has previously been developed for tracking the response of individual voxels, both quantitatively, and qualitatively, in the form of colorization of the ADC map to create a functional diffusion map reflecting these voxel-wise changes (Hamstra, Chenevert et al. 2005). In brain cancer, the functional diffusion map has been shown to predict clinical outcomes earlier than conventional imaging methods (Hamstra, Galban et al. 2008); functional diffusion mapping has also been extended to other organs (Lee, Bradley et al. 2007; Galban, Mukherji et al. 2009).

At the time of this writing, voxel-to-voxel tracking of ADCs has been performed in a limited number of patients in the breast (Ma, Meyer et al. 2009). Implementation of this technique in the breast is challenging due to deformability of the breast tissue, fluidity of breast size and breast contents, anatomic location of the breast and presence of multiple air-tissue interfaces, creating regions of magnetic susceptibility and vulnerability to artifacts arising from distortion. Distortion is especially a problem in the EPI sequences used for DW-MRI on most commercially available systems (Kuroki and Nasu 2008). These sources of motion and artifacts can present difficulties in registering voxels from image series to image series within one exam; registering voxels from exam to exam, such as would be required in longitudinal tracking, would be expected to be even
more difficult in the breast. Due to these significant technical and anatomic challenges, a method able to track heterogeneity in longitudinal treatment response without requiring accurate image registration may be valuable in monitoring tumor response to chemotherapy in the breast.

The goal of this study was to develop and implement a method for monitoring response heterogeneity that did not require registration of images at the voxel-level. Based on both prior studies of the functional diffusion map and the fact that tumor response to treatment can be heterogeneous, it was hypothesized that quantifying heterogeneity with this method would be useful in predicting response to chemotherapy in patients with breast cancer.

9.2. Materials and Methods

Patients: Patients with locally advanced breast cancer (Group 2, as described in Chapters 2 and Appendix 2 and referred to in Chapters 4, 5, and 8) were enrolled at our institution as part of the multi-center trial ACRIN 6657. All patients signed informed consent and the protocol was approved by our institutional review board. Patients were scanned with magnetic resonance imaging (MRI) before, during, and after treatment with neoadjuvant chemotherapy.

Imaging

Conventional MRI: Sagittal DCE-MRI data was acquired in accordance with the required study protocol for ACRIN 6657.
**Diffusion-weighted MRI:** Bilateral, axial diffusion-weighted MRI (DW-MRI) was acquired with a fat-suppressed, 2D, single shot fast-spin echo (SSFSE) sequence using parameters described in Chapter 8. Non fat-suppressed data was also acquired, but only fat-suppressed data was included in this analysis.

**Image Processing**

**ADC Maps:** Maps of the measured apparent diffusion coefficient (ADC) were created offline, based on an assumption of monoexponential signal decay of the original signal ($S_{b0}$) to $S_{b600}$ with application of the diffusion gradients.

**Tumor Delineation:** Tumor delineation on ADC maps was guided by identification of regions of abnormal enhancement on DCE-MRI, using methods described in Chapter 8. Abnormal enhancement was defined by signal enhancement ratio (SER) (Partridge, Gibbs et al. 2002; Li, Partridge et al. 2008). Regions with SER>1 were converted to region of interests and automatically registered with ADC maps in IDL (Version 7.0, ITT Visual Information Solutions, Boulder Colorado).

**Development of Color Map:** The mean and standard deviation for the tumor ADC distribution as baseline were calculated for each VOI. As described in Chapter 8, tumor ROIs were converted to tumor masks using in-house software programmed in IDL (Version 7.0, ITT Visual Information Solutions, Boulder Colorado). Colorization of tumor ADCs was then performed in Matlab. The tumor voxels of each VOI (each visit) post-chemotherapy were compared to the baseline ADC distribution. Since each post-
chemotherapy voxel was compared to the pre-chemotherapy distribution and not each pre-chemotherapy voxel, exact image registration was not required (voxel to voxel comparisons were not performed). Gray-level tumor voxels were reassigned to a standard color scheme of blue, pink, and teal, or to user-defined alternative colors. Using the standard color scheme, tumor voxels more than 1 standard deviation above the baseline mean ADC were colored blue, representing increased ADC. Tumor voxels more than 1 SD below the baseline mean ADC were colored pink, representing decreased ADC. Tumor voxels within 1 SD of the baseline mean ADC were colored teal, representing no change in ADC as compared to the baseline distribution.

This method was initially applied to manually delineated tumor ROIs in Group 1 patients; application of this method to manually defined ROIs was discontinued due to concerns for reproducibility and subsampling of tumor. An illustration of this method in a Group 1 patient is provided to illustrate the longitudinal color mapping that is applied to Group 2 patients in this study (Figure 9-1).

Figure 9-1. Color Mapping. Changes in tumor ADC relative to baseline are spatially mapped (blue=increased, teal=no change, pink=increased). The absolute numbers and percentages of voxels in each color change category are also calculated.
Colorization of the ADC map provided both quantitative and non-quantitative output. The total numbers and percentages of blue, pink, and teal voxels at each visit, as well as visit to visit changes in these parameters, were calculated. The option of overlaying the colored tumor ROIs with the original ADC map was also provided. As a part of the color map processing, the user was shown colorized tumor maps for each slice at pre- and post-chemotherapy visits, allowing for an overall impression to be made regarding voxel changes, as well as identification of regions lower or higher than the baseline distribution.

**Statistical Analysis:** Normality of parameters was tested with the Lilliefors test at alpha=.05 and the t-test (parametric) or Wilcoxon rank-sum (nonparametric) was used to evaluate the significance of differences in MR parameters (MR volume, MR LD, mean tumor ADC, colorized ADC variables) between patients recurring and not recurring at 3 years. Univariate Cox regression was used to evaluate MR parameters as predictors for recurrence free survival time (RFST). For both tests, a p<.05 was considered significant, and a p<.15 was considered as a possible trend. Correlation between selected variables was tested by computing the linear correlation coefficient, $R^2$, and p value for the null hypothesis of no correlation. All statistical tests were performed in Matlab (Matlab Version R2007A, The Mathworks, Natick, MA).
9.3. Results

Patient Accrual

As described in Chapter 8, 66 patients in Group 2 were enrolled in this study and underwent follow-up. Diffusion was measured both at baseline and following 1 cycle of chemotherapy in 16 patients. 1 patient was excluded from further analysis due to lack of surgery, leaving a final study size of 15 patients.

Predictors of 3-year Recurrence-Free Survival

No variable was a statistically significant (p<.05) predictor of 3-year RFS at baseline, but baseline mean tumor ADC showed a trend towards prediction of RFS (p=.058), with a higher median ADC measurement in the group of patients recurring within 3 years (Table 9-1). Early change and early percent change in MR tumor volume were statistically significant predictors at alpha = .05 (p=.022 and .005, respectively). No colorized ADC variables were statistically significant predictors of RFS, but teal variables showed a marginal trend, with greater decreases in teal ADC voxels (representing no change) in the nonrecurring patients.
Table 9-1. MR Size, ADC, and Colorized ADC Variables as Predictors of RFS

<table>
<thead>
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<th>N recur</th>
<th>N non-recur</th>
<th>Median recur</th>
<th>Range Recur</th>
<th>Median nonrecur</th>
<th>Range nonrecur</th>
<th>p-value</th>
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</thead>
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<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>38.8</td>
<td>5.31 - 54.61</td>
<td>13.7</td>
<td>8.54 - 89.39</td>
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<td>37.0</td>
<td>5.14 - 51.05</td>
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<td>1.11 - 67.94</td>
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<td>-42.88 - -2.05</td>
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<td>11</td>
<td>-3.2</td>
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<td>-88.13 - -13.4</td>
<td>0.005</td>
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<tr>
<td>Visit 1</td>
<td>4</td>
<td>11</td>
<td>70.0</td>
<td>29 – 108</td>
<td>60.0</td>
<td>31 - 90</td>
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<td>26 - 88</td>
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<td>-8 – 8</td>
<td>-5.0</td>
<td>-29 - 6</td>
<td>0.237</td>
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<td>-50 - 7.69</td>
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<td></td>
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<td>1052.7 - 1787.5</td>
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<td>1338.6</td>
<td>909.52 - 2312.7</td>
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<td>84.3</td>
<td>-4.2 - 237.3</td>
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<td>-877.98 - 901.9</td>
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<td>5.1</td>
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<td>-49.12 - 63.93</td>
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<td><strong>Blue (increasing)</strong></td>
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<td>4</td>
<td>11</td>
<td>548.5</td>
<td>105 - 1184</td>
<td>287.0</td>
<td>62 - 1136</td>
<td>0.343</td>
</tr>
<tr>
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<td>11</td>
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<td>116 – 714</td>
<td>224.0</td>
<td>0 - 2848</td>
<td>0.571</td>
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<td>-470 - 168</td>
<td>-62.0</td>
<td>-336 - 1712</td>
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<td>-14.6</td>
<td>-67.39 - 37.09</td>
<td>-20.9</td>
<td>-100 - 150.7</td>
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</tr>
<tr>
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<td>20.3</td>
<td>18.34 - 24.33</td>
<td>20.5</td>
<td>16.38 - 47.33</td>
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</tr>
<tr>
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<td>11</td>
<td>26.6</td>
<td>20.3 - 47.95</td>
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</tr>
<tr>
<td>change, proportion</td>
<td>4</td>
<td>11</td>
<td>4.0</td>
<td>0.49 - 29.61</td>
<td>2.2</td>
<td>-47.33 - 81.52</td>
<td>0.970</td>
</tr>
<tr>
<td>% change, proportion</td>
<td>4</td>
<td>11</td>
<td>17.4</td>
<td>2.46 - 161.47</td>
<td>12.1</td>
<td>-100 - 497.53</td>
<td>0.753</td>
</tr>
<tr>
<td><strong>Pink (decreasing)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#, visit 1</td>
<td>4</td>
<td>11</td>
<td>589.0</td>
<td>84 – 920</td>
<td>211.0</td>
<td>31 - 1217</td>
<td>0.291</td>
</tr>
<tr>
<td>#, visit 2</td>
<td>4</td>
<td>11</td>
<td>167.5</td>
<td>10 – 639</td>
<td>102.0</td>
<td>3 - 325</td>
<td>0.274</td>
</tr>
<tr>
<td>change, #</td>
<td>4</td>
<td>11</td>
<td>-255.5</td>
<td>-613 - 74</td>
<td>-98.0</td>
<td>-1135 - 130</td>
<td>0.603</td>
</tr>
<tr>
<td>% change, #</td>
<td>4</td>
<td>11</td>
<td>-65.2</td>
<td>-96.69 - 30.54</td>
<td>-47.4</td>
<td>-98.58 - 129.03</td>
<td>0.346</td>
</tr>
<tr>
<td>proportion, visit 1</td>
<td>4</td>
<td>11</td>
<td>17.3</td>
<td>15.4 - 29.22</td>
<td>17.7</td>
<td>10.01 - 25.59</td>
<td>0.697</td>
</tr>
<tr>
<td>proportion, visit 2</td>
<td>4</td>
<td>11</td>
<td>10.0</td>
<td>1.98 - 18.17</td>
<td>21.5</td>
<td>0.64 - 100</td>
<td>0.295</td>
</tr>
<tr>
<td>change, proportion</td>
<td>4</td>
<td>11</td>
<td>-13.6</td>
<td>-14.65 - 2.77</td>
<td>0.5</td>
<td>-21.31 - 76.34</td>
<td>0.226</td>
</tr>
<tr>
<td>% change, proportion</td>
<td>4</td>
<td>11</td>
<td>-60.6</td>
<td>-88.07 - 18.02</td>
<td>2.1</td>
<td>-97.07 - 322.58</td>
<td>0.259</td>
</tr>
<tr>
<td><strong>Teal (no change)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#, visit 1</td>
<td>4</td>
<td>11</td>
<td>1549.5</td>
<td>316 - 3872</td>
<td>669.0</td>
<td>38 - 4577</td>
<td>0.571</td>
</tr>
<tr>
<td>#, visit 2</td>
<td>4</td>
<td>11</td>
<td>750.5</td>
<td>207 - 2164</td>
<td>250.0</td>
<td>0 - 1586</td>
<td>0.211</td>
</tr>
<tr>
<td>change, #</td>
<td>4</td>
<td>11</td>
<td>-823.0</td>
<td>-2027 - 258</td>
<td>-364.0</td>
<td>-3305 - 16</td>
<td>0.661</td>
</tr>
<tr>
<td>% change, #</td>
<td>4</td>
<td>11</td>
<td>-12.2</td>
<td>-90.73 - 29.83</td>
<td>-72.2</td>
<td>-100 - 3.62</td>
<td>0.143</td>
</tr>
<tr>
<td>proportion, visit 1</td>
<td>4</td>
<td>11</td>
<td>63.1</td>
<td>46.46 - 64.79</td>
<td>59.6</td>
<td>29.01 - 73.6</td>
<td>0.949</td>
</tr>
<tr>
<td>proportion, visit 2</td>
<td>4</td>
<td>11</td>
<td>58.0</td>
<td>47.26 - 75</td>
<td>42.9</td>
<td>0 - 61.81</td>
<td>0.107</td>
</tr>
<tr>
<td>change, proportion</td>
<td>4</td>
<td>11</td>
<td>2.4</td>
<td>-16.35 - 12.43</td>
<td>-11.6</td>
<td>-72.97 - 11.92</td>
<td>0.133</td>
</tr>
<tr>
<td>% change, proportion</td>
<td>4</td>
<td>11</td>
<td>8.1</td>
<td>0.75 - 46.55</td>
<td>3.1</td>
<td>-163.15 - 110.7</td>
<td>0.804</td>
</tr>
</tbody>
</table>

223
Predictors of Recurrence-free Survival Time

In univariate Cox regression (Table 9-2), MR tumor volume at visit 2 and early percent change in MR volume were statistically significant predictors of RFST (p=.039 and .029, respectively and hazard ratio, HR, = 1.040 and 1.071, respectively). MR volume at baseline showed a trend towards prediction of RFST (p=.146, HR= 1.021). MR LD at visit 2 was the only variable related to MR LD that showed a trend towards prediction (p<.15). Mean tumor ADC was not a predictor of recurrence free survival time (RFST) and did not show a trend towards prediction of RFST. Colorized ADC variables at baseline were statistically significant predictors of RFST (p=.034 for number blue voxels and p=.037 for number of pink voxels). Number of teal voxels at visit 1 and visit 2 showed a trend, but the HR was approximately 1 for both measurements. Early change in the number of pink voxels showed a trend towards prediction of RFST (p=.109, HR=.998).
Table 9-2. Univariate Cox Regression

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>N</th>
<th>Range</th>
<th>Median</th>
<th>HR</th>
<th>95% CI of HR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MR Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>15</td>
<td>5.3101-89.3917</td>
<td>18.208</td>
<td>1.021</td>
<td>0.99271-1.0507</td>
<td>0.146</td>
</tr>
<tr>
<td>Visit 2</td>
<td>14</td>
<td>1.106-67.9359</td>
<td>9.073</td>
<td>1.040</td>
<td>1.0021-1.0786</td>
<td>0.039</td>
</tr>
<tr>
<td>Early change</td>
<td>14</td>
<td>-42.8751-7.2461</td>
<td>-7.397</td>
<td>1.096</td>
<td>0.93571-1.2845</td>
<td>0.255</td>
</tr>
<tr>
<td>% Early change</td>
<td>14</td>
<td>-88.1285-24.3179</td>
<td>-39.783</td>
<td>1.071</td>
<td>1.0072-1.1382</td>
<td>0.029</td>
</tr>
<tr>
<td><strong>MR LD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>15</td>
<td>29-108</td>
<td>63.000</td>
<td>1.031</td>
<td>0.98062-1.0838</td>
<td>0.233</td>
</tr>
<tr>
<td>Visit 2</td>
<td>15</td>
<td>26-108</td>
<td>51.000</td>
<td>1.033</td>
<td>0.99811-1.0768</td>
<td>0.117</td>
</tr>
<tr>
<td>Early change</td>
<td>15</td>
<td>-29-8</td>
<td>-5.000</td>
<td>1.087</td>
<td>0.95367-1.2389</td>
<td>0.212</td>
</tr>
<tr>
<td>% Early change</td>
<td>15</td>
<td>-50-10.3896</td>
<td>-10.714</td>
<td>1.060</td>
<td>0.97442-1.1527</td>
<td>0.175</td>
</tr>
<tr>
<td><strong>Mean Tumor ADC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>15</td>
<td>1052.7-1787.5</td>
<td>1429.500</td>
<td>1.002</td>
<td>0.99772-1.0065</td>
<td>0.350</td>
</tr>
<tr>
<td>Visit 2</td>
<td>15</td>
<td>909.52-2312.7</td>
<td>1503.400</td>
<td>1.001</td>
<td>0.99923-1.0035</td>
<td>0.210</td>
</tr>
<tr>
<td>Early change</td>
<td>15</td>
<td>-837.2-897.9</td>
<td>68.200</td>
<td>1.001</td>
<td>0.99853-1.0029</td>
<td>0.525</td>
</tr>
<tr>
<td>% Early change</td>
<td>15</td>
<td>-48.6235-63.6809</td>
<td>3.895</td>
<td>1.010</td>
<td>0.97976-1.0413</td>
<td>0.519</td>
</tr>
<tr>
<td><strong>Blue (increasing)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#, Visit 1</td>
<td>15</td>
<td>62-1184</td>
<td>291.000</td>
<td>1.002</td>
<td>1.0002-1.0046</td>
<td>0.034</td>
</tr>
<tr>
<td>#, Visit 2</td>
<td>15</td>
<td>0-2848</td>
<td>224.000</td>
<td>1.000</td>
<td>0.99969-1.0013</td>
<td>0.233</td>
</tr>
<tr>
<td>Change, #</td>
<td>15</td>
<td>-470-1712</td>
<td>-62.000</td>
<td>1.000</td>
<td>0.99893-1.0017</td>
<td>0.648</td>
</tr>
<tr>
<td>% Change, #</td>
<td>15</td>
<td>-100-150.7042</td>
<td>-20.883</td>
<td>1.006</td>
<td>0.99547-1.0171</td>
<td>0.315</td>
</tr>
<tr>
<td>Proportion, visit 1</td>
<td>15</td>
<td>16.384-47.328</td>
<td>20.481</td>
<td>0.939</td>
<td>0.77688-1.1356</td>
<td>0.518</td>
</tr>
<tr>
<td>Proportion, visit 2</td>
<td>15</td>
<td>0-97.899</td>
<td>23.153</td>
<td>1.006</td>
<td>0.97586-1.0351</td>
<td>0.707</td>
</tr>
<tr>
<td>Change, proportion</td>
<td>15</td>
<td>-47.328-81.515</td>
<td>2.224</td>
<td>1.006</td>
<td>0.98127-1.0322</td>
<td>0.621</td>
</tr>
<tr>
<td>% change, proportion</td>
<td>15</td>
<td>-100-497.5281</td>
<td>12.071</td>
<td>1.001</td>
<td>0.99603-1.0055</td>
<td>0.750</td>
</tr>
<tr>
<td><strong>Pink (decreasing)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#, Visit 1</td>
<td>15</td>
<td>31-1217</td>
<td>224.000</td>
<td>1.002</td>
<td>1.0001-1.004</td>
<td>0.037</td>
</tr>
<tr>
<td>#, Visit 2</td>
<td>15</td>
<td>3-639</td>
<td>102.000</td>
<td>1.004</td>
<td>0.99829-1.0106</td>
<td>0.158</td>
</tr>
<tr>
<td>Change, #</td>
<td>15</td>
<td>-1135-130</td>
<td>-154.000</td>
<td>0.998</td>
<td>0.99656-1.0003</td>
<td>0.109</td>
</tr>
<tr>
<td>% Change, #</td>
<td>15</td>
<td>-98.5782-129.0323</td>
<td>-47.472</td>
<td>0.986</td>
<td>0.96327-1.0089</td>
<td>0.226</td>
</tr>
<tr>
<td>Proportion, visit 1</td>
<td>15</td>
<td>10.014-29.216</td>
<td>17.720</td>
<td>1.043</td>
<td>0.86104-1.2633</td>
<td>0.667</td>
</tr>
<tr>
<td>Proportion, visit 2</td>
<td>15</td>
<td>0.6424-100</td>
<td>15.258</td>
<td>0.954</td>
<td>0.88095-1.0321</td>
<td>0.239</td>
</tr>
<tr>
<td>Change, proportion</td>
<td>15</td>
<td>-21.3136-76.336</td>
<td>-7.852</td>
<td>0.934</td>
<td>0.8454-1.0311</td>
<td>0.176</td>
</tr>
<tr>
<td>% Change, proportion</td>
<td>15</td>
<td>-97.0741-322.5828</td>
<td>-47.323</td>
<td>0.990</td>
<td>0.97467-1.0056</td>
<td>0.207</td>
</tr>
<tr>
<td><strong>Teal (no change)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#, visit 1</td>
<td>15</td>
<td>38-4577</td>
<td>793.000</td>
<td>1.000</td>
<td>0.99993-1.001</td>
<td>0.092</td>
</tr>
<tr>
<td>#, visit 2</td>
<td>15</td>
<td>0-2164</td>
<td>378.000</td>
<td>1.001</td>
<td>0.99998-1.0028</td>
<td>0.053</td>
</tr>
<tr>
<td>Change, #</td>
<td>15</td>
<td>-3305-258</td>
<td>-364.000</td>
<td>1.000</td>
<td>0.99986-1.0003</td>
<td>0.285</td>
</tr>
<tr>
<td>% Change, #</td>
<td>15</td>
<td>-100-29.8266</td>
<td>-47.917</td>
<td>1.014</td>
<td>0.99914-1.0376</td>
<td>0.223</td>
</tr>
<tr>
<td>Proportion, visit 1</td>
<td>15</td>
<td>29.008-73.602</td>
<td>62.574</td>
<td>1.015</td>
<td>0.93044-1.1066</td>
<td>0.741</td>
</tr>
<tr>
<td>Proportion, visit 2</td>
<td>15</td>
<td>0-75</td>
<td>50.246</td>
<td>1.035</td>
<td>0.97747-1.0949</td>
<td>0.241</td>
</tr>
<tr>
<td>Change, proportion</td>
<td>15</td>
<td>-72.9718-12.426</td>
<td>-9.327</td>
<td>1.030</td>
<td>0.97451-1.0882</td>
<td>0.297</td>
</tr>
<tr>
<td>% Change, proportion</td>
<td>15</td>
<td>-163.155-110.7511</td>
<td>3.554</td>
<td>1.005</td>
<td>0.99024-1.021</td>
<td>0.482</td>
</tr>
</tbody>
</table>
With the exception of the pink category at visit 2, the number of pixels in each color group was highly correlated with the total lesion volume at the corresponding timepoint (Table 9-3). The correlation between volume and number of colorized pixels is depicted in Figure 9-2. Note that the number of pink (decreased ADC) pixels did not correlate with lesion volume and the change in pink pixels showed a trend towards significance in predicting RFST.

Table 9-3. Correlation between Tumor Volume and ADC Color Variables

<table>
<thead>
<tr>
<th>Parameter 1</th>
<th>Parameter 2</th>
<th>R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>volume, visit 1</td>
<td># blue, visit 1</td>
<td>0.839</td>
<td>1.63E-06</td>
</tr>
<tr>
<td>volume, visit 1</td>
<td># teal, visit 1</td>
<td>0.898</td>
<td>8.00E-08</td>
</tr>
<tr>
<td>volume, visit 1</td>
<td># pink, visit 1</td>
<td>0.795</td>
<td>8.09E-06</td>
</tr>
<tr>
<td>volume, visit 2</td>
<td># blue, visit 2</td>
<td>0.767</td>
<td>4.03E-05</td>
</tr>
<tr>
<td>volume, visit 2</td>
<td># teal, visit 2</td>
<td>0.718</td>
<td>1.29E-04</td>
</tr>
<tr>
<td>volume, visit 2</td>
<td># pink, visit 2</td>
<td>0.208</td>
<td>0.10</td>
</tr>
<tr>
<td>% change in volume</td>
<td>change in # pink</td>
<td>0.054</td>
<td>0.42</td>
</tr>
<tr>
<td>change in volume</td>
<td>change in # pink</td>
<td>0.186</td>
<td>0.12</td>
</tr>
</tbody>
</table>

![Graphs showing correlation](image)

Figure 9-2. Correlation between MR2 Volume and ADC Color Variables. Scatterplots with color variables on the x-axis and volume at MR 2 on the y-axis demonstrate the correlation between color variables and MR volume. At visit 2, the number of teal (far left plot) and number of blue (middle plot) pixels correlated with lesion volume; however, the number of pink pixels did not correlate with lesion volume (far right plot). Pink, dashed lines correspond to linear regression lines.
**Example Case**

Example images for this analysis are shown for one patient (Figure 9-3). In this patient, the tumor ADC distribution increased following chemotherapy as compared to baseline. This increase is reflected in an increased proportion of blue pixels post-treatment as compared to baseline. In addition, there is a decrease in pink pixels post-treatment as compared to baseline. The patient did not recur during the study period (censored RFST=3.9 years).

The mean ADC for this patient increased 26% from visit 1 to visit 2. MR volume decreased 24% from visit 1 to 2. (In Chapter 3, the general pattern of a good response was described as increased ADC and decreased volume). Normalized histograms are also shown for the entire tumor volume of interest (Figure 9-4). The changes observed on the representative slice are echoed by the histogram changes for the whole tumor. The histograms provide quantitative but not spatial information about the ADC distribution; in contrast, the color-coded ADC map provides information regarding the spatial distribution of these changes within the tumor.
Figure 9-3. Example Case. Color-coding of a representative ROI at visit 1 and visit 2 shows an increase in the proportion of blue pixels following chemotherapy and a decrease in the proportion of pink pixels. The percentage of voxels assigned to each color is listed for the tumor VOI.
In this study, a method for tracking regional tumor differences in ADC change, that did not require longitudinal DW-MRI registration at the voxel-level, was developed and tested in patients with locally advanced breast cancer undergoing treatment with

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**Figure 9-4. Normalized histograms for example case.** Normalized histograms for tumor ADC distributions at baseline (left) and following 1 cycle chemotherapy (right) are shown. The lowest occupied ADC bins are less occupied on visit 2 (thin arrow) as compared to visit 1; conversely, higher ADC bins are occupied on visit 2 than on visit 1 (thick arrow).

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**9.4. Discussion**

In this study, a method for tracking regional tumor differences in ADC change, that did not require longitudinal DW-MRI registration at the voxel-level, was developed and tested in patients with locally advanced breast cancer undergoing treatment with
neoadjuvant chemotherapy. The tumor burden of voxels increasing, decreasing, or not changing in ADC relative to the baseline ADC distribution was calculated for each patient, and parameters related to these colorized ADC measurements were tested for prediction of long-term patient outcomes (3-year recurrence free survival, RFS, and recurrence-free survival time, RFST). While colorized ADC measurements at baseline were statistically significant, these measurements were highly correlated with MR tumor volume, which has previously been shown to predict RFST (Partridge, Gibbs et al. 2005). In contrast, the change in decreasing (pink) voxels did not correlate with MR volume changes and showed a trend towards prediction of RFST. Measurements related to teal (no change) voxels showed trends towards prediction of recurrence-free survival.

A decreased proportion of visit 2 teal voxels was associated with 3-year RFS and this is consistent with the expectation that successful therapies will increase tumor diffusivity. Accordingly, failure of tumor ADC to increase – represented by the color teal (no change in ADC) and pink (decreased ADC) in the colorized ADC map – would predict a poor response and poorer long-term outcomes. While the proportion of teal voxels at visit 2 showed only a trend towards significance in prediction of 3-year RFS, this study was limited in sample size (N=15) and colorized ADC parameters should be tested in larger studies.

The results of this study do not clearly indicate that colorized ADC parameters are valuable; however, these colorized ADC parameters could be assessed in future studies along with more standard MR measurements. The processing method that allows for quantification of colorized parameters requires DICOM images, a standard image format, and is based in Matlab, a common software program, and should be compatible with data
acquired on a variety of scanners, with a variety of DW-MRI sequences. The processing method could be integrated into both retrospective and prospective studies. Currently, assessment of the colorized ADC parameters does require user-interaction in selection of DICOM images, but this step could be automated. The output of the processing algorithm can be easily incorporated into spreadsheets and compilation of the output from processing of multiple patients’ data should also be amenable to automation.

A major strength of the method used in this study is the fact that it does not require longitudinal registration of ADC map voxels. In the breast, nonlinear registration methods are often utilized due to the deformability of the breast (Guo, Sivaramakrishna et al. 2006; Ma, Meyer et al. 2009). Not only are these methods technically challenging, but they also may not be entirely accurate or their implementation straightforward, especially in the context of treatment with chemotherapy. Recently, density in the contralateral breast has been shown to decrease in response to treatment with neoadjuvant chemotherapy (Chen, Nie et al.). Changes in breast composition could impair registration; for example, if tumor voxels at one exam were compared to adipose voxels at the next exam, the ADC change in the particular voxel may be erroneously interpreted as a decrease in ADC. Similarly, changes in total breast volume could impair accurate voxel registration: voxels imaged at one exam may no longer contain breast tissue at the next exam. In this method, voxels are compared to the baseline ADC distribution, and not corresponding baseline voxels, reducing the chance for these erroneous comparisons.

This new method is, however, still prone to errors resulting from inaccurate tumor region of interest (ROI) delineation. If the tumor ROIs at baseline include significant amounts of adipose or normal tissue, the baseline distribution may not be accurately
measured, impacting colorized ADC calculations at subsequent visits. In addition, ROI delineation may be limited by spatial resolution of the DW-MRI acquisition. If an acquisition provides poor enough resolution to allow for significant partial volume effects, the ADC distribution may be similarly misrepresented and errors could impact the longitudinal colorized ADC measurements.

This study was also limited in that colorized parameters derived from later MR timepoints were not tested. It should however be noted that as the tumor decreases in size at later timepoints, delineation of the tumor on ADC maps and subsequent analysis of the ADC becomes increasingly more difficult. The colorized analysis further subdivides the tumor volume into three color groups and thus at later timepoints a colorized analysis could be more difficult to implement than a more standard quantitative ADC analysis. Voxels were assigned to color groups in this study based on whether or not they fell above, below, or within one standard deviation from the mean ADC at baseline and more optimal cut-off points for voxel assignment may exist. An additional limitation of this study was that combinations of MR parameters were not tested for prediction of RFS. It is possible that the colorized parameters would add valuable adjunct information to MR volume or mean tumor ADC. However, this study was small, limiting analyses. Additional study is needed.

9.5. Conclusions

While some ADC measurements of tumor response heterogeneity showed trends towards prediction of patient outcomes, the most statistically significant measurements were highly correlated with MR tumor volume. Early change in decreasing (pink) voxels
was not correlated with MR tumor volume but showed only a trend towards prediction of 3-year RFS. Study in larger patient cohorts, with longer follow-up time is needed. In addition, the predictive value of parameters derived from this method should be compared with the predictive value of parameters derived from methods utilizing image registration. Monitoring tumor response heterogeneity using DW-MRI has been shown to be valuable in other types of cancers and determining its value in the breast requires further study.

9.6. Acknowledgements

This analysis relied on data acquired through the study ACRIN 6657. Funding for this analysis was provided by the California Breast Cancer Research Program.

9.7. References


Chapter 10. Tumor Segmentation: Comparison of Different Methods for Defining Breast Cancer Regions on DW-MRI

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.1. Introduction</td>
<td>236</td>
</tr>
<tr>
<td>10.2. Materials and Methods</td>
<td>237</td>
</tr>
<tr>
<td>Patients</td>
<td>237</td>
</tr>
<tr>
<td>Imaging</td>
<td>238</td>
</tr>
<tr>
<td>Image Processing</td>
<td>239</td>
</tr>
<tr>
<td>Image Analysis</td>
<td>239</td>
</tr>
<tr>
<td>Method Comparisons and Statistics</td>
<td>244</td>
</tr>
<tr>
<td>10.3. Results</td>
<td>244</td>
</tr>
<tr>
<td>Patient accrual</td>
<td>244</td>
</tr>
<tr>
<td>Quantitative Analysis</td>
<td>244</td>
</tr>
<tr>
<td>Qualitative Analysis</td>
<td>248</td>
</tr>
<tr>
<td>Case studies</td>
<td>250</td>
</tr>
<tr>
<td>10.4. Discussion</td>
<td>252</td>
</tr>
<tr>
<td>10.5. Conclusion</td>
<td>258</td>
</tr>
<tr>
<td>10.6. References</td>
<td>258</td>
</tr>
</tbody>
</table>
10.1. **Introduction**

Diffusion-weighted magnetic resonance imaging (DW-MRI) provides an in-vivo, non-ionizing method of measuring the random motion of water molecules, or diffusion, quantified as the apparent diffusion coefficient (ADC). ADC reflects cell density, apoptosis, and necrosis and could be valuable in characterizing breast tumors and monitoring tumor response to treatment (Chenevert, Meyer et al. 2002; Iacconi 2010). Multiple studies have reported changes in the ADC in patients with locally advanced breast cancer treated with neoadjuvant chemotherapy (Pickles, Gibbs et al. 2006; Yankeeelov, Lepage et al. 2007; Sharma, Danishad et al. 2008; Iacconi, Giannelli et al. 2009) and these changes have correlated with tumor size reduction, a surrogate outcome (Iacconi, Giannelli et al. 2009).

Despite the progress made in monitoring breast tumor response to treatment with DW-MRI, this technique has not yet been validated for routine clinical use. In fact, nearly the same number of studies finding value in DW-MRI has found it not to be valuable in predicting tumor response to treatment (Manton, Chaturvedi et al. 2006; Nilsen, Fangberget et al. 2010; Tozaki, Oyama et al. 2010). Different studies have used different methods of acquiring diffusion data and the impact of these differences on the ADC measurement was explored in Part III, Technical Developments. However, different studies have also used different methods of defining the tumor on DW-MRI. No standard exists. A common method of defining tumor on DW-MRI data is to manually delineate the tumor on one slice in the DW-MRI volume for each exam (Partridge, Mullins et al. 2010). The mean tumor ADC at baseline is compared to the on subsequent exams (Iacconi, Giannelli et al. 2009). This method is relatively fast, but in restricting the tumor...
delineation to one MR slice, it subsamples the tumor. Large tumors may encompass 10 or more MR slices. In addition, user variability in manually outlining the tumor is expected to reduce repeatability of this method. In the clinical setting, the ability to manually define the tumor with irregular contours is not always available and regularly shaped regions of interest (ROIs) (circle or square) may be used.

It is not known how ROI shape impacts the ADC measurement. In addition, an automated method of defining tumor that compares well with the commonly used method – manual delineation – has not yet been made available to the analysis of DW-MRI data of the breast. The purpose of this study was two-fold: 1) to develop an alternative method of tumor delineation requiring less user interaction, and 2) to compare tumor ADC measurements obtained with this processing method, and others, to measurements obtained with a reference method. It was hypothesized that because tumors often have an abnormal contrast profile as well as abnormal diffusivity, combining information from both contrast-enhanced and diffusion-weighted magnetic resonance imaging could provide an adequate approximation of tumor boundaries.

10.2. Materials and Methods

Patients: The study population consisted of patients enrolled in a neoadjuvant study at our institution, approved by the Institutional Review Board and compliant with HIPAA. All patents signed informed consent. Only patients with invasive breast cancer, confirmed by pathology, age 18 years or older, and treated with neoadjuvant chemotherapy were enrolled. Patients were scanned with MRI prior to treatment with
chemotherapy between October 2008 and June 2010. Only patients scanned with DCE- and DW-MRI at our institution were included in the study.

**Imaging:** All scanning was performed on a 1.5 T GE Signa scanner (GE Medical Systems, Milwaukee, Wisconsin). Eight-channel bilateral coils were used (Sentinelle Medical, Inc, Toronto, Canada). The diffusion sequences were acquired following contrast-enhanced imaging.

**Contrast-enhanced imaging:** Contrast-enhanced imaging data was acquired using a fat-suppressed, 3D, T1-weighted, fast gradient recalled-echo (3DFGRE) sequence with the following parameters: TR = 9 ms, TE = 4 ms, flip angle = 10°, field of view = variable, slice thickness = 2-2.2 mm, and acquisition matrix = 512 x 320. 124 slices were acquired in the axial orientation to cover both breasts and the number of averages was 0.75. Gadopentetate dimeglumine (Magnevist, Schering AG, Berlin, Germany) was administered as a contrast agent and MR volumes were acquired prior to contrast injection, and at two or more timepoints following contrast injection.

**Diffusion imaging:** Our standard diffusion sequence, a 2D, T2-weighted echo planar diffusion-weighted sequence, was acquired bilaterally in the axial orientation. The parameters were: TR/TE= 6000/108.5 ms, FOV= 400mm, acquisition matrix= 128 x 128, in-plane resolution= 3.125 x 3.125mm, number of slices= 20, slice thickness= 3mm, number of excitations= 6. Diffusion-weighted gradients were applied sequentially in 6 directions with b=600. Images were also acquired with b=0.
Image Processing:

**ADC Maps:** ADC maps were constructed off-line from diffusion-weighted imaging data using in-house software and an assumption of mono-exponential decay of the original signal, $S_0$ to $S_D$, with application of diffusion-weighted gradients (Equation 1).

Equation 1 \[ S_D = S_0 e^{-bD} \]

**SER Maps:** Signal enhancement ratio (SER) maps were calculated using in-house software programmed in Interactive Data Language (ITT Visual Information Solutions, Boulder, CO) by applying the relationship in Equation 2 using the signal acquired at each voxel pre-contrast ($S_0$), post-contrast ($S_1$), and later post-contrast ($S_2$):

Equation 2 \[ SER = \frac{S_1 - S_0}{S_2 - S_0} \]

Image Analysis:

**ROI Delineation:**

For each exam, tumor regions of interest (ROIs) were defined using each of 6 different methods:

- **Method #1: Manual Whole-tumor Delineation (Reference Method):** Regions of high-intensity on $b=600$ images were manually outlined using in-house software programmed in IDL (ITT Visual Information Solutions, Boulder, CO), taking care to avoid inclusion of adipose and necrotic tissue by verifying that the corresponding intensity on the ADC map was low, but not as low as that of fat. Regions were defined on all slices in the MR volume with identifiable tumor. The
DCE-MRI data was re-sampled to the resolution of the DW-MRI data to guide identification of the general location of tumor in the breast; however, DCE-MRI was not used to guide exclusion of particular regions. This method was considered to be the reference method for DW-MRI analysis because it included all identified tumor regions based on DW-MRI (Figure 10-1)

![Figure 10-1. Manual and Volumetric ROIs. As seen at the far left, ROIs are manually drawn on b=600 images for Method #1. Manual ROIs correspond to bright regions on b=600 (far left) and dark areas on ADC (2nd image from left). In the volumetric method (Methods #4 and #5), regions of abnormal enhancement (3rd image from left), are automatically selected. Volumetric ROIs are shown on the last image.](image)

- **Method #2: Single Slice Delineation:** A single contiguous ROI was delineated on one slice of the b=600 DW-MRI volume: the slice with the largest contiguous surface area of tumor, assessed by visual inspection. The ROI was drawn so as to include regions that were generally hyperintense on the b=600 image, and hypointense on ADC. A subtraction DCE MRI volume (early post- minus pre-contrast) was used to limit inclusion of tissue to enhancing regions, allowing for exclusion of necrotic tissue. Manual tumor delineation on a single slice is a common method of breast tumor delineation on DW-MRI data.
• **Method #3: Circular ROI:** A circular ROI of a fixed size (5mm) was placed in a contiguous, homogeneous region of the tumor on the slice with the largest contiguous surface area of tumor, assessed by visual inspection.

• **Method #4: Volumetric ROIs:** An SER map was automatically generated from pre and post-contrast DCE-MRI data using previously published methods (Partridge, Gibbs et al. 2005; Li, Partridge et al. 2008). SER map voxels with intensity >1 were considered to be tumor and tumor regions were re-sampled to the DW-MRI resolution and automatically placed on ADC maps using in-house software programmed in our laboratory in Interactive Data Language (IDL, ITT Visual Information Solutions) (Figure 10-1).

• **Method #5: Shifted Volumetric ROIs:** ROIs derived using Method 4 were manually shifted into spatial alignment on the ADC map to mitigate misregistration due to EPI distortion and motion between DCE- and DW-MRI exams. This method also allowed for optional manual removal of ROIs that did not appear to correspond to tumor.

• **Method #6: Combined Method:** Tumor regions automatically generated using Method 4 were intersected with low diffusing fibroglandular tissue regions selected using a semi-automated method based on clustering with a fuzzy-c-means algorithm (Figure 10-2). Fibroglandular tissue was identified on the b=600 T2-weighted DW-MRI series, and not the pre-contrast DCE-MRI, to reduce the risk of misregistration between DCE- and DW-MRI series. To further restrict the selected tissue to low diffusing tissue of interest (tumor), b=600 images were selected for fuzzy clustering (Figure 10-2). Whereas high intensity regions on b=0
images would have corresponded to normal fibroglandular tissue and tumor, high
intensity regions on b=600 images corresponded more closely to low diffusing
tissue, most likely to be tumor. Intersection of this low diffusing tissue map with
ROIs derived from the unshifted SER map further restricted ROIs to tumor
(Figure 10-3).

Figure 10-2. Fuzzy clustering. Control points were placed to select the original
image data (far left), the number of clustering groups was then selected and tissue
was clustered (middle image) with a fuzzy-c-means algorithm. The cluster best
matching the low diffusing region was then selected (far right).

Figure 10-3. Intersection of Tissue and SER Map. ROIs derived from fuzzy
clustering (far left) were intersected with regions identified on the SER map using
Method #4 (2nd image from left). The result of the intersection (3rd image from left)
was considered tumor in the combined method, method #6. In the last image,
regions selected by the combined method are in green, regions selected only by the
tissue mask are pink and regions selected only by the SER method are blue.
Quantitative ROI Analysis:

For each of the above methods, tissue contained in the ROIs was extracted by assigning all voxels outside the ROIs to 0; the resulting segmented volume was considered to be tumor, or a “tumor mask.” The ADC distribution for each set of tumor masks was then analyzed in Matlab (Matlab Version R2007a, The Mathworks Inc, Natick, MA). The mean was considered to be the primary measure of the tumor ADC distribution. Median, skew, kurtosis, standard deviation, peak height location, proportion of total voxels contained in the peak height, number of occupied histogram bins, number of total voxels, and 12.5th, 25th, 75th, and 87.5th percentile ADCs were also calculated.

Qualitative Analysis:

Tumor ADC histograms from Methods #2-6, derived as described above, were qualitatively compared to the tumor ADC histogram derived from Method #1, manual delineation of the full tumor volume, considered the reference method in this study. Tumor ADC histograms were rated on a scale from 1-3. A rating of 1 was assigned if the histogram poorly approximated the distribution for Method #1, a rating of 2 was assigned if the histogram somewhat approximated the distribution, and a rating of 3 was assigned if the approximation was judged to be good. The rating system considered both histogram shape (occupied bins) and bin height. The histograms from the 5 methods were also directly compared and the histogram that most closely approximated the histogram for Method #1 was identified.
**Method Comparisons and Statistics:** The primary tumor ADC distribution measurement assessed was mean tumor ADC. For each case, the percent change in mean tumor ADCs derived from each method (#2-6) relative to that derived from Method #1 was calculated and the method most closely approximating that of Method #1 was identified. ANOVA was used to compare the quantitative tumor ADC distribution parameters for Methods #1-6, across patients. All p-values less than .05 were considered to be statistically significant. A multiple comparison procedure was used to determine which methods were statistically significantly different. Statistical analyses were performed in Matlab (R2007a, The Mathworks, Natick, MA) and relevant functions were anova1 and multcompare. For the qualitative analysis, scores for each method were averaged across patient exams.

**10.3. Results**

**Patient accrual:**

During the study period, 9 patients were enrolled. Of these 9 patients, 3 were excluded from analysis due to lack of baseline DW-MRI and the 6 remaining patients were included in the analysis.

**Quantitative Analysis:**

The mean tumor ADC was compared across the 6 methods (Figure 10-4).
Figure 10-4. Comparison of Six Methods in Measuring Mean Tumor ADC. The bar chart displays the mean tumor ADC calculated using each method, in six cases. Cases are ordered by mean tumor ADC derived from manual whole-tumor ROIs (Method #1). For all cases, means very by method. In all but case #4 (mucinous cancer), the mean tumor ADC derived from the manual whole tumor ROIs is lower than the mean of the shifted SER ROI.

The mean tumor ADCs derived from Methods #2-6 were compared to the mean tumor ADC derived from Method #1, considered the reference method. The percent change for each method relative to Method #1 was calculated and is depicted Figure 10-5.
Figure 10-5. Comparison of Mean Tumor ADC Calculated with Alternative Methods versus the Whole-Tumor Manual Method. The percent change in mean tumor ADC calculated from each method, relative to the reference Method 1 was calculated. For each case, the sum of all percent changes is scaled to 100% for display. As seen in the figure, the combined method provides a good approximation of the mean when the mean tumor ADC is low; however, the single slice method provides the most consistent best approximation of the mean tumor ADC derived from the manual method. In all but one case, the SER methods (blue and purple), do not provide an adequate approximation of the manually-derived mean.

The distributions of the ADC histogram parameters across the 6 patients were compared for the different methods. Notably, despite observable differences in individual patients, the distribution of the mean tumor ADC did not differ by method (Figure 10-6, Table 10-1). ADC parameters (Table 10-1) that were significantly different between methods were: standard deviation (Figure 10-7), kurtosis, maximum ADC, ADC range, peak height %, and the number of occupied histogram bins.
Figure 10-6. Mean. Box plots correspond to the distribution of mean tumor ADC measurements obtained with each method, across the 6 cases. The distribution did not vary significantly by method.

Figure 10-7. Standard Deviation. Box plots correspond to the distribution of the standard deviation of tumor ADC measurements obtained with each method, across the 6 cases. The distribution differed significantly by method.
Table 10-1. Comparison of ADC Parameters for 6 Methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>p-value</th>
<th>Differing methods</th>
</tr>
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<tbody>
<tr>
<td>Mean ADC</td>
<td>0.98127</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.00000</td>
<td>1,3</td>
</tr>
<tr>
<td>Skew</td>
<td>0.79071</td>
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<tr>
<td>Kurtosis</td>
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<td>1,3</td>
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<tr>
<td>12.5th percentile</td>
<td>0.90103</td>
<td></td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.99291</td>
<td></td>
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<tr>
<td>50th percentile</td>
<td>0.99526</td>
<td></td>
</tr>
<tr>
<td>75th percentile</td>
<td>0.62955</td>
<td></td>
</tr>
<tr>
<td>87.5th percentile</td>
<td>0.16603</td>
<td></td>
</tr>
<tr>
<td>Minimum ADC</td>
<td>0.14375</td>
<td></td>
</tr>
<tr>
<td>Maximum ADC</td>
<td>0.00018</td>
<td>1,3</td>
</tr>
<tr>
<td>ADC range</td>
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<td>2,3 and 1</td>
</tr>
<tr>
<td># Occupied bins</td>
<td>0.00101</td>
<td>1,3</td>
</tr>
<tr>
<td># Voxels</td>
<td>0.06968</td>
<td></td>
</tr>
<tr>
<td>Peak height %</td>
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<td>1,3</td>
</tr>
<tr>
<td>Peak height location</td>
<td>0.99654</td>
<td></td>
</tr>
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</table>

Qualitative Analysis

Differences in the overall tumor ADC distribution derived from each method were qualitatively evaluated. Each method resulted in a poor approximation of the distribution derived from Method #1 in at least one case (Figure 10-8). The single slice, shifted volumetric, and combined methods compared well (rating 2-3) with Method #1 in a similar proportion of cases.
Figure 10-8. Qualitative Assessment of ADC Distributions. The ADC distribution from the single slice method compared well (rating 2-3) with the manual method in all but one case. Similarly, the distribution from the combined method compared well (rating 2-3) in 4 of the 6 cases; in 1 case it resulted in a tumor mask of 0 voxels. The circle method consistently compared poorly (rating=1), and the SER method compared poorly in half of cases; the shifted SER compared better than the SER method, and similarly to the combined method on the three-point rating system.

The method that resulted in the best qualitative approximation of the tumor ADC distribution derived from the whole tumor manual method was selected for each case. In one case, no method provided a good approximation. In the remaining cases, the combined method provided the best approximation in the greatest number of cases (3/5 cases, Figure 10-9).

The method that resulted in the best approximation of the tumor ADC mean derived from the whole tumor manual method was also selected; the manual, single slice, and combined methods all provided the best approximation in an equal number of cases (Figure 10-9).
Figure 10-9. Best Method. The pie chart at left illustrates the proportion of cases in which each method provided the best approximation of the tumor ADC distribution derived from Method #1, the reference method. The pie chart at right illustrates the proportion of cases in which each method provided the best approximation of the tumor ADC mean derived from Method #1. As seen in the figures, no method scored as well as the combined method in approximating both tumor ADC distribution and mean.

Case studies:

To illustrate differences in ROIs placed with the 6 methods, results are shown for one case in which alternative methods did provide a reasonable approximation of the ADC distribution (Figure 10-10). Method #6 improves approximation of the manual tumor distribution by reducing very low ADC voxels selected by the SER method, likely to be adipose tissue due to misregistration. Method #2 closely compares with Method #1 and includes manual delineation of tumor, but on one slice instead of multiple slices.
Figure 10-10. Results for one case. Tumor ADC distributions for ROIs placed using the six methods are shown. The thick arrow denotes very low ADC regions selected by volumetric analysis in Method #4. The thin arrow shows that this region is reduced when the SER Map used in Method #4 is intersected with the DW-imaging tissue map in Method #6. As seen in the distribution for Method #3, placement of a circular ROI results in subsampling of the tumor distribution. Method #2 and Method #1 result in similar distributions.

To illustrate the potential discordance between volumetric and manual methods, results for a patient with mucinous cancer are shown (Figure 10-11). In this case, only manual methods approximated the ADC distribution. The tumor did not exhibit an abnormal contrast profile; therefore, Methods #4 and #5 which included only contrast information did not isolate the tumor. A strength of the combined method is that it reduces inclusion of noncancerous regions. In this case it resulted in a tumor volume of 0 because the ROIs selected with the volumetric methods did not correspond to low diffusing tissue and were therefore not included in the final intersection.
Figure 10-11. Mucinous cancer. In the case of a patient with mucinous cancer, only manual methods (#1-3) captured the tumor and only #2-3 approximated the distribution obtained with the reference method, Method #1. The results for the volumetric methods are inconsistent with that for the manual. Because the regions selected with the volumetric method did not correspond to the tumor, intersection with diffusion-weighted tissue mask resulted in a tumor volume of 0, and no tumor ADC distribution for Method #6.

10.4. Discussion

This study investigated the impact of different tumor delineation methods on measured tumor ADC values, with the goal of identifying a delineation method that would reduce user interaction required for tumor delineation and subsequent tumor ADC measurement. It was found that while the mean tumor ADC did not vary significantly between methods (p=.98), other measurements of the tumor ADC distribution, such as range and peak height, varied significantly (p<.05) between methods. Notably, methods with reduced user interaction - - the method involving delineation on a single slice and the semi-automated method combining diffusivity and contrast information - -
approximated the tumor ADC distribution derived from the reference method. These findings are important because the single slice and combined methods could provide reliable and less labor-intensive alternatives to measuring tumor ADC, facilitating research and clinical application of DW-MRI to the monitoring of treatment response in breast tumors.

The fact that the mean ADC was not significantly different between methods may be initially surprising because methods differed in included regions of tumor, but this finding is also compatible with the fact that all methods measured the same tumor, in the same patient, at the same timepoint. In general, areas of necrosis (high ADC) were avoided by all methods and abnormal regions (low diffusivity or abnormal contrast) were targeted for inclusion by all methods. In addition, while the differences in mean ADC were not significantly different across the 6 cases for the 6 methods, differences for individual patients may be clinically significant, especially in studies assessing treatment response by tracking a mean ADC.

Histogram parameters, when statistically significantly different, usually differed between the circle and reference method, and this is compatible with the fact that the circle method targeted a small, homogeneous region of tumor that was unlikely to capture tumor heterogeneity.

In general, the purely volumetric methods did not capture tumor as well as the manual methods in this study, and this may be surprising as breast tumors are associated with abnormal enhancement kinetics. However, some cancers, such as the mucinous cancer in this study, may not have abnormal contrast enhancement and therefore may not be identified with DCE-MRI-derived volumetric methods. In addition, areas identified as
tumor with the volumetric methods may have increased signal attenuation on DW-MRI and therefore may not be identified as tumor using a manual method, leading to further discrepancies between volumetric and manual methods. On low b-value DW-MRI, diffusion measurements are more vulnerable to overestimation due to perfusion. Highly vascular areas identified by volumetric but not manual analysis may be associated with increased signal attenuation on low b-value DW-MRI. Signal attenuation would lead to these regions not being bright on DW-MRI and not being identified as tumor.

Sources of variability could have impacted implementation of the six methods in the six cases. All methods, except for Method #4, involve some degree of user interaction and are therefore prone to differences due to user variability. In addition, Method #6 required the user to select the number of tissue groups needed for fuzzy clustering. This number varied by cases and may depend on presence of normal fibroglandular tissue. For example, in one case, most of the fibroglandular tissue present was tumor and only 2 clustering groups were needed; conversely, in another case with more normal tissue, more than 2 groups were needed. Method #6 also required the user to define a volume in which to apply fuzzy clustering. To reduce time, the region of interest was defined on one slice and pasted throughout the volume. Individual variation in tumor location, skin hyperintensity on DW-MRI, and presence of other hyperintense regions (implants, other lesions) resulted in case by case differences in the shape and location of this region of interest. Automation of this region of interest placement could reduce this source of variability in Method #6.

Variability in tumor biology also accounts for the differing performance of particular methods in particular cases. In the patient with mucinous cancer, Methods #4-6
did not capture the tumor, whereas Methods #1-3 did capture the tumor. This can be explained by the fact that the cancer had an atypically high ADC, in contrast to the low ADC typically encountered by tumor. Visual assessment by a user was therefore needed to identify the tumor. In addition, this tumor did not exhibit abnormal contrast kinetics, resulting in the failure of Methods #4-6 to capture the tumor. This case illustrated a strength of Method #6. Whereas Methods #4 and #5 selected regions that were ultimately not tumor, Method #6 intersected these erroneous regions with tissue regions identified by fuzzy clustering and the intersection resulted in a tumor volume of 0. While the tumor ADC was not able to be measured and tracked, the risk of tracking an erroneous value was also reduced. Therefore, failure of Method #6 to capture tumor could serve as a warning that volumetric methods are not appropriate in particular cases.

Due to patient motion and EPI distortion, the purely volumetric methods risk inclusion of normal fibroglandular and adipose tissue in the tumor ROI. Methods of image registration have been explored to help correct for distortion and motion (Partridge, Demartini et al. 2010). Image registration is difficult due to lack of consistent presence of a stable landmark in breast images. In addition, misregistration due to EPI distortion is likely due to nonlinear image shifts and implementation of nonlinear image registration algorithms may be difficult to implement and validate in clinical breast MRI. The combined method reduces the impact of misregistration without requiring use of a nonlinear registration algorithm. Method #6 restricts volumetric regions of interest to fibroglandular tissue. Use of the diffusion-weighted images for fuzzy clustering increases the ability of this method to restrict regions to cancerous fibroglandular tissue. In all but
one case, Method #6 reduced the volume of tumor that would have been selected by purely volumetric methods.

Based on a qualitative analysis of tumor ADC distributions, and individual quantitative differences in mean tumor ADC, the combined method, Method #6, may be most optimal for measurement of tumor ADC in non-mucinous breast cancers. The single slice method, Method #2, also performed equally well in its measurement of tumor ADC and nearly equally well in its depiction of the tumor ADC distribution and could be a viable alternative to whole-tumor delineation in mucinous and non-mucinous breast cancers.

Despite these findings, this study was limited in its small sample size and in the limitations inherent to each of the six methods that were tested. The single slice method was limited by biological differences in tumors. For tumors with large necrotic cores surrounded by a smaller amount of viable tissue, it was particularly difficult to delineate viable tissue (bright on DCE-MRI subtraction) from nearby necrotic regions (dark on DCE-MRI subtraction); on DW-MRI, both of these regions could be hyperintense. Similarly, a 5mm circular ROI may be too large for some tumors. It was also assumed that areas that were bright on diffusion and dark on DCE-MRI subtraction images were fibrotic regions that did not warrant inclusion in the tumor ROI; however, these regions could have represented viable tumor with normal contrast kinetics.

The volumetric methods were limited in their selection of tumor based on abnormal contrast kinetics; tumors without abnormal enhancement kinetics may not be adequately captured by a purely volumetric method. In addition, the results obtained with the volumetric methods may have been improved if minimum ROI size requirements had
been instituted; this would have reduced inclusion of scattered regions that may or may not have been tumor. However, a strength of an imaging method is its spatial resolution and minimum ROI size requirements would reduce the applicability of diffusion analysis to smaller tumors.

Selection of tumor based on fuzzy clustering alone would avoid the need for dependence on DCE-MRI data, and was needed in Method #6. However, in preliminary analysis, use of the diffusion-weighted tissue map alone resulted in tumor ADCs that were too high relative to those derived from the reference method and tumor volumes that were much higher than those derived using the volumetric methods. This suggests that normal fibroglandular tissue is likely to be included in a method relying solely on the diffusion-weighted tissue mask, limiting its utility in measuring ADC. However, combining the tissue mask with the contrast profile map resulted in a tumor delineation method that compared well relative to the reference method.

This study compared different methods of tumor delineation in their ability to capture the tumor ADC distribution. At the time of this writing, multiple methods are used in the literature and evidence of comparisons between methods is not apparent. Prior to this study, it was not known if methods other than whole-tumor manual delineation could adequately capture the tumor ADC distribution. Based on the findings in this small cohort, a method combining diffusivity and contrast information shows promise in capturing the variation in diffusivities present within a tumor. This combined method also involves less user-interaction and manual delineation than other methods. Delineation on a single slice also compared well with the reference method and would reduce time involved in ROI placement. In addition, in clinical settings in which tumor can only be
delineated with a circular ROI, the measured mean tumor ADC is not expected to
different significantly from that measured using more extensive delineation. The finding
that less user-intensive delineation methods may be adequate could improve the ability to
analyze diffusion data in large, prospective studies and facilitate the clinical adaptation of
DW-MRI in the breast.

10.5. Conclusion

This study found that less user- and time-intensive methods of tumor delineation
on DW-MRI data could serve as alternatives to manual delineation of all regions
encompassed by the tumor. This study also developed and tested a new method
combining diffusivity and contrast information and this new method compared well with
the reference method. Validation of the single slice and combined methods is needed in a
larger patient cohort, in which more tumor variation is present. Future work should also
compare the ability of the parameters extracted from these methods to predict patient
outcomes.

10.6. References

Iacconi, C., M. Giannelli, et al. (2009). "The role of mean diffusivity (MD) as a
predictive index of the response to chemotherapy in locally advanced breast


Part V: Clinical Evaluation

In this section, the research question investigated in Part II -- whether diffusion is valuable in predicting treatment response in patients with locally advanced breast cancer -- is revisited, using methods tested and optimized in Parts III and IV. In Chapter 4 of Part III, fat suppression was shown to greatly impact measured ADC. In Chapter 5, echo planar imaging was shown to have advantages over the single shot fast spin echo sequence used in Part II. And in Chapter 6, a promising fat-suppressed, reduced field of view diffusion-weighted echo planar sequence was optimized for use in the breast. In Chapter 11 of this section, a study is described in which this new sequence was acquired in patients and subsequent diffusion measurements were compared to measurements obtained with a standard sequence.

Based on the findings Chapter 8, parameters derived from the ADC distribution, in addition to the mean tumor ADC, were tested in this section, and a method of tumor segmentation investigated in Chapter 10 was used. In Chapter 12, a study is presented in which the new sequence is acquired prospectively, and longitudinally, in patients treated with neoadjuvant chemotherapy and ADC changes are correlated with changes in tumor size. The ability to measure ADC in tumors (Chapter 11) and to monitor tumor response to treatment (Chapter 12) may be greatly improved with alternative methods in image acquisition and image processing.
Chapter 11. High-Resolution DW-MRI of Invasive Breast Cancer

11.1. Introduction

Diffusion-weighted magnetic resonance imaging (DW-MRI) is a promising tool for characterizing micro-structural properties of breast lesions, with applications to both the diagnosis of breast cancer as well as to the detection of tumor response in patients treated with neoadjuvant chemotherapy. DW-MRI provides a non-invasive, non-contrast,
three-dimensional method to measure the random motion of water molecules, or
diffusion, in vivo.

Diffusion is limited by properties of the tissue such as cell density, and also by
cellular properties such as intracellular water fraction, and presence of organelles and cell
membranes. In cancer, changes in cellular and tissue properties are reflected in changes
in diffusion. Using DW-MRI, diffusion is measured as the apparent diffusion coefficient
(ADC). Decreased ADC has been reported in malignant tumors relative to normal breast
tissue, and the value of DW-MRI relative to mammography and dynamic-contrast
enhanced magnetic resonance imaging (DCE-MRI) in the diagnostic setting has been
explored (Park, Cha et al. 2007; Yoshikawa, Ohsumi et al. 2007; Partridge, Demartini et
al. 2010). In prognostic studies, increases in tumor ADC have been shown to occur
earlier than changes in tumor size during treatment with neoadjuvant chemotherapy
(Pickles, Gibbs et al. 2006).

Despite these findings, DW-MRI of the breast has yet to be fully integrated into
clinical practice. Some prognostic studies have found little value in breast DW-MRI in
monitoring treatment response (Manton, Chaturvedi et al. 2006; Nilsen, Fangberget et al.
2010; Tozaki, Oyama et al. 2010). Tumor response to treatment is likely to be
heterogeneous and capturing heterogeneity in diffusivity requires high spatial resolution.
High resolution DW-MRI could improve prognostic and diagnostic applications in the
breast, but obtaining higher resolution is technically challenging with current
commercially available sequences.

Most commercially available DW-MRI sequences are echo planar imaging (EPI)-
based and resolution is limited by the imaging field-of-view and number of phase and

262
frequency encoding steps that can be acquired before the signal decays. Acquired in-
plane resolution in DW-MRI of the breast is generally 2 mm or worse. EPI sequences are
also prone to distortion. In breast EPI, distortion and other artifacts are particularly a
problem due to changes in magnetic susceptibility at the air-tissue interfaces at the
anterior and lateral borders of both breasts. The posterior location of the lungs can also
result in susceptibility effects.

Imaging with a reduced field-of-view (rFOV) has the potential to reduce
distortion by decreasing the required readout duration for imaging and by allowing for
air-tissue interfaces to be excluded from the shim volume, reducing susceptibility-
induced artifacts. rFOV DWI-MRI has been applied to other anatomic regions such as the
spine (Wheeler-Kingshott, Hickman et al. 2002; Jeong, Kim et al. 2005; Wilm, Svensson
et al. 2007) and brainstem (Karampinos, Van et al. 2008), but to our knowledge it has not
been reported in breast tumors. An alternative approach to reducing distortion is the use
of parallel imaging (Kuroki, Nasu et al. 2004; Sinha and Sinha 2008). However, parallel
imaging requires estimating the coil sensitivities, a process that may lead to artifacts in
the resulting images due to motion (for example, breathing, cardiac motion). An rFOV
DW-MRI sequence with higher in-plane resolution was developed for the spine utilizing
a novel 2D RF pulse (Saritas, Cunningham et al. 2008), and was later used for evaluating
diffusion outside the central nervous system (Saritas, Shankaranarayanan et al. 2009). We
aimed to apply this sequence to the breast for quantitative DW-MRI of breast tumors.

In this study we investigated the impact of rFOV DWI-MRI on quantified and
clinically interpreted DW-MRI data in invasive breast tumors, with the goal of improving
tumor characterization. We compared quantitative and qualitative measures of tumor
heterogeneity derived from rFOV versus standard FOV DW-MRI acquisitions. We also compared overall image quality. We hypothesized that the increased in-plane resolution in rFOV DW-MRI would improve characterization of tumor heterogeneity while the rFOV would reduce distortion and improve image quality.

11.2. Materials and Methods

Patients: The study population consisted of patients enrolled in imaging studies approved by the Institutional Review Board and compliant with HIPAA. All patients signed informed consent. Only patients with invasive breast cancer and scanned with rFOV DW-MRI, standard diffusion, and DCE-MRI at our institution were included in the study. Additional inclusion criteria were adequate image quality for quantitative analysis and MR tumor volume \( \geq 0.1 \text{ cm}^3 \) (Partridge, Gibbs et al. 2002; Li, Partridge et al. 2008). Patients were scanned between December 22, 2009 and July 6, 2010.

Imaging: All scanning was performed on a 1.5 T GE Signa scanner (GE Healthcare, Waukesha, WI). An eight-channel bilateral coil was used (Sentinelle Medical, Inc, Toronto, Canada). The diffusion sequences were acquired following contrast-enhanced imaging.

Contrast-enhanced imaging: Bilateral, axial, dynamic-contrast enhanced (DCE) MR images were acquired using a fat-suppressed T1-weighted three-dimensional fast gradient recalled echo (3DFGRE) sequence. Parameters were as follows: TR = 5.9-9.2 ms, TE = 2.6-4.5 ms, flip angle = 10°, FOV = 260-340 mm, acquisition matrix = 512 x
260, 512 x 192, or 384 x 224, slice thickness = 2 mm, number of slices = 92-124, and number of averages = .8. Gadopentetate dimeglumine (Magnevist, Schering AG, Berlin, Germany) was administered as a contrast agent at a dose of 0.1 mmol/kg body weight, followed by a saline flush of 10 mL saline. Images were acquired prior to contrast injection and at up to four timepoints post-injection.

**Diffusion imaging:** Our standard diffusion sequence, a 2D, fat-suppressed, echo planar diffusion-weighted sequence, was acquired bilaterally in the axial orientation. The imaging parameters were: TR/TE = 6000/108.5 ms, FOV = 400 mm x 400 mm, acquisition matrix = 128 x 128, in-plane resolution = 3 mm x 3 mm, slice thickness = 3 mm, number of slices = 20 (with the exception of 1 patient who had 8 slices), number of excitations = 6, acquisition time = 4.4 min. Diffusion-weighting gradients were applied in 6 non-collinear directions with b = 600. Images were also acquired with b = 0.

A reduced field-of view (rFOV), 2D, fat-suppressed, echo planar diffusion-weighted sequence (Saritas, Cunningham et al. 2008) was acquired unilaterally in the axial and/or sagittal orientation (in the final cohort, only 2 patients were scanned exclusively with sagittal rFOV). The parameters were: TR/TE = 4000/ 64.8 ms, FOV = 140 x 70 mm, acquisition matrix = 128 x 64, in-plane resolution = 1 mm x 1 mm, slice thickness = 4 mm, number of slices = 8, number of excitations = 16, acquisition time = 4 min. Diffusion-weighting gradients were applied sequentially in 3 orthogonal directions with b = 600. Images were also acquired with b = 0. A 16-slice option was developed for use in patients with large tumors. Additional parameter changes were as follows: TR = 3000, acquisition time = 6.6 min.
**Image Processing:** ADC maps for standard FOV diffusion were constructed offline using in-house software. The apparent diffusion coefficient (ADC, or D) for each voxel was calculated under the assumption of monoexponential signal decay of the original signal ($S_{b0}$) to $S_{b600}$ with application of the diffusion gradients using the relationship in Equation 1 (Neil 1997):

$$S_{b600} = S_{b0}e^{-bD}$$

Acquired images for rFOV diffusion were first complex averaged using an automated method, and then ADC maps were constructed automatically at the scanner using the relationship in Equation 1 and previously reported methods (Saritas, Cunningham et al. 2008).

**Image Analysis:**

*Quantitative Analysis:* Tumor regions-of-interests (ROIs) were manually delineated on a central slice of rFOV images in collaboration with a board-certified radiologist specializing in breast imaging. ROIs were placed using in-house software programmed in Interactive Data Language (Version 7.0, ITT Visual Information Solutions, Boulder, Colorado). Care was taken to capture areas that were hyperintense on DCE-MRI subtraction images (pre-contrast subtracted from post-contrast), and generally hyperintense on DW-MRI and hypointense on ADC. ROIs were drawn to approximate tumor boundaries, so as to capture as much tumor as possible and to avoid inclusion of surrounding tissue. Areas of necrosis, based on lack of enhancement on DCE-MRI data, were excluded. Areas with obvious partial voluming averaging of adjacent structures
were also excluded by visual assessment. The same ROIs were mapped to the standard FOV diffusion ADC maps. In cases of misregistration between the two acquisitions, ROIs were adjusted so as to cover the tumor. In some cases, differences in slice thickness between the rFOV and standard FOV diffusion led to mapping of the ROI to more than one slice on standard FOV diffusion; in these cases, the slice with the most tumor was selected. In the few cases in which rFOV and standard FOV were acquired in different orientations, the standard FOV images were reformatted and resampled to match the rFOV image series. The standard FOV images were selected for reformatting due to the fact that voxels were approximately isotropic.

For the tumor regions delineated by ROIs on rFOV and standard FOV ADC maps, histograms were constructed for the tumor ADCs, with bins of width 100*10^{-6} mm²/s (Yankeelov, Lepage et al. 2007), from 0-5000 x10^{-6} mm²/s. Despite the fact that 5000 x10^{-6} mm²/s is above the diffusivity of free water at body temperature, this value was chosen as the upper limit to allow for exploratory analysis. Since the two sequences differed in resolution and FOV, histogram frequencies were normalized to the total number of tumor voxels (Ge, Kolson et al. 2003). The number of occupied bins (nbins) for tumor ADCs obtained from standard and rFOV diffusion sequences was calculated. The mean, median, minimum and maximum ADC, skew, kurtosis, and 12.5th, 25th, 75th, and 87.5th percentile ADCs were calculated. Measurements of lower (12.5th, 25th) and upper (75th, 87.5th) ADC percentiles were included in the analysis due to the fact that viable, invasive cancers have lower ADCs than normal tissue and changes in lower and upper ADC ranges may correlate with treatment response. All histograms were
constructed and analyzed in MATLAB (Version R2007a, MathWorks, Natick, Massachusetts).

**Qualitative Analysis:** Two board-certified radiologists specializing in breast imaging (and who did not take part in the ROI delineation above) qualitatively compared anonymized images from rFOV and standard FOV DW-MRI sequences for each patient. Reviewers were blinded to tumor histology, patient outcomes, and sequence type. Standard and rFOV images were referred to as sequence 1 and sequence 2. Sequence number was varied to improve blinding; however, complete blinding to sequence type was not possible due to the visual differences between images. For each patient, reviewers were shown 1 slice from rFOV and standard diffusion, covering the tumor and matched in spatial coordinates between sequence 1 and sequence 2. Reviewers were asked to rate 5 characteristics for each exam. On DW-images (b=600), tumor morphologic detail, tumor heterogeneity, lesion conspicuity, and overall image quality were rated; on T2-weighted images (b=0), overall image quality was rated. For each characteristic, readers determined if the characteristic appeared better on sequence 1, better on sequence 2, or if it appeared to be similar between the two sequences (Al-Saeed, Ismail et al. 2009). Reviewers were given the option of requesting additional slices, dynamic-contrast enhanced MRI information, or re-windowing of the images.

**Statistics:** Tumor ADC means and histogram parameters were compared pairwise for rFOV and standard FOV acquisitions. The paired Wilcoxon sign rank test was used. Tumor ADC means were compared between rFOV and standard FOV acquisitions.
with a Bland-Altman plot (Bland and Altman 1986) and the 95% confidence interval for the agreement between the two methods was calculated.

11.3. Results

**Patient accrual and tumor characteristics:** During the study period, 17 patients with invasive breast cancer >0.1 cm³ were scanned with rFOV and standard diffusion. Of these patients, 3 did not meet initial inclusion criteria due to image quality problems for which we have since instituted measures to help prevent (in one exam fat suppression failed, one exam had a wrap-like artifact and one had significant noise at the level of the tumor). Of the remaining 14 patients meeting inclusion criteria, two were excluded from analyses due to small tumor size and difficulty in identifying tumor on a single slice (Figure 11-1). The remaining 12 patients were included in both quantitative and qualitative analyses.

All 12 patients were diagnosed with invasive breast cancer on pathology; 1 patient had invasive lobular carcinoma and all other patients had invasive ductal carcinoma. In 6 patients, the cancer was grade 2 (Scarf-Bloom-Richardson); in 5 patients, grade 3; in 1 patient grade was 1-2. Mean MR tumor volume was 15.5 cm³ (SD = 16, range 0.4-46.6 cm³) and mean MR tumor longest diameter was 4.6 cm (SD=2.6, range 1.3-9.3 cm). Analysis of diffusion in tumors with longest diameter as small as 0.5 cm has previously been reported (Partridge, Mullins et al. 2010). Mean age was 52 (SD=9.2).
Figure 11-1. Flowchart of patient accrual. Of the initial 14 patients meeting inclusion criteria, two of the patients were later excluded from the quantitative analysis due to small tumor size (<.3 cm³). These 2 patients were retrospectively excluded from the qualitative analysis, for a total size of 12.
Quantitative assessment of tumor ADC distributions:

In the 12 patients included in this analysis, the mean tumor ADC calculated from rFOV DW-MRI was 1116.1 *10^{-6} \text{ mm}^2/\text{s} (SD=136.2) and mean tumor ADC calculated from standard FOV DW-MRI was 1154.5 *10^{-6} \text{ mm}^2/\text{s} (SD=134.8). Tumor ADC was not significantly different between methods (p=.52, Table 11-1). When the difference between the mean ADCs were plotted against the average between the two methods (Figure 11-2), the limits of agreement were -195.51 to 272.35 *10^{-6} \text{ mm}^2/\text{s} and more than 95% of the differences in the data were within these limits, suggesting that measurements of mean tumor ADC with the two methods were comparable.

In further analysis of the tumor ADC distributions obtained with the two methods, the 12.5^{th} percentile tumor ADC and minimum tumor ADC were significantly different between rFOV and standard FOV DW-MRI (p=.027, .001, respectively), with a trend towards a lower 12.5^{th} percentile and minimum ADC in the rFOV acquisition (Table 11-1). The maximum ADC (p=.003), ADC range (p<.001), number of occupied bins (p<.001), and number of tumor pixels (p=.002) were also significantly different in the rFOV acquisition relative to the standard FOV. All other variables, including skew and kurtosis, were not significantly different between acquisitions.
Figure 11-2. Bland-Altman Plot of Mean Tumor ADC Calculated with Two Acquisitions. The Bland-Altman plot illustrates that the rFOV and standard diffusion acquisitions provide comparable measures of mean tumor ADC. Differences in measurements of mean ADC (10-6 mm²/s) are within 1.96 SD (dotted lines) of the mean difference (dashed lines). The 95% confidence interval for the limits of agreement (dotted lines) is -195.5 to 272 10-6 mm²/s.
Table 11-1. Comparison of ADC Distribution Parameters between Two Acquisitions

<table>
<thead>
<tr>
<th>Tumor ADC Variable</th>
<th>Mean, rFOV (SD)</th>
<th>SD, rFOV</th>
<th>Mean, standard FOV</th>
<th>SD, standard FOV</th>
<th>Mean % change in rFOV relative to standard FOV</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ADC</td>
<td>1116.1</td>
<td>136.16</td>
<td>1154.5</td>
<td>134.8</td>
<td>-2.8592082</td>
<td>0.51855</td>
</tr>
<tr>
<td>Skew</td>
<td>0.42722</td>
<td>0.48453</td>
<td>0.41366</td>
<td>0.58834</td>
<td>17.058804</td>
<td>0.96973</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>3.4866</td>
<td>1.0449</td>
<td>3.4083</td>
<td>1.2901</td>
<td>10.5131726</td>
<td>0.42383</td>
</tr>
<tr>
<td>Minimum ADC</td>
<td>267.33</td>
<td>285.42</td>
<td>629.67</td>
<td>305.85</td>
<td>-37.664873</td>
<td>0.0014648</td>
</tr>
<tr>
<td>12.5th percentile ADC</td>
<td>775.12</td>
<td>183.85</td>
<td>892.17</td>
<td>198.84</td>
<td>-9.7207694</td>
<td>0.026855</td>
</tr>
<tr>
<td>25th percentile ADC</td>
<td>901.25</td>
<td>156.7</td>
<td>981.95</td>
<td>158.69</td>
<td>-7.3540112</td>
<td>0.063965</td>
</tr>
<tr>
<td>50th percentile ADC</td>
<td>1092.3</td>
<td>133.39</td>
<td>1122.5</td>
<td>132.13</td>
<td>-2.245478</td>
<td>0.62207</td>
</tr>
<tr>
<td>75th percentile ADC</td>
<td>1307.1</td>
<td>140.64</td>
<td>1315.6</td>
<td>118.82</td>
<td>-0.3375362</td>
<td>0.79102</td>
</tr>
<tr>
<td>87.5th percentile ADC</td>
<td>1486.4</td>
<td>151.87</td>
<td>1472.4</td>
<td>115.21</td>
<td>1.2995803</td>
<td>0.90967</td>
</tr>
<tr>
<td>Maximum ADC</td>
<td>2164.6</td>
<td>265</td>
<td>1835.3</td>
<td>263.83</td>
<td>19.7041988</td>
<td>0.003418</td>
</tr>
<tr>
<td>ADC range</td>
<td>1897.2</td>
<td>431.82</td>
<td>1205.7</td>
<td>393.16</td>
<td>72.5223474</td>
<td>0.00048828</td>
</tr>
<tr>
<td># Occupied bins</td>
<td>19.583</td>
<td>4.1661</td>
<td>12.25</td>
<td>3.7929</td>
<td>71.5923312</td>
<td>0.00048828</td>
</tr>
<tr>
<td># Tumor pixels</td>
<td>1255.3</td>
<td>1029.4</td>
<td>382.92</td>
<td>816.49</td>
<td>680.150958</td>
<td>0.0024414</td>
</tr>
</tbody>
</table>
Qualitative analysis:

Two readers independently rated 12 cases in 5 categories. Ratings from both readers showed a preference for rFOV in all categories (Figure 11-3); however, Reader 2 showed a stronger preference for rFOV than Reader 1 and rated rFOV as the preferred series for all cases and all categories (Figure 11-3).
Figure 11-3. Charts showing qualitative ratings assigned by each reader in 12 cases.
Case studies:

Images are shown for two cases in which readers rated rFOV better than standard FOV DW-MRI in all qualitative areas (Figure 11-4 and Figure 11-5). In the first case, despite qualitative improvements with rFOV, histograms (Figure 11-4) appear similar, although differences in skew and nbins can be measured. In the second case, histograms (Figure 11-5) appear markedly different, congruent with the qualitative differences identified by the radiologists.
Figure 11-4. Images and histograms for Case 1. Overall image quality is improved on b=0 and b=600 combined images for rFOV DW-MRI (top row, first and second images, respectively), compared to standard FOV b=0 and b=600 images (middle row, first and second images, respectively). Both radiologists also rated rFOV diffusion (top row, 2nd image) as superior to standard (middle row, 2nd image) in depiction of morphologic detail, tumor heterogeneity, lesion conspicuity. Despite improvements in tumor depiction, tumor ADC distributions (last row) derived from ADC maps for rFOV and standard diffusion, respectively (third image of top and middle row, respectively) are similar.
Figure 11-5. Images and histograms for Case 2. Overall image quality is improved on $b=0$ and $b=600$ combined images for rFOV DW-MRI (top row, first and 2nd images, respectively), compared to standard FOV $b=0$ and $b=600$ images (middle row, 1st and 2nd images, respectively). Both radiologists also rated rFOV diffusion as superior to standard in depiction of morphologic detail, tumor heterogeneity, and lesion conspicuity. Tumor ADC distributions for rFOV and standard (last row, 1st and 2nd images, respectively) derived from ADC maps (last images of top and middle row, respectively) differ between acquisitions, with a decreased number of occupied bins in standard FOV vs rFOV.
11.4. Discussion

The results of this study indicate that rFOV DW-MRI improves tumor depiction as compared to standard FOV DW-MRI, and that these improvements are reflected in both quantitative and qualitative differences in tumor representation on DW-MRI. A significant finding of this study was that two radiologists specializing in breast MRI independently assigned higher image quality and tumor depiction ratings to rFOV DW-MRI than to standard FOV diffusion. Radiologists saw improvements in clinically significant image quality characteristics, consistent with the fact that rFOV diffusion restricted the image FOV, resulting in increased image resolution and simultaneously decreased susceptibility to artifacts due to distortion.

Our results also show that the qualitative improvements with rFOV DW-MRI can be related to statistically significant differences in the tumor ADC distribution. The fact that the distributions differed in the lower ADC range is compatible with the fact that a higher resolution would yield less partial voluming. Decreased partial voluming of low-ADC tumor with high-ADC normal tissue would result in lower ADCs in rFOV than standard diffusion. Similarly, the maximum ADC differed and decreased partial voluming of tumor with adipose tissue (ADC tumor > ADC adipose tissue), would result in higher ADCs in rFOV than standard diffusion.

The fact that the mean ADC did not differ significantly between the two sequences relates to the fact that both sequences were measuring the same tumor, at the same timepoint, and regardless of resolution, the bulk tumor would be presumed to have the same mean ADC. The statistically non-significant difference in the mean ADCs supports the validity of the new sequence: it is capable of measuring the same mean
diffusivity as the standard DW-MRI technique. Since most treatment monitoring studies track a mean tumor ADC, use of rFOV DW-MRI instead of standard FOV diffusion would not negatively impact such studies. On the contrary, our qualitative results suggest that such studies could be improved with use of rFOV DW-MRI. Furthermore, in addition to mean ADC, other ADC histogram parameters have been shown to have value in tracking treatment response in the breast (Yankeelov, Lepage et al. 2007), as well as outside the breast (Pope, Kim et al. 2009), and in distinguishing different disease subtypes (Tozer, Jager et al. 2007). The fact that rFOV diffusion resulted in statistically significant differences in particular parameters related to the ADC distribution is important because it means that rFOV diffusion could potentially improve measurements in applications relying on a quantitative assessment of the ADC distribution. The significant difference in lower ADCs is important because tumors are expected to have lower ADCs and improved measurement could be beneficial in diagnostic and treatment monitoring applications.

The promising results of this study are tempered by the variable image quality, seen especially early in the study. Even though image quality with rFOV diffusion was qualitatively better than with standard FOV diffusion, image quality was not consistent within rFOV exams. Three exams suffered from poor quality and needed to be excluded from all analyses. We have since optimized rFOV DW-MRI to help reduce variable image quality. This sequence utilizes a narrow-bandwidth water-selective excitation, underscoring the importance of accurate identification of the center frequency of water. In phantom studies, inaccurate center frequency identification resulted in the same artifacts as seen in patient scans. We have since instituted a protocol of manually
identifying the center frequency of water. Subsequently, the appearance of artifacts has decreased and quality has improved.

Another source of potential variability in the data results from the fact that the tumor was represented by 1 region of interest (ROI) on one slice in the image volume. The same volume of tumor imaged with standard diffusion cannot always be imaged with rFOV diffusion because rFOV diffusion is limited to a 140 mm x 70 mm x 32-64 mm acquisition volume. Representing the tumor on 1 slice, and defining this slice first on the rFOV DW-MRI was therefore a compromise between the FOV differences and the manual, time-consuming nature of the ROI delineation. Single-slice ROI delineation is not uncommon (Nilsen, Fangberget et al. 2010; Partridge, Mullins et al. 2010). ROIs defined on rFOV diffusion were automatically registered with standard FOV diffusion images, but the ROI location and morphology were verified on standard FOV diffusion and adjusted as needed. In one case, the ROI required erosion on the standard FOV diffusion image, but in all other cases either no changes were needed or the ROI only needed to be shifted in position. The need for a shift can be traced to the fact that DW-EPI acquisitions suffer from distortion in the breast (Sinha and Sinha 2008). Patient motion may also have occurred, although the timing of the standard and rFOV diffusion sequences and the good fit of the ROI with both sequences imply that any contributions of patient motion were likely to have been small.

This study was limited in that the parameters for rFOV and standard FOV diffusion were not exactly matched. Due to study and practical constraints, the slice thickness for rFOV diffusion was 4 mm while the slice thickness for standard FOV diffusion was 3 mm. Despite this difference, rFOV voxels were still over 6 times smaller
than standard FOV voxels. This is illustrated in the statistically significant difference in the number of voxels in the rFOV and standard FOV tumor masks for the same ROI; rFOV tumor masks had more voxels, consistent with the fact that resolution was 6 times better and image reconstruction for both acquisitions was in the form of 256 x 256 pixel images, resulting in the ability of rFOV to cover the tumor with the same number of pixels utilized by standard FOV diffusion to cover both breasts. Partial voluming in the rFOV due to the 4 mm slice thickness would still be small compared to the partial voluming in the standard FOV diffusion sequence.

It should be mentioned that the in-plane resolution of the rFOV acquisition used in this study (approximately 1 mm x 1 mm in-plane) was still 1000 times larger than the diameter of a cell. The true, biological ADC heterogeneity for the tumors in this study is not known and would require complete pathological examination of the entire tumor, which was not possible due to the fact that for most patients, surgery followed completion of neoadjuvant treatment, not completion of the DW-MRI exam, and treatment effect would impair radiographic-pathological correlation. In one prognostic study, pathological markers were not predicted by ADC (Kim, Cha et al. 2009). ADC depends on a variety of molecular and cellular factors.

Despite the limitations of this study, the quantitative and qualitative results strongly suggest that, compared to standard FOV diffusion, rFOV diffusion is able to better depict the diffusivity of invasive breast tumors at a sub-centimeter level. This improvement is important because changes in diffusion are thought to occur before changes in tumor size (Pickles, Gibbs et al. 2006). Improved identification of treatment-induced diffusivity changes could allow for better assessment of tumor response and
better tailoring of therapies to a patient’s response. In addition, a higher-resolution sequence could potentially have value in identifying smaller areas of high cellularity, which could represent tumors that may not have otherwise been detected. Prognostic studies evaluating tumor response to treatment and diagnostic studies evaluating the presence of in situ cancer or smaller lesions may benefit from rFOV diffusion. These benefits must however be substantiated by assessing the ability of rFOV diffusion measurements to predict clinical outcomes. Large, prospective studies comparing the predictive abilities of rFOV diffusion prognostic and diagnostic markers to those of standard FOV diffusion are needed.

11.5. Conclusion

In conclusion, the depiction of invasive breast tumors with rFOV DW-MRI is qualitatively and quantitatively different than the depiction with standard FOV DW-MRI. Our results show that image quality and tumor depiction is improved with rFOV diffusion as compared to standard FOV diffusion. These finds are consistent with the fact that rFOV diffusion decreases artifacts due to distortion and improves resolution, resulting in decreased partial voluming. This decrease in partial voluming was reflected in statistically significant differences in maximum tumor ADC, the number of occupied bins, and importantly, in minimum and 12.5th percentile tumor ADC, measures of the low ADCs expected to be found in most invasive tumors. High resolution DW-MRI of the breast is promising and further study relating measurements to clinical outcomes is needed.
11.6. Acknowledgements

This study was made possible through a collaboration with GE Healthcare and Stanford University.

11.7. References


12.1. Introduction

DW-MRI is a non-ionizing, non-contrast, three-dimensional method of measuring water mobility in vivo. Because water mobility relates to biological properties such as cell density that differ between cancerous and normal tissue, measurement of water
mobility may have important diagnostic and prognostic value in oncology (Chenevert, Meyer et al. 2002; Hamstra, Rehemtulla et al. 2007). In breast cancer, DW-MRI has been shown to add positive predictive value to diagnostic studies (Park, Cha et al. 2007; Partridge, DeMartini et al. 2009; Partridge, Demartini et al. 2010), and it has been shown to predict tumor response to neoadjuvant chemotherapy (Pickles, Gibbs et al. 2006; Yankeeelov, Lepage et al. 2007; Sharma, Danishad et al. 2008; Iacconi, Giannelli et al. 2009). Accurate prediction of tumor response could allow for ineffective treatment regimens to be discontinued, sparing patients potentially toxic side effects, and allow for more effective therapies to be introduced, potentially improving overall survival from breast cancer.

Despite the promise of DW-MRI for improving the in vivo monitoring of response to chemotherapy in patients with locally advanced breast cancer, DW-MRI has been technically limited. The echo planar imaging sequences on today’s commercially available scanners are prone to distortion (Jezzard and Balaban 1995; Sinha and Sinha 2008), potentially impairing water mobility measurements. In-plane spatial resolution is also typically 2 mm x 2 mm or worse, resulting in potential partial voluming between responding and non-responding regions of a tumor, and potential mischaracterization of tumor response to treatment. While many studies have found value in DW-MRI in predicting response to treatment, many studies have also found that DW-MRI does not have value (Manton, Chaturvedi et al. 2006; Nilsen, Fangberget et al. 2010; Tozaki, Oyama et al. 2010).

A reduced field of view (rFOV) DW-MRI sequence was developed for use in the spine (Saritas, Cunningham et al. 2008). The reduced field of view provides two
significant advantages for breast imaging: it improves in-plane resolution and has the potential to reduce artifacts due to distortion. In Chapter 6, early studies involving optimization of rFOV DW-MRI for use in patients with breast tumors were presented. In Chapter 11, it was shown that relative to standard FOV diffusion, rFOV DW-MRI improves image quality and tumor characterization of invasive breast tumors, and that these improvements are accompanied by statistically significant differences in the tumor ADC distribution.

It was hypothesized that because rFOV DW-MRI improves image quality and results in quantitative changes in ADC measurements that are likely to reflect tumor heterogeneity, measurements of tumor response with rFOV diffusion would differ from measurements with standard FOV diffusion, and rFOV diffusion measurements would better correlate with patient outcomes. To test this hypothesis, patients with locally advanced breast cancer undergoing treatment with neoadjuvant chemotherapy were longitudinally monitored with both rFOV and standard FOV diffusion. In this chapter, longitudinal differences between rFOV and standard FOV diffusion measurements are assessed in an early series of patients scanned under this protocol.

12.2. Materials and Methods

Overview: Patients with locally advanced breast cancer and treated with neoadjuvant chemotherapy were enrolled in IRB-approved studies at our institution and scanned with MRI before, during, and after treatment with neoadjuvant chemotherapy. All patients gave informed consent. A subset of patients was scanned with both standard FOV and rFOV diffusion and these patients are included in this analysis.
**Patients:** All patients were determined to have locally advanced breast cancer and were treated with neoadjuvant chemotherapy. All patients had pathology-confirmed invasive breast cancer. Additional criteria for inclusion in this study were: 1) baseline rFOV and standard diffusion, 2) rFOV and standard diffusion following initiation of chemotherapy, 3) tumor size $\geq 0.1$ cm$^3$ at all included MR timepoints, and 4) image quality sufficient for quantitative analysis.

**Imaging**

*Conventional MR imaging:* Fat-suppressed, T1-weighted dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) data was acquired with a three-dimensional fast gradient echo (3D FGRE) sequence using parameters described in Chapter 11.

*Diffusion-weighted MR imaging:* Standard diffusion was acquired post-contrast using a diffusion-weighted echo planar imaging (DW-EPI) sequence. Parameters were as previously described, although in one case the b values were equal to 0 and 800 instead of 0 and 600. Reduced field of view (rFOV) diffusion was acquired post-contrast using an rFOV diffusion-weighted echo planar imaging (rFOV DW-EPI) sequence, with $b=0$ and 600. Parameters were as previously described.

**Image Processing**

ADC maps for standard FOV diffusion were calculated off-line using previously described methods. Acquired image volume for rFOV diffusion were automatically
complex averaged and ADC maps were then constructed automatically from complex averaged images using previously published methods (Saritas, Cunningham et al. 2008).

Pre- and early post-contrast image volumes from dynamic contrast enhanced (DCE) MR data were reformatted twice: once to match rFOV diffusion resolution and slice prescription and once to match standard diffusion resolution and slice prescription. A subtraction image was then created from each set of pre- and early post-contrast images (early post-contrast – pre-contrast). Processing was done using in-house software programmed in IDL (Version 7.0, ITT Visual Information Solutions, Boulder, CO).

Signal enhancement ratio (SER) maps were constructed for all dynamic-contrast enhanced imaging exams by registering pre-contrast (S0), early post-contrast (S1), and late post-contrast (S2) DCE volumes and calculating (S1-S0)/(S2-S0) for each voxel (Partridge, Gibbs et al. 2002). SER maps were used to define MR tumor volume; change in MR tumor volume was the outcome variable used in this study.

**Image Analysis**

**ROI Delineation**: One or more regions of interest (ROIs) were placed on the rFOV diffusion slice estimated to contain the largest tumor surface area. Tumor surface area was estimated from enhancing areas on DCE subtraction images (early post-contrast – pre-contrast volumes) and generally hyperintense areas on DW-MRI and hypointense areas on ADC map. The ROIs were similarly placed on standard diffusion images. When the slice with the largest tumor surface area corresponded to the slice with the same patient coordinates in rFOV and standard diffusion image volumes, the rFOV
ROIs were used as a starting point for ROI placement on standard diffusion. The ROIs were manually shifted and eroded or redrawn as necessary on standard-diffusion.

**Quantitative analysis:** Tumor ROIs were automatically registered with ADC maps (IDL, ITT Visual Information Solutions, Boulder, CO). The ADC distribution of the voxels between 0 and $5000 \times 10^{-6}$ mm$^2$/s contained in the ROIs was quantitatively assessed in Matlab (Version R2007a, The Mathworks, Natick, MA). The mean, median, 12.5th, 25th, 75th, 87.5th quartile ADC, minimum ADC, maximum ADC, skew, kurtosis, and number of occupied histogram bins (bin width = $100 \times 10^{-6}$ mm$^2$/s) were calculated from the ADC distribution.

**Statistics**

Longitudinal changes in histogram parameters were calculated as percent changes and absolute changes. Differences in changes between rFOV and standard FOV diffusion were assessed with the paired Wilcoxon sign rank test.

**Case Studies**

Patient cases were retrospectively reviewed for relationship between early tumor ADC histograms and early percent change in MR tumor volume (final percent change in MR tumor volume was not available for all patients at the time of the analysis). Tumor response was defined as a percent change in MR volume by extending Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (Eisenhauer, Therasse et al. 2009) to volume changes (Prasad, Jhaveri et al. 2002). A response was defined as MR volume change > 65% (partial response, PR and complete response, CR categories) and
all other changes (stable disease, SD and progressive disease, PD) in MR volume were considered to be a non-response. Volumetric and unidimensional RECIST categories are summarized in Table 12-1. ADC distribution parameters were calculated from tumor regions in Matlab (Version R2007A, The Mathworks, Natick, MA) and changes in ADC distribution parameters (normalized peak height, peak height location, mean, median, minimum, maximum tumor ADC) were assessed. Changes were compared between DW-MRI acquisitions.

<table>
<thead>
<tr>
<th>RECIST category</th>
<th>Percent Change in Tumor Longest Diameter</th>
<th>Percent Change in Tumor Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Response (CR)</td>
<td>No disease remaining</td>
<td>No disease remaining</td>
</tr>
<tr>
<td>Partial Response (PR)</td>
<td>&gt;30% decrease</td>
<td>&gt;65% decrease</td>
</tr>
<tr>
<td>Stable Disease (SD)</td>
<td>Intermediate Changes</td>
<td>Intermediate changes</td>
</tr>
<tr>
<td>Progressive Disease (PD)</td>
<td>&gt;20% increase</td>
<td>&gt;44% increase</td>
</tr>
</tbody>
</table>

12.3. Results

Patient Accrual

Between December 2009 and July 2010, only three patients were scanned with both post-contrast rFOV DW-MRI and post-contrast standard DW-MRI at baseline and at MR2. Two patients were enrolled in Group 5 and MR2 therefore followed the third taxane cycle; one patient was enrolled in Group 4 and MR2 therefore followed the first
taxane cycle. All patients were diagnosed with pathology-confirmed invasive ductal carcinoma. Tumor characteristics are summarized in Table 12-2.

**Table 12-2. Tumor Characteristics and Response Classification by Volume**

<table>
<thead>
<tr>
<th>Patient</th>
<th>SBR Tumor Grade</th>
<th>MR tumor volume at MR1 (cm³)</th>
<th>MR tumor LD at MR1 (cm²)</th>
<th>MR tumor volume at MR2 (cm³)</th>
<th>MR tumor LD at MR2 (cm²)</th>
<th>Early % change in MR volume</th>
<th>Response category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>9.0</td>
<td>5.2</td>
<td>2.2</td>
<td>5.2</td>
<td>-75.6</td>
<td>Partial response</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>25.5</td>
<td>4.6</td>
<td>30.4</td>
<td>4.8</td>
<td>19.3</td>
<td>Stable Disease</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>17.3</td>
<td>3.1</td>
<td>16.3</td>
<td>3.5</td>
<td>-5.8</td>
<td>Stable Disease</td>
</tr>
</tbody>
</table>

* LD=longest diameter; SBR=Scarf-Bloom-Richardson

**Longitudinal Change in rFOV and Standard FOV ADC Parameters**

Due to the small number of patients accrued for this study, statistical analyses were underpowered. No p-values were less than 0.05.

When the early changes in ADC distribution parameters were compared between standard FOV and rFOV acquisitions, no statistically significant differences were seen; however, differences between acquisitions were seen in the absolute change in maximum tumor ADC. The median value was -184 *10^{-6} \text{ mm}^2/\text{s} in the rFOV acquisition (range -240- -35) and the median value was 295*10^{-6} \text{ mm}^2/\text{s} in the standard FOV acquisition (range 124-380) (p=0.25). Differences were also seen in the absolute change in 12.5^{th} percentile tumor ADC (median in rFOV= -15 *10^{-6} \text{ mm}^2/\text{s}, range -84.5-269.9; median in standard FOV= 94.75*10^{-6} \text{ mm}^2/\text{s}, range=-11.5-311.12; p=0.25).
While not statistically significant, differences between acquisitions were seen in three percent change metrics: early percent change in tumor ADC distribution kurtosis (median in rFOV= -4%, median in standard FOV= 26%; p=.25), percent change in maximum ADC (median in rFOV=-7.11, median in standard FOV=20%; p=.25), and early percent change in 12.5\textsuperscript{th} percentile ADC (median in rFOV=-1.7%, and median in standard FOV=11%; p=0.25).

**Case Studies**

In Case 1, the patient was a partial responder after 1 cycle of chemotherapy (MR2). In Case 2 and Case 3, the patients had stable disease following three cycles of chemotherapy (MR2).

In Case 1, analysis of normalized histograms for the tumor ADC distribution shows that volumetric tumor response is not clearly predicted by standard or rFOV DW-MRI (Figure 12-1).
Figure 12-1. Case 1: Partial Response. For both rFOV and standard FOV MRI, the minimum ADC increases with treatment, hallmark of a response. In standard FOV, the maximum ADC increases (21%), hallmark of a response, but the maximum ADC changes negligibly (-7%) in rFOV DW-MRI. In rFOV DW-MRI, the normalized peak height increases (28%), and the peak height location increases (21%), reflecting an increase in high ADC values. In standard FOV, the normalized peak height changes negligibly (3%), but the peak height location is increased (21%), suggesting a shift towards higher ADCs. Of note, the peak height location for standard and rFOV DW-MRI acquisitions is the same at both timepoints.
In Case 2, the patient’s volumetric tumor response is classified as stable disease by MR2. The tumor ADC distribution measured by rFOV DW-MRI better reflects the patient’s poor response than the distribution measured by standard DW-MRI (Figure 12-2).

![Figure 12-2. Case 2: Stable Disease. The minimum ADC in rFOV DW-MRI is decreased (-29%) with treatment, suggesting a poor response. The minimum ADC increases (18%) in standard FOV DW-MRI, suggesting a good response. The normalized peak height in rFOV DW-MRI decreases (-20%) in the mid-ADC range (location change 10%), also suggesting a poor response; conversely, the peak height](image_url)
minimally changes (2%) in standard FOV DW-MRI with a minimal change in location (-9%). The mean and median of both rFOV and standard DW-MRI-derived ADC distributions change negligibly (-2-3%).

The changes reflected in the tumor ADC distribution are not apparent from a qualitative assessment of diffusion-weighted images and ADC maps (Figure 12-3).

![Figure 12-3. Visual Assessment of Tumor Changes in Case 2. In Case 2, the signal attenuation does not appear to change from MR1 to MR2 in diffusion-weighted images following chemotherapy for either rFOV and standard DW-MRI; similarly, ADC maps look similar.](image)

In Case 3, the patient’s volumetric response was classified as stable disease at MR2. The tumor ADC distribution measured by rFOV DW-MRI appears to better predict the patient’s poor response than the distribution measured by standard FOV DW-MRI (Figure 12-4).
Figure 12-4. Case 3: Stable Disease. With rFOV DW-MRI, the peak height location for the tumor ADC distribution decreases (-24%), suggesting a poor response; conversely, this location increases (12%) for standard DW-MRI, suggesting a good response. The maximum ADC increases (20%) with standard, also suggesting a good response; however, maximum ADC decreases (-11%) with rFOV DW-MRI, consistent with the patient’s poor response.
12.4. Discussion

In this study, ADC measurements derived from rFOV and standard FOV DW-MRI data were compared in a group of patients with locally advanced breast cancer undergoing longitudinal monitoring with MRI during the course of treatment with neoadjuvant chemotherapy. The cases presented in this study suggest that early changes in the distribution of tumor ADC values may reflect response to chemotherapy, and that changes in measurements from the rFOV acquisition may better reflect tumor response than changes in measurements from a standard FOV acquisition. While statistical analyses in this case series were severely underpowered, retrospective analyses of individual cases illustrated differences between rFOV and standard DW-MRI monitoring of tumor response to treatment. In non-responders, changes in ADC distribution parameters measured with rFOV DW-MRI better correlated with patient response than changes in parameters measured with standard FOV DW-MRI. While continued analysis in patients is needed, these early cases studies suggest that rFOV DW-MRI measurements may correlate with tumor response.

In Case 1, some ADC distribution changes seemed to conflict in prediction of response. Statistical significance of the ability of particular parameters to predict response should be assessed in large patient cohorts. Decision algorithms are needed for cases of conflicting changes in ADC measurements, and conflicting changes in ADC and volume measurements. While the ability of rFOV DW-MRI parameters to better predict response as compared to standard DW-MRI could not be assessed in this study, rFOV DW-MRI has been previously shown to improve image quality and better depict breast tumor heterogeneity and tumor morphology.
In processing rFOV and standard FOV DW-MRI exams it was also noted that the rFOV seemed to better preserve tumor morphology as captured on DCE-MRI, facilitating ROI placement. Because ROIs were first drawn on rFOV images, some bias may have existed in analysis of standard FOV DW-MRI. The rFOV slice with the largest tumor surface area was selected prior to selection of the standard FOV slice and this may have resulted in a bias towards selection of the slice with the same coordinates on standard DW-MRI. At the same time, because rFOV and standard FOV DW-MRI imaged the same tumor at the same point in time, it follows that the same tumor region would be selected. rFOV DW-MRI acquisitions were centered over the largest area of the tumor, further facilitating this similarity.

Despite trends seen in individual cases, this study was limited by a small sample size. Case numbers were too small for sufficient statistical power. The small sample numbers occurred despite acquisition with both rFOV and standard FOV DW-MRI over a 7 month time period. This illustrates an additional limitation of the study: acquisition with two different diffusion sequences adds time to the MRI exam and diffusion is not currently standard of care in breast MRI. Patient tolerance and time constraints limit the ability to scan patients with additional MRI sequences.

Due to time and practical constraints, it may not be possible to compare the predictive ability of rFOV with standard FOV DW-MRI in the same patient group. However, based on the fact that rFOV DW-MRI provides improved image quality, it may still be justified to monitor diffusion changes with rFOV DW-MRI in research settings. Depending on the quality of the DW-MRI data acquired in larger studies with each sequence, replacement of standard FOV with rFOV could be considered. rFOV DW-MRI
does not provide coverage of the contralateral breast, but standard FOV DW-MRI has been associated with both inadequate fat suppression in the contralateral breast and severe distortion in the ipsilateral breast, highlighting the need for high quality diffusion imaging.

Unilateral rFOV DW-MRI may be a much needed compromise between an interest in scanning the contralateral breast with DW-MRI and a need for high quality imaging. Improved image quality may allow for better assessment, better understanding, and better utilization of the true potential of DW-MRI in predicting breast cancer treatment response.

12.5. Acknowledgments

This work was made possible through a collaboration with General Electric Healthcare and Standard University.

12.6. References


Appendix 1: Breast Cancer Staging

Breast cancer stage is determined by properties related to the tumor (T) and involved nodes (N), and presence of metastases (M). The staging criteria was updated in 2010 and the T, N, and M combinations corresponding to particular stages are listed in the table below.

AJCC Staging Criteria for Breast Cancer, 7th Edition

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>Tis</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage I</td>
<td>Stage IA</td>
<td>T1</td>
<td>N0</td>
</tr>
<tr>
<td></td>
<td>Stage IB</td>
<td>T0</td>
<td>N1mi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T1</td>
<td>N1mi</td>
</tr>
<tr>
<td>Stage II</td>
<td>Stage IIA</td>
<td>T0</td>
<td>N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T1</td>
<td>N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T2</td>
<td>N0</td>
</tr>
<tr>
<td></td>
<td>Stage IIB</td>
<td>T2</td>
<td>N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3</td>
<td>N0</td>
</tr>
<tr>
<td>Stage III</td>
<td>Stage IIIA</td>
<td>T0</td>
<td>N2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T1</td>
<td>N2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T2</td>
<td>N2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3</td>
<td>N1 or N2</td>
</tr>
<tr>
<td></td>
<td>Stage IIIB</td>
<td>T4</td>
<td>N0, N1, or N2</td>
</tr>
<tr>
<td></td>
<td>Stage IIIC</td>
<td>Any T</td>
<td>N3</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Stage IV</td>
<td>Any T</td>
<td>Any N</td>
</tr>
</tbody>
</table>

Note that T1 includes T1mi and T0 and T1 with only micrometastatic involvement of nodes are Stage IB. Based on p. 360, AJCC Criteria, American Joint Committee on Cancer, 2010

Each T, N, and M stage corresponds to particular clinical or pathological features.

The descriptions of the clinical criteria for each T, N, and M category are described on the next page (figures from Breast chapter of AJCC 7th edition).
**Primary Tumor (T)**
The T classification of the primary tumor is the same regardless of whether it is based on clinical or pathologic criteria, or both. Size should be measured to the nearest millimeter. If the tumor size is slightly less than or greater than a cutoff for a given T classification, it is recommended that the size be rounded to the millimeter reading that is closest to the cutoff. For example, a reported size of 1.1 mm is reported as 1 mm, or a size of 2.01 cm is reported as 2.0 cm. Designation should be made with the subscript “c” or “p” modifier to indicate whether the T classification was determined by clinical (physical examination or radiologic) or pathologic measurements, respectively. In general, pathologic determination should take precedence over clinical determination of T size.

<table>
<thead>
<tr>
<th>TX</th>
<th>Primary tumor cannot be assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>Tis (DCIS)</td>
<td>Ductal carcinoma in situ</td>
</tr>
<tr>
<td>Tis (LCIS)</td>
<td>Lobular carcinoma in situ</td>
</tr>
<tr>
<td>Tis (Paget’s)</td>
<td>Paget’s disease of the nipple NOT associated with invasive carcinoma and/or carcinoma in situ (DCIS and/or LCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget’s disease are categorized based on the size and characteristics of the parenchymal disease, although the presence of Paget’s disease should still be noted.</td>
</tr>
</tbody>
</table>

**Regional Lymph Nodes (N)**

**Clinical**
- NX: Regional lymph nodes cannot be assessed (e.g., previously removed)
- N0: No regional lymph node metastases
- N1: Metastases to movable ipsilateral level I, II axillary lymph node(s)
- N2: Metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in clinically detected ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases
- N2a: Metastases in ipsilateral level I, II axillary lymph nodes fixed to one another (matted) or to other structures
- N2b: Metastases only in clinically detected ipsilateral internal mammary nodes and in the absence of clinically evident level I, II axillary lymph node metastases
- N3: Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s) with or without level I, II axillary lymph node involvement; or in clinically detected ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph node metastases; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
- N3a: Metastases in ipsilateral infraclavicular lymph node(s)
- N3b: Metastases in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
- N3c: Metastases in ipsilateral supraclavicular lymph node(s)

**Distant Metastases (M)**
- M0: No clinical or radiographic evidence of distant metastases
- cM0(+) : No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are not larger than 0.2 mm in a patient without symptoms or signs of metastases
- M1: Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven larger than 0.2 mm

**Note:** Invasion of the dermis alone does not qualify as T4

- T4a: Extension to the chest wall, not including only pectoralis muscle adherence/invagination
- T4b: Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d’orange) of the skin, which do not meet the criteria for inflammatory carcinoma
- T4c: Both T4a and T4b
- T4d: Inflammatory carcinoma (see “Rules for Classification”)

# Appendix 2: Patients with Locally Advanced Breast Cancer Treated with Neoadjuvant Chemotherapy and Scanned with MRI in Clinical Studies at UCSF

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Study Name</th>
<th>Number of patients</th>
<th>Number of sites</th>
<th>Dates</th>
<th>Inclusion criteria</th>
<th>Chemotherapy Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Pilot Neoadjuvant</td>
<td>N= 68-72</td>
<td>1</td>
<td>1998 - 2002</td>
<td>Locally advanced breast cancer treated with neoadjuvant chemotherapy</td>
<td>4 cycles AC 4 cycles AC, followed by a taxane</td>
</tr>
<tr>
<td>Group 2</td>
<td>ACRIN 6657/ I-SPY TRIAL</td>
<td>N= 237</td>
<td>9</td>
<td>May 2002 - March 2006</td>
<td>Breast cancer ≥ 3 cm treated with neoadjuvant chemotherapy</td>
<td>AC–only regimen or 4 cycles AC followed by 4 cycles of a taxane</td>
</tr>
<tr>
<td>Group 3</td>
<td>ACRIN 6657 extension/ I-SPY TRIAL</td>
<td>N→ 140</td>
<td>8</td>
<td>Fall 2007 - current</td>
<td>Breast cancer ≥ 3 cm, Stage III, treated with neoadjuvant chemotherapy</td>
<td>Taxane-only regimen or 12 cycles of a taxane followed by 4 cycles AC</td>
</tr>
<tr>
<td>Group 4</td>
<td>Hylton Neoadjuvant Study</td>
<td>N→ 75</td>
<td>1</td>
<td>October 2008 - current</td>
<td>Breast cancer treated with neoadjuvant chemotherapy</td>
<td>Variable</td>
</tr>
<tr>
<td>Group 5</td>
<td>ACRIN 6693/ I-SPY TRIAL 2</td>
<td>N→ 800</td>
<td>17</td>
<td>March 2010 - current</td>
<td>Breast cancer ≥ 2.5 cm, treated with neoadjuvant chemotherapy, meeting biomarker requirements* and meeting at least one of the following: Stage II/III, T4/M0, regional stage IV with supraclavicular nodes as the only metastatic sites</td>
<td>12 cycles of a taxane + study drug (Figitumumab, Neratinib, or ABT-888), with trastuzumab if HER-2+, followed by 4 cycles AC</td>
</tr>
</tbody>
</table>

AC=adriamycin(doxorubicin)+cyclophosphamide
*Biomarker requirement for Group 5: Cancer must be considered high risk according to MammoPrint or low risk according to MammoPrint and either ER- or ER+/HER2+
Appendix 3: MR Parameters as Predictors for 3-year RFS

**Background:** In a pilot study (Chapter 3), parameters derived from MRI data were tested for their ability to predict recurrence-free survival (RFS). Receiver operator characteristic (ROC) can be used to assess the ability of a parameter to predict an outcome such as RFS.

**Methods:** ROC analysis was performed in STATA (IC 11.1, StataCorp LP, College Station Texas) for the MR size and diffusion variables that showed the strongest trends towards prediction of the binary outcome 3-year recurrence-free survival. Early percent change in normalized mean tumor ADC, baseline volume, and early percent change in longest diameter were selected for this analysis. The binary outcome variable was 3-year recurrence-free survival (or 3 year recurrence). The AUC for the three ROC curves was compared.

**Results:**

Early percent change had the highest AUC:

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>ROC AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Percent Change nADC</td>
<td>15</td>
<td>.8036</td>
</tr>
<tr>
<td>Baseline Volume</td>
<td>30</td>
<td>.7143</td>
</tr>
<tr>
<td>Early Percent Change LD</td>
<td>21</td>
<td>.7692</td>
</tr>
</tbody>
</table>

However, when the AUC was compared for the 15 patients with all 3 variables, LD performed slightly better (AUC=.8393, versus .75 for volume and .8036 for nADC), and the AUCs were not significantly different (p=0.8275).
ROC curves for the prediction of 3 year recurrence-free survival (or recurrence, in the case of nADC):

![ROC curve for Early Percent Change in nADC](image1)

*Area under ROC curve = 0.8036*

![ROC curve for Baseline Volume](image2)

*Area under ROC curve = 0.7143*
Conclusion: MR volume, MR LD, and MR nADC all predicted RFS better than chance, but prediction for the three variables was similar.
### Appendix 8.1. Histogram Analysis, Predictors of Recurrence-free Survival

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<th>N non-recur</th>
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### Appendix 8.2. Histogram Analysis, Univariate Cox Regression

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