Dynamic Contrast-Enhanced MRI and Diffusion-Weighted MRI for the Diagnosis of Bladder Cancer

DISSERTATION

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By

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Abstract

It is estimated by the American Cancer Society that there will be about 72,570 newly diagnosed cases of bladder cancer (about 54,610 in men and 17,960 in women) and about 15,210 (about 10,820 in men and 4,390 in women) deaths from bladder cancer in 2013. Early detection and accurate staging of bladder cancer are crucial to stratify the treatment plan and ensure the best patient prognosis. In the treatment of bladder cancer patients with chemotherapy, it is important to predict a patient as a chemotherapeutic responder or non-responder to both maximize the patient benefit from chemotherapy and avoid unnecessary delay of cystectomy. However, these clinical needs in bladder cancer management are still unmet in clinical tests, cystoscopy, and bladder imaging. This study is aimed at evaluating the abilities of 3T dynamic contrast-enhanced MRI (DCE-MRI) and diffusion-weighted MRI (DWI) to improve the detection and staging and to enable the prediction of chemotherapeutic response in bladder cancer.

A total of fifty-three patients were enrolled in the study. Different inclusion criteria were established for three different types of data assessment: (1) Improving
bladder cancer detection with DCE-MRI; (2) early prediction of chemotherapeutic response in bladder cancer with DCE-MRI; and (3) quantitative assessment of T staging of bladder cancer with DWI.

Thirty-six patients were included in the analysis of DCE-MRI for bladder cancer detection. The results demonstrated that the maps of DCE-MRI pharmacokinetic parameters can better visualize small malignant tumors and the tumors within bladder wall thickenings to improve the detection of bladder cancer compared to conventional T2-weighted MRI alone. Twenty-five patients were included in the analysis of DCE-MRI for early prediction of chemotherapeutic response in bladder cancer. Using k-means clustering of DCE-MRI pharmacokinetic parameters, each bladder tumor was divided into three clusters of different microcirculation characteristics, thus, different chemotherapeutic responses. These differences enabled to classify a bladder cancer patient as a responder or a non-responder at the mid-cycle time point of therapy. Eighteen patients were included in the DWI data for T staging of bladder cancer. The ADC referenced to the individual bladder urine was found to be associated with the tumor stage. The group of stage T1 or lower (Ta, Tis, T1) had a significantly lower relative ADC than the group of stage T2 (P<0.03), stage T3 (P<0.05), and stage T4 (P<0.03). The relative ADC of the group of stage T2 was also found to be significantly lower than that of the group of stage T3 (P<0.03) and stage T4 (P<0.05).

In conclusion, 3T MRI with functional DCE-MRI and DWI can substantially improve the diagnosis of bladder cancer by enabling better visualization of
malignant bladder tumors and the quantitative assessment to classify tumor stage and chemotherapeutic response.
Dedication

Dedicated to my most beloved ones: my son, my husband, and my parents.
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My biggest gratitude is to my parents whose unconditional love, full trust, and constant caring have nurtured and heartened me ever since I was born.

To my husband, I am thankful for his love, understanding, and endless support. His calmness, advice and reliability have always led me through all hardships I have had in the past 8 years. To my son, I am grateful for being in my life. He has been my strongest happiness, motivation, and strength that continuously push me forward to the completion of my Ph.D. study.

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Fields of study

Major Field: Biophysics
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1 Introduction

1.1 Bladder Cancer Overview

1.1.1 Epidemiology and Pathophysiological Features

Bladder cancer is the fourth most common cancer in men and the tenth most common cancer in women in the United States and is three to four times more common in men than in women (1). It is estimated by American Cancer Society that there will be about 72,570 newly diagnosed cases of bladder cancer (about 54,610 in men and 17,960 in women) and about 15,210 (about 10,820 in men and 4,390 in women) deaths from bladder cancer in 2013(2). Histological types of bladder cancer include:

Transitional (urothelial) cell carcinoma: This is the most common type of bladder cancer and accounts for 90% of bladder cancers. 25% of urothelial cancers exist with a histological mixture of small cell neuroendocrine, micro-papillary, sarmatoid, and pasmacytoid components (1, 3). Etiologic factors for urothelial bladder cancers are divided in three categories: genetic and molecular abnormalities, chemical or environmental exposures such as cigarette smoking, and chronic irritation (3).
**Squamous cell carcinoma:** This accounts for 5% of bladder cancers (3).

**Adenocarcinoma:** Less than 2% of bladder cancer is in this type. Nearly all cases of squamous carcinoma and adenocarcinoma are muscle-invasive at the time of diagnosis. So, the prognosis of the two types of bladder cancer is worse than that of urothelial bladder cancer (3).

**Small cell carcinoma:** A small number of bladder cancer cases are in this type.

### 1.1.2 Diagnostic Classification Systems

Two different classification systems are used in bladder cancer diagnosis: grading system and staging system.

**Grading system:**

Tumor grading is based on the level of cell differentiation and appearance in bladder tumors. In low grade bladder tumors, cells are still well differentiated and tend to grow slowly. In high grade cancers, cells are poorly differentiated, growing quickly, and more likely to spread. The World Health Organization and International Society of Urological Pathology agreed a new grading system for bladder cancer in 1998. According to this system, urothelial bladder tumors are classified into four grades (3):

- Papilloma: noncancerous (benign) tumor.
- Papillary urothelial neoplasm of low malignant potential: tumor with very slow growth and unlikely to spread.
Figure 1.1: TNM classification system for bladder cancer staging

Low grade carcinoma: tumor with slow growth and unlikely to spread.

High grade carcinoma: tumor with quick growth and likely to spread.

**TNM staging system:**

The tumor (T) staging of the primary bladder tumor indicates how far the primary bladder cancer invades into the bladder wall (Figure 1.1 and Table 1.1).

Node (N) staging indicates how the cancer spreads to regional lymph nodes near the bladder (4) (Table 1.2).

Metastasis (M) staging indicates whether or not the cancer has metastasized (spread to distant and distinguished sites) (Table 1.3).
Table 1.1: T staging in bladder cancer

<table>
<thead>
<tr>
<th>T stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>The primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumor is found</td>
</tr>
</tbody>
</table>
| Ta/Tis  | Ta: non-invasive papillary carcinoma  
Tis: non-invasive flat carcinoma (carcinoma in situ-CIS) |
| T1      | The primary tumor invades the lamia propria (mucosa), but not into the muscularis propria |
| T2      | The primary tumor invades the muscularis propria (sub-mucosa), but not into the fat tissues surrounding the bladder wall  
  T2a: the invasion is only in the half (inner) layer of muscularis propria  
  T2b: the invasion is more than half of muscularis propria |
| T3      | The primary tumor invades the surrounding fat tissues  
  T3a: the invasion is only microscopic  
  T3b: the invasion is macroscopic (extravesical mass). |
| T4      | The primary tumor invades one of the nearby organs: prostate, uterus, vagina, pelvic wall, and abdominal walls.  
  T4a: the primary tumor invades prostate, uterus, or vagina  
  T4b: the primary tumor invades pelvic or abdominal wall |
Table 1.2: N staging in bladder cancer

<table>
<thead>
<tr>
<th>N stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>The cancer has not spread to any regional lymph nodes</td>
</tr>
<tr>
<td>N1</td>
<td>The cancer spreads to a single lymph node, 2 cm or less in the greatest dimension</td>
</tr>
<tr>
<td>N2</td>
<td>The cancer spreads a single lymph node more than 2 cm but not more than 5 cm in the greatest dimension, or multiple lymph nodes, none more than 5 cm in the greatest dimension</td>
</tr>
<tr>
<td>N3</td>
<td>The cancer spread to a lymph node more than 5 cm in the greatest dimension</td>
</tr>
</tbody>
</table>

Table 1.3: M staging in bladder cancer

<table>
<thead>
<tr>
<th>M stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MX</td>
<td>Distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

1.1.3 Patient Management and Treatment

Four types of standard treatment for bladder cancer have been established (5):

Surgery

Transurethral resection of bladder tumor (TURBT): A surgery to remove or burn (with high electricity) a bladder tumor with assistance of a cystoscope and a slender tube put through the urethra.
Radical cystectomy (RC): A surgery to remove the bladder, nearby lymph nodes and organs invaded by the cancer. The surgery can be cystoprostatectomy for men or hysterectomy for women, followed by urinary diversion procedure.

Partial (segmental) cystectomy: A surgery is done to remove part of the bladder.

**Chemotherapy**

Systemic chemotherapy: The drug used to kill the cancer cells is taken by mouth or injected into a vein or muscle to enter the bloodstream.

Regional chemotherapy: The drug is placed into the cerebrospinal fluid, an organ, or a body cavity to mainly kill cancer cells in those areas.

Intravesical chemotherapy: The drug in chemotherapy is inserted right into bladder through the urethra.

**Radiation therapy**

External radiation therapy: A high-energy radiation such as x-rays to kill cancer was sent towards the cancer by a machine outside the body.

Internal radiation therapy: The radiation is from a radioactive substance sealed in needles, seeds, wires, or catheters that are placed near or into the cancer.

**Biologic therapy (immunotherapy)**

The treatment exploits the immune system in the patient’s body to kill the cancer by using substances made by the body or made in a laboratory to boost, direct, or restore the natural defenses against cancer.
In addition to those standard treatments, chemoprevention and photodynamic therapy are being studied in clinical trials for the clinical use in the future.

Histological features of bladder cancer that are based on to stratify a treatment plan include the tumor stage, grade, size, and configuration. But, first and foremost, selection of treatment options depends on the stage of bladder cancer:

**Non-muscle-invasive bladder cancer**

Non-muscle-invasive bladder cancer (NMIBC) includes bladder tumors of stage Ta, Tis, and T1. The standard treatments for NMIBC are TURBT and adjuvant intravesical chemotherapy or immunotherapy. Low grade Ta bladder tumors are treated with TURBT alone. High grade non-muscle-invasive tumors at the high risk of recurrence and progression to invasive tumors are considered to have repeated TUR and adjuvant intravesical chemotherapy. Radical cystectomy might be considered for recurrent high-grade T1 tumors (6).

**Muscle-invasive bladder cancer**

Muscle-invasive bladder cancer (MIBC) is of stage T2 or higher. The standard treatment for MIBC is radical cystectomy with bilateral lymph node dissection and with the consideration of neoadjuvant and adjuvant chemotherapy.

The rationale for chemotherapy is to treat micro-metastasis and to help reduce the tumor burden such as downstaging and shrinking the bladder tumor for better outcome of cystectomy (7). However, only about half of the bladder cancers respond to chemotherapy. For resistant tumors, the treatment can cause an
unnecessary delay of the surgery and result in the surgery complication such as tumor progression during chemotherapy (7).

The adjuvant chemotherapy can take advantage of pathological diagnosis to select the patients at the highest risk of disease for the treatment. The clinical trials showed that adjuvant chemotherapy significantly improved the survival for patients with the highest risk of disease such as high stage and nodal involvement (7).

**Metastatic bladder cancer**

The standard treatment for metastatic bladder cancer is systemic chemotherapy.

### 1.1.4 Unmet clinical needs in bladder cancer diagnosis

Patient management of bladder cancer depends on the accuracy of the cancer diagnosis and the early prediction of chemotherapeutic response. However, this is still an unmet clinical need in both clinical tests and bladder imaging. The current standard for bladder cancer diagnosis is cystoscopic imaging. Due to its invasiveness, cystoscopy cannot be performed on some patients due to intense discomfort, bleeding, or local implications such as infections or mechanical obstructions (8). Furthermore, cystoscopic imaging is limited in providing information on the perivesical invasion of bladder malignancies and identifying flat tumors or tumors behind the bladder wall (8). Current imaging modalities such as CT and ultrasound have been brought forward to provide additional tools to improve bladder cancer diagnosis (9-15). However, CT has not overcome its
limitations such as understaging of advanced bladder tumors and its inability to detect microscopic bladder lesions or the lesions at the bladder base adjacent to the prostate (1, 12, 14, 15). In addition to CT, PET/CT also has been evaluated to improve those limitations: catheter-assisted 18F-FDG-PET/CT imaging using standardized bladder flushing and filling presented with a sensitivity 63% (95% CI: 0.36-0.84) (9); C-acetate PET/CT revealed a sensitivity of 80% (8 out of 10 malignant tumors) (13). While multidetector CT with multiplanar reformatted imaging and virtual cystoscopy had a high sensitivity (94%) in comparison with conventional cystoscopy, it was shown in the study that conventional cystoscopy had discrepancies with histopathology (8). Ultrasound, as an alternative methodology for bladder imaging, was limited in detecting bladder tumors on the bladder floor and fundus (10, 11). Ultrasound was reported to have a sensitivity of 66% with micro-bubble contrast enhancement vs. 61% without contrast (11).

The assessment of chemotherapeutic response is essential to avoid unnecessary delay of definitive cystectomy and surgical complications arising during chemotherapy. Nonetheless, there are currently no effective method to meet this critical need in both clinical tests and bladder imaging such as Computed Tomography (CT) and Ultrasound (US). Extensive research on gene expression of bladder cancer cells is being performed to seek a biomarker that can effectively classify chemo-resistant and chemo-sensitive bladder tumors (7, 16). However, these studies still need more validation of their findings before a biomarker can be established in clinical diagnosis.
1.2 Advances in Bladder Cancer MR Imaging

Magnetic Resonance Imaging can bring the advantages of no ionizing radiation, high spatial resolution, high soft tissue contrast, multi-planar imaging, and functional imaging such as diffusion-weighted MRI (DWI) and dynamic-contrast enhanced MRI (DCE-MRI) altogether to allow more accurate staging of bladder cancer than other imaging modalities (1, 17-26).

1.2.1 Detection and Staging of Bladder Cancer

Using pathological findings from cystoscopic biopsy as a reference standard, bladder cancer detection with MRI has been improved and had high sensitivity, specificity, and overall accuracy (17, 24, 27-29) (Table 1.4). T2-Weighted (T2W) MRI and additional DWI have been reported to have overall accuracy of 94% and 97%, respectively (17, 27). The reference standard used in the two studies is cystoscopy (27) or both cystoscopy and pathological findings (17). However, it was shown that cystoscopy is not able to differentiate malignant from benign tumors (8). Therefore, a study with pathological examination as the reference standard would be more reliable.

With the addition of DWI and DCE-MRI, the accuracy of T staging has been significantly improved compared to conventional MRI as well as other imaging modalities including CT and US (21, 23, 24, 26, 30-34) (Table 1.5). The highest overall accuracy of 92% was achieved with the combination of conventional T2W MRI, DWI, and DCE-MRI (32). However, the study mostly used pathological
findings of biopsy as the reference standard. The most recent studies that fully used pathological findings of surgical specimens as a reference standard had a high sensitivity of 86%, but low specificity of 48%, and accuracy of 74% (35), or had low overall accuracy (36, 37).

Several studies have used MRI to identify positive lymph nodes (N staging) and metastasis (M staging) and showed that MRI had good accuracy and high specificity (26, 35, 38). Nonetheless, further study needs to be done to establish a reference standard, a standardized protocol, and image interpretation (26, 35, 38) (Table 1.6).

DWI and DCE-MRI of bladder are also potentially useful in providing a quantitative assessment in the diagnosis (20, 24, 27-29, 32, 33, 39) (Table 1.7). Apparent diffusion coefficient (ADC) from DWI has shown its potential in differentiating malignant from benign tumors (20, 27-29), assessing the tumor aggressiveness characterized by tumor grade and tumor stage (24, 28, 32, 33). However, overstaging of bladder cancer is still a prevalent drawback of current MR imaging of bladder cancer, and current MRI studies have not resolved the challenge in differentiating malignant tumors from inflammatory or reactive changes of bladder walls (19, 21-23, 26, 32).

### 1.2.2 Assessment of therapeutic response

A small number of MRI studies have assessed bladder cancer’s response to different types of therapy at different times of such treatment (25, 40-43) (Table
Dobson used conventional MRI and DCE-MRI to detect residual tumors after 4 months and 12 months of the radiotherapy and showed that DCE-MRI had a high negative predictive value (100% at 4 months and 93% at 4 months) and improved the detection with conventional MRI only, and that the accuracy of residual tumor detection was better at 4 months than at 12 months (40). This showed that longer time of radiotherapy could have effect on the accuracy of tumor detection. Nishimura also concluded that inflammatory changes in neoadjuvant chemotherapy caused the difficulty in accurately staging bladder cancer (41). DWI was shown to have higher specificity and accuracy than conventional MRI and DCE-MRI in assessing the residual bladder malignant tumors after chemoradiotherapy.

A study by Schrier et al (42) of the feasibility of MRI in early prediction of chemotherapeutic response in patients with regionally metastatic or unresectable transitional cell carcinoma (TCC) bladder cancer found that fast DCE-MRI had a high sensitivity, specificity, and accuracy in distinguishing responders from non-responders after 2 cycles of chemotherapy. This indication of the capability of DCE-MRI in early prediction of bladder cancer’s chemotherapeutic response suggests that further study needs to be done to validate the robustness of DCE-MRI in other cohorts of bladder cancer patients.
<table>
<thead>
<tr>
<th>Study (Number) (Ref.)</th>
<th>Techniques</th>
<th>Ref. Standard</th>
<th>Tumor size (mm)</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsuki et al (15) (29)</td>
<td>DWI</td>
<td>Cystoscopic biopsy</td>
<td>30 (6-58)*</td>
<td>100% (17)**</td>
</tr>
<tr>
<td>Abou-El-Ghar et al (130) (17)</td>
<td>T2W DWI</td>
<td>Cystoscopy and pathological finding from RC</td>
<td>29 (3-85)*</td>
<td>96% 98% 86% 92% 94% 97%</td>
</tr>
<tr>
<td>Kobayashi et al (104) (24)</td>
<td>T2w DWI</td>
<td>Pathological findings from TUR</td>
<td>18 (2-63)*</td>
<td>92%/91%*** 91%/89%***</td>
</tr>
<tr>
<td>Avcu et al (83) (27)</td>
<td>DWI</td>
<td>Cystoscopy</td>
<td>100%</td>
<td>77% 94%</td>
</tr>
<tr>
<td>Daggulli et al (45) (28)</td>
<td>DWI</td>
<td>Pathological findings from TUR</td>
<td>37 (10-75)*</td>
<td>94% 85% 89%</td>
</tr>
</tbody>
</table>

* Mean (range). ** The number in the parenthesis is the number of tumors. *** Sensitivity of T2W-MRI/sensitivity of DWI.
## Table 1.5: T staging of Bladder Cancer with MRI

<table>
<thead>
<tr>
<th>Study (Number) (Ref.)</th>
<th>Techniques</th>
<th>Tumor size (mm)</th>
<th>Ref. Standard</th>
<th>Stage ≤ T1 vs. ≥ T2</th>
<th>Stage ≤ T2 vs. ≥ T3</th>
<th>Stage-by-stage Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al (36) (30)</td>
<td>T1W T2W DCE-MRI CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hayashi et al (71) (23)</td>
<td>CE-MRI TUUS</td>
<td></td>
<td></td>
<td>91%/78% 87%/84%/67%/66%</td>
<td></td>
<td>83%/60%</td>
</tr>
<tr>
<td>Tekes et al (71) (26)</td>
<td>T1W+T2W +DCE-MRI</td>
<td>25 (5-73) Pathological staging from biopsy/RC</td>
<td>97%/95% 67%/55%/83%/76%/86%/79% 84%/79%/85%/79% 62%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>El-Assmy et al (106) (21)</td>
<td>T2W DWI</td>
<td>29 (3-85) Pathological findings from biopsy</td>
<td>6%/64%</td>
<td>15%/70% 39.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takeuchi et al (40) (32)</td>
<td>T2W DWI DCE-MRI</td>
<td>10 (1-85) Pathological staging from TUR/RC</td>
<td>50%/70%/95%/97%/85%/92%/67%/88%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuncbilek et al (24) (33)</td>
<td>DCE-MRI</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Watanabe et al (19) (34)</td>
<td>T1W+T2W T1W+T2W+C E-MRI T1W+T2W+DWI</td>
<td>Cystoscopic and patho. findings</td>
<td>80%/80%/79%/79%/79%/79%/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rajesh et al (100) (31)</td>
<td>T2w+CE-MRI</td>
<td>78% 93% 85%</td>
<td>91% 60% 89%</td>
<td>63%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kobayashi et al (104) (24)</td>
<td>T2w DWI</td>
<td>18 (2-63) Pathological findings from TURBT</td>
<td>68%/76% 91%/80%/83%/79%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daneshmand et al (122) (35)</td>
<td>DCE-MRI</td>
<td>86% 48% 74%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liedberg et al (53) (36)</td>
<td>Conventional MRI CE-MRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>38%</td>
</tr>
<tr>
<td>Vargas et al (16) (37)</td>
<td>Conventional MRI CE-MRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56%</td>
</tr>
</tbody>
</table>

*Mean (range).

* Mean (range).

1 T1W/T2W/DCE-MRI/CT. 2 CE-MRI/TUUS. 3 Reviewer 1/reviewer 2. 4 T2W/DWI. 5 Overall accuracy of T2W. 6 Accuracy of DWI for (stage T1)/(stage T2)/(stage T3)/(stage T4). 7 T2W/(T2W+DCE)/(T2W+DCE)(T2W+DCE+DCE).

1 T1W+T2W/(T1W+T2W+CE-MRI)/(T1W+T2W+DWI). 2 Reviewer 1/reviewer 2 of T2W. 3 Reviewer 1/reviewer 2 of DWI.
<table>
<thead>
<tr>
<th>Study (Number) (Ref.)</th>
<th>Techniques</th>
<th>Ref. Standard</th>
<th>N staging</th>
<th>M staging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tekes et al (71) (26)</td>
<td>T1W+T2W +DCE-MRI</td>
<td>Pathology</td>
<td>96% (10) 78%</td>
<td>98%</td>
</tr>
<tr>
<td>Rosenkrantz et al (17) (38)</td>
<td>DWI CE-CT and MR imaging</td>
<td></td>
<td></td>
<td>ADC of metastasis vs. non-metastasis: significant M1/2: 1.07 ± 0.18 M0: 1.45 ± 0.22 (×10^-3 mm²/sec)</td>
</tr>
<tr>
<td>Daneshmand et al (122) (35)</td>
<td>DCE-MRI</td>
<td>Pathological findings of RC</td>
<td>41% 92%</td>
<td>80%</td>
</tr>
</tbody>
</table>

* The number in the parenthesis is the number of lymph nodes.
<table>
<thead>
<tr>
<th>Study (Number) (Ref.)</th>
<th>Ref. Standard</th>
<th>Tumor size (mm)</th>
<th>Parameter</th>
<th>Malignant vs. Benign</th>
<th>Histologic Types/Grades</th>
<th>TNM Stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsuki et al (15) (29)</td>
<td>Cystoscopy</td>
<td>30 (6-58)*</td>
<td>ADC</td>
<td>Carcinoma (17)**:</td>
<td>1.18±0.21</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Urine: 3.28±0.20</td>
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<td></td>
<td>NBW: 2.27±0.24</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PZ prostate: 1.85±0.22</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>TZ prostate: 1.57±0.09</td>
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<td></td>
<td></td>
<td>SV: 2.01±0.22</td>
<td>(×10^{-3} mm²/sec)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All significant</td>
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</tr>
<tr>
<td>El-Assmy et al (43) (20)</td>
<td>Cystoscopy</td>
<td></td>
<td>ADC</td>
<td>Carcinoma (43)*:</td>
<td>0.57–2.39 (1.40±0.51)*</td>
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<td></td>
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<td></td>
<td>Urine: 2.81–4.10 (3.50±0.43)*</td>
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<td></td>
<td>NBW: 0.91–3.89 (2.29±0.78)*</td>
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<td></td>
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<td></td>
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<td>PZ prostate: 0.84–2.7 (1.77±0.44)*</td>
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<td></td>
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<td></td>
<td>TZ prostate: 0.81–2.90 (1.88±0.54)*</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SV: 1.24–3.02 (2.12±0.43)*</td>
<td>(×10^{-3} mm²/sec)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All significant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takeuchi et al (40) (32)</td>
<td>Pathological 10 (1-85)*</td>
<td>Pathological staging from TUR/RC</td>
<td>ADC</td>
<td>Carcinoma (41)*:</td>
<td>1.07±0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>G1: 0.91–1.56 (1.29±0.21)*</td>
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<td></td>
<td>G2: 0.74–1.61 (1.13±0.24)*</td>
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<td></td>
<td></td>
<td>G3: 0.56–0.99 (0.81±0.11)*</td>
<td>(×10^{-3} mm²/sec)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significant:</td>
<td></td>
<td></td>
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<tr>
<td>Kobayashi et al (104) (24)</td>
<td>Pathological findings from TUR</td>
<td>ADC</td>
<td>Tumors (121)*:</td>
<td>0.39-2.07 (0.86)</td>
<td>High (50)<em>: 0.79 (0.69-0.88)</em></td>
<td></td>
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<tr>
<td>Avcu et al (83) (27)</td>
<td>Cystoscopy</td>
<td></td>
<td>ADC</td>
<td>Malignant (46)*:</td>
<td>1.06±0.26</td>
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<tr>
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<td></td>
<td></td>
<td>Low (17)*: 1.28±0.18</td>
<td>(×10^{-3} mm²/sec)</td>
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<td></td>
<td></td>
<td>Control (20)*:</td>
<td>2.01±0.11</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cutoff: 1.135 (×10^{-3} mm²/sec)</td>
<td>sensitivity: 78.9%; specificity: 85.2%</td>
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<tr>
<td>Daggulli et al (45) (28)</td>
<td>Pathological findings from TUR</td>
<td>ADC</td>
<td>Malignant vs. BWT:</td>
<td>not significant</td>
<td>TCC vs. SCC: significant</td>
<td>Superficial vs Invasive:</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Malignant vs. NBW:</td>
<td>significant</td>
<td></td>
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<tr>
<td>Tuncbilek et al (24) (33)</td>
<td>Pathological findings from TUR and Cystectomy</td>
<td>DCE-MRI parameter:</td>
<td>E_{max1}, E_{max2}</td>
<td>Positive Correlation between:</td>
<td>Positive Correlation between:</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>E_{max1} and micro- vessel density</td>
<td></td>
</tr>
<tr>
<td>Naish et al (12) (17/38)</td>
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<td></td>
<td>K^{trans}</td>
<td>Agreement between</td>
<td></td>
<td>DCE-MRI and DCE-CT</td>
</tr>
<tr>
<td>Rosenkrantz et al (17) (38)</td>
<td>ADC</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Mean (range). ** The number in the parenthesis is the number of tumors. * Range (mean ± standard deviation).

b Mean (Interquartile range)
<table>
<thead>
<tr>
<th>Study (Number) (Ref.)</th>
<th>Techniques</th>
<th>Ref. Standard Type of Therapy</th>
<th>Time of Imaging</th>
<th>Quantitative Assessment</th>
<th>Assessment of tumor presence or early prediction of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dobson et al (40)</td>
<td>Conventional DCE-MRI at 0.5T</td>
<td>Cystoscopy and Biopsy</td>
<td>Radiotherapy</td>
<td>Before 4 months</td>
<td>75%/100%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12 months (after)</td>
<td>63%/48%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>63%/76%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Schrier et al (36)</td>
<td>Conventional DCE-MRI</td>
<td>Pathological findings from TUR (n=20) or RC (n=16) after chemotherapy</td>
<td>Chemotherapy</td>
<td>Before 2 cycles</td>
<td>81%/91%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
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<td>4 cycles</td>
<td>50%/93%&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>6 cycles</td>
<td>69%/92%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Roe et al (26)</td>
<td>DCE-MRI</td>
<td>Biological image-adapted radiotherapy</td>
<td></td>
<td>Before</td>
<td>Extracted DCE-MRI parameters:</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>- independent of tumor volume</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>- correlated with stage</td>
</tr>
<tr>
<td>Nishimura et al (27)</td>
<td>MRI (sequence not specified)</td>
<td>Pathological findings from RC</td>
<td>Neoadjuvant Chemo</td>
<td>After</td>
<td>60%/75%/30%/78%&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yoshida et al (20)</td>
<td>T1W+T2W DCE DWI</td>
<td>Pathological findings of RC</td>
<td>Chemoradiotherapy</td>
<td>After</td>
<td>43%/57%/57%/45%/18%/90%&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48%/33%/80%&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Residual tumor at 4 months. <sup>b</sup> Residual tumor at 12 months. <sup>c</sup> Early prediction of responder or non-responder after 2 cycles of treatment by conventional MRI/DCE-MRI. <sup>d</sup> Staging tumor after therapy for Group A (8 patients) who had both chemotherapy and staging biopsy, group B (10 patients) who had chemo-radiation therapy and staging biopsy, and group C (9 patients) who had only staging biopsy: (all patients)/(group A)/group B/ group C.
2 Materials and Methods

2.1 Subjects

This study was approved by the local Institutional Review Board. With the goal to use the pathological findings of cystectomy bladder specimen as a reference standard, enrollment criteria include: (1) patient is 18 years or older; (2) patient is with known bladder cancer; (3) patient is scheduled for radical cystectomy; (4) patient is able and willing to give valid written informed consent; (5) patient has no contraindications to MRI. From July 2009 to August 2012, fifty patients (44 males, 9 females; age range: 38-86 years, median: 68 years; weight range: 52-159 kg, median: 89 kg) have been enrolled in this ongoing study.

Out of the fifty patients, eighteen patients had only one baseline (pre-chemotheraphy) MRI exam due to their tumor progression or specific co-morbidity. Thirteen of these eighteen patients had cystectomy. The other thirty-two patients were treated with two cycles of cisplatin-based neoadjuvant chemotherapy before having their second (mid-cycle) MRI. Five patients were then sent to the cystectomy. The remaining twenty-seven patients completed the next two cycles
Table 2.1: The numbers of patients with 1, 2, or 3 MRIs with or without cystectomy.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>1 MRI</th>
<th>2 MRIs</th>
<th>3 MRIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystectomy</td>
<td>13</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>No cystectomy</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

of chemotherapy before their post-chemotherapy MRI. Twenty-five of these twenty-seven patients had cystectomy. Totally, forty-three patients had cystectomy; seven patients did not have cystectomy. All cystectomy bladder specimens were examined by pathology. The time interval from the last MRI to cystectomy ranged from 7 to 74 days with the median of 20 days (average ± standard deviation: 27 ± 18 days). The details are summarized in Table 2.1.

2.2 MRI Protocol

All patients were scanned on a 3T MRI system (Achieva; Philips Healthcare, Cleveland, Ohio, USA) using a 32-channel phased-array surface coil as well as a 2-channel RF transmit coil. All patients were imaged in the supine position with feet going first in the MRI scanner. All scans were performed with conventional T2-weighted MRI in coronal, sagittal, and axial orientations prior to DWI and DCE-MRI with the contrast agent (CA) administration.

T2w MRI was performed with a Turbo spin echo sequence in the coronal (repetition time msec/echo time msec, 4928/80; matrix, 300×300; spatial resolution (RL/AP/FH), 1.00×1.00×3.00 mm; number of slices, 32; acquisition time, 4 minutes; field of view (RL/AP/FH), 300x300x105 mm; sensitivity encoding
factor, 2), sagittal (repetition time msec/echo time msec, 4623/80; matrix, 300×299; spatial resolution (RL/AP/FH), 1.00×1.00×3.00 mm; number of slices, 42; acquisition time, 5.5 minutes; field of view (RL/AP/FH), 300x300x138 mm; sensitivity encoding factor, 2), and axial (repetition time msec/echo time msec, 4264/80; matrix, 292×323; spatial resolution (RL/AP/FH), 0.99×1.06×3.00 mm; slice gap, 0.3 mm; number of slices, 40; acquisition time, 5 minutes; field of view (RL/AP/FH), 290x341x130 mm; sensitivity encoding factor, 2) orientations.

DWI was performed with a single-shot Diffusion-Weighted Echo Planar Imaging (DW-EPI) in axial plane (repetition time msec/echo time msec, 2000/58; matrix, 96×159; spatial resolution (RL/AP/FH), 2.25×2.25×3.00 mm; b-values, 0, 333, 667, 1000 s/mm²; slice gap, 0.3 mm; number of slices, 20; acquisition time, 4 minutes; field of view (RL/AP/FH), 220x358x66 mm; sensitivity encoding factor, 2) planes.

DCE-MRI was performed using a 3D-spoiled gradient echo sequence (repetition time msec/echo time msec, 5/2; flip angle, 20°; matrix, 212×213; field of view (RL/AP/FH), 360x360x95 mm; spatial resolution (RL/AP/FH), 1.70×1.68×5.00 mm; number of slices, 19; number of signal average (NSA), 1; temporal resolution, 8.25 sec; acquisition time, 8.5 minutes; number of dynamic scans, 60) in the axial orientation. A single dose (0.2 mmol per kilogram body weight) of Gd-based contrast agent (Magnevist, Bayer Healthcare) was intravenously injected at a constant rate of 0.5 ml/s after the fifth dynamic scan of acquisition, followed
by a flush of 25 ml saline at the flow rate of 2ml/s. Depending on the patient’s weight, infusion time ranged from 21 to 64s with the median of 35s.

2.3 Image Processing

2.3.1 DWI Data

Four different b-values (0, 333, 667, 1000 s/mm²) were used acquire a signal intensity curve. Apparent Diffusion Coefficient (ADC) was calculated from signal intensity by using the equation \( \frac{S}{S_0} = e^{-b \cdot ADC} \) where \( S_0 \) and \( S \) are the signal intensities at b-value of 0 and a non-zero b-value (333, 667, or 1000) s/mm². The ADC value was the parameter obtained from the curve fitting. In addition, three different ADC values corresponding to b-values of 333, 667, and 1000 s/mm² were calculated.

DWI data was processed using IDL (Exelis VIS)-based software to calculate ADC with the above described method and ADC maps. ADC maps were color-coded with the same color table for all cases.

2.3.2 DCE-MRI Data

This study applied a modified Brix’s linear two-compartment pharmacokinetic model (44, 45) to quantitatively assess the dynamic characteristics of the signal enhancement in body tissues. The detailed description of the model can be found in the paper by Yang (45). Here, only a brief summary of the model is presented. The model uses the linear relationship between the relative signal enhancement and tissue CA concentration: \( \frac{S_{CA}(t) - S_0}{S_0} = FC_T(t) \) where \( S_{CA}(t) \) and \( S_0 \) are
respectively the signal intensities with and without CA; $C_T(t)$ is the CA concentration in the tissues; and $F$ is a constant and specific to the type of tissues. Note that both $S_{CA}(t)$ and $C_T(t)$ are time-dependent. It is also assumed in the linear two-compartment model (45) that the CA concentrations $C_p$(plasma) and $C_e$ (extravascular extracellular space –EES) are instant and homogeneous in the same compartment and that exchange rates (between plasma and EES - $k_{pe}$; between EES and plasma - $k_{ep}$; and elimination rate - $k_{el}$) are constant.

With these assumptions and a constant CA injection rate of $K_{in}$, two coupled differential equations are established as follows:

$$
\begin{align*}
\frac{dC_p}{dt} &= -(k_{pe} + k_{el})C_p + \frac{V_e}{V_p}k_{ep}C_e + \frac{K_{in}}{V_p}, \quad 0 \leq t \leq \tau \\
\frac{dC_e}{dt} &= \frac{V_p}{V_e}k_{pe}C_p - k_{ep}C_e \\
\frac{dC_p}{dt} &= -(k_{pe} + k_{el})C_p + \frac{V_e}{V_p}k_{ep}C_e, \quad t > \tau \\
\frac{dC_e}{dt} &= \frac{V_p}{V_e}k_{pe}C_p - k_{ep}C_e
\end{align*}
$$

where $V_p$ and $V_e$ are the compartment volumes of the plasma and the EES space.

The general solutions ($C_p$ and $C_e$) to these equations are found to be dependent of the compartment volumes $V_p$ and $V_e$ and exchange rates $k_{pe}, k_{ep}$ and $k_{el}$. Using the above described linear relationship of the relative signal intensity and CA concentrations $C_p$ and $C_e$, the signal intensity can be shown to be only dependent of $V_p, V_e, k_{pe}, k_{ep}$ and $k_{el}$. The compartment volumes $V_p$ and $V_e$ are then replaced
with amplitude parameters $A_p$ and $A_e$ with a factor difference from $V_p$ and $V_e$.

Finally, the amplitude parameters and exchange rates can be obtained from the relative signal intensity in the tissues with the aid of the arterial input function (AIF) in the common case of DCE-MRI data.

Applying the modified Brix's linear two-compartment pharmacokinetic model (45), DCE-MRI data were processed using an IDL (Exelis VIS)-based software environment. An AIF was manually selected for each dataset by placing an arterial region of interest (ROI) on the right common femoral artery. Pharmacokinetic parameters amplitude (Amp) and the AIF-adjusted exchange rate of the contrast agent between EES and the plasma space ($k_{ep}$) were quantified, and their pharmacokinetic maps were acquired. The pharmacokinetic parameters are voxel-base values. The maps of Amp and $k_{ep}$ were coded with the same color table and on the same parameter scale for all cases. The display of color maps followed the standard display method for Brix’s model (46). A threshold value of 1.0 (a.u.) was chosen for Amp. The color pharmacokinetic maps were overlaid on original DCE (T1-weighted) MR images.
3 Improving Bladder Cancer Detection with DCE-MRI

3.1 Introduction

Cystoscopy is the standard for the diagnosis and local management of bladder cancer. However, it is invasive and limited in assessing the surface and the fat infiltration of bladder malignancies (3). CT is the imaging modality that is the most commonly used to initially assess bladder cancer. However, CT has not overcome its limitations including the risk of ionized radiations, low accuracy (4), and high interobserver variability in the staging of bladder cancer (5). As a result, accurate diagnosis of bladder cancer which is essential to patient management and treatment strategy is still an unmet clinical need in cystoscopy and CT. Without any risk of ionized radiations and with the capabilities of tissue characterization and multi-planar functional imaging, MRI has been shown to be the most accurate technique to assess the depth of tumor infiltration (6), and useful in evaluating the chemotherapy in bladder cancer (7) to resolve this unmet clinical need. Thus, it is essential to conduct a multi-modal MRI study that systematically assesses the capabilities of conventional and functional MRI in the detection, staging, and assessment of therapeutic response of bladder cancer in which detection with MRI must be firstly evaluated.
Functional dynamic contrast-enhanced MRI (DCE-MRI) can assess the microcirculation of malignant tissues, therefore, visualizes the neoangiogenesis via the signal enhancement of contrast agent. DCE-MRI has already demonstrated good reproducibility and high accuracy in the differentiation of superficial from muscle-invasive bladder cancer (8). High field 3T MRI has been shown to be superior to lower field MRI (such as 1.5T) in the spatial resolution, signal-to-noise ratio, and the delineation of the depth of tumor invasion in different types of cancer (9-11). However, there has been only one study that used 3T MRI in bladder cancer diagnosis and showed that 3T DCE-MRI could make improvement in T and N staging of muscle-invasive bladder cancer (12). Furthermore, no study has been done to evaluate the capability of 3T MRI in the detection of bladder cancer.

The impact of dielectric artifacts such as shaded areas on MR images increases with the field strength. It was reported that multi-transmit technology helps reduce the dielectric effects at high field, improve homogeneity of RF field, and decrease scan time at 3T MRI (13).

The purpose of this study is to evaluate the capability of conventional T2-weighted MRI (T2w-MRI) and the additional diagnostic value of functional DCE-MRI using 3T multi-transmit in the detection of bladder cancer.
3.2 Materials and Methods

3.2.1 Patient Inclusion

Seven patients did not have cystectomy and two patients did not complete their last pre-surgical MRI scan due to their specific co-morbidity. Five patients had poorly distended bladder volume on their last MRI via the visual inspection by two radiologists (with more than 10 years of experience) who were blinded to clinical and pathological findings. These fourteen patients were excluded from this data analysis.

Out of thirty-six included patients, seven patients with aggressive bladder tumors which required immediate cystectomy and six patients with non-muscle-invasive tumors which did not have chemotherapy were directed to cystectomy after their baseline MRI. The other twenty-three patients were treated with two cycles (21 days per cycles) of chemotherapy prior to the first follow-up MRI. Three patients were subsequently sent to cystectomy due to their tumor resistance to chemotherapy. The remaining twenty patients completed the other two cycles of chemotherapy and had the second follow-up MRI, followed by cystectomy. All cystectomy bladder specimens were examined by pathology. The pathological findings of cystectomy bladder specimens were used a reference standard. Study flow chart is described in Figure 3.1.
Figure 3.1: Flow chart of the DCE-MRI study on bladder cancer detection. Thirty-six patients were included in the study. All patients had cystectomy after their last MRI. All surgical bladder specimens were examined by pathology.

### 3.2.2 Data Interpretation

A Philips Extended Brilliance Workspace (EBW) workstation was used for data review. The two radiologists independently reviewed MRI data. Each radiologist localized bladder malignant tumors using conventional T2W MR images, and then using additional pharmacokinetic maps (DCE maps). On color DCE maps, malignant tumors were identified with continuous color pixels on the bladder wall that show signal enhancement (see Color Table on Figure 3.2). The location of bladder cancer was identified as right lateral, left lateral, anterior, posterior, dome, and trigone/apex on the bladder wall in both radiological read of the last
pre-surgical MRI and pathological findings of cystectomy bladder specimens. An independent investigator was tasked to match the radiological read with the pathological findings of the same patient. A true positive finding of radiological read was confirmed when the location of a malignant bladder tumor was matched between radiological read and pathological finding. A true negative finding of radiological read was confirmed when there was no malignant tumor found in the bladder by both radiological read and pathological finding.

### 3.2.3 Statistical Analysis

Descriptive statistics (mean and standard deviation or median and range for continuous variables; frequency and proportion with 95% confidence interval for categorical variables) were used to summarize the data. Exact binominal method was used to calculate the 95% confidence interval of proportion. Kappa values were calculated to evaluate the interobserver agreement for the interpretation of T2w-MRI alone and additional DCE-MRI. Agreement was considered moderate, good, and excellent for kappa values of 0.41-0.60, 0.61-0.80, and greater than 0.80, respectively (51). For each observer, diagnostic sensitivity, specificity, and accuracy were calculated for T2w-MRI only as well as with additional DCE-MRI. McNemar test was performed on a commercial statistical package (SAS 9.2; SAS Institute Inc., Cary, NC, USA) to evaluate the differences in these diagnostic values. P < 0.05 was considered statistically significant.
3.3 Results

3.3.1 Tumor Characteristics
Pathological examination confirmed bladder malignant tumors found in twenty-eight patients and no malignancy identified in the other eight patients at the time of cystectomy. Of the eight negative patients, two did not have chemotherapy, and six completed all four cycles of chemotherapy. Of the twenty-eight positive patients, eleven patients did not have chemotherapy, and seventeen had chemotherapy. Four positive patients were found with two or more malignant tumors (Table 1). A total of thirty-six malignant tumors were found in the twenty-eight positive patients. In the four patients with more than one malignant tumor, the T stage was confirmed only for the most invasive tumor. All malignant tumors were found to be of high grade by pathology except three tumors whose grades were not reported. The largest measurable tumor diameter ranged from 1 mm to 100 mm with the median of 26 mm.

3.3.2 Localization of malignant tumors
On T2w MR Images, twenty-nine malignant tumors were identified by observer 1 (radiologist 1) and twenty-six found by observer 2 (radiologist 2) (Figures 2 and 3). Both observers confirmed five out of eight negative cases. In the other three negative cases, the benign bladder wall thickening shown on T2w MR images was misdiagnosed as a malignant tumor. The sensitivity, specificity, and accuracy of the localization of bladder malignant tumors using T2w-MRI alone
Table 3.1: Tumor characteristics

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tis/Ta</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>T1</td>
<td>3 (11%)</td>
</tr>
<tr>
<td>T2</td>
<td>11 (39%)</td>
</tr>
<tr>
<td>T3</td>
<td>8 (29%)</td>
</tr>
<tr>
<td>T4</td>
<td>4 (14%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of malignant tumors per case</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 (86%)</td>
</tr>
<tr>
<td>2</td>
<td>1 (3.5%)</td>
</tr>
<tr>
<td>3</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>4</td>
<td>1 (3.5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Size</th>
<th>Number of malignant tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1cm</td>
<td>7 (19%)</td>
</tr>
<tr>
<td>&gt; 1cm</td>
<td>20 (56%)</td>
</tr>
<tr>
<td>NA*</td>
<td>9 (25%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Number of malignant tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid</td>
<td>19 (53%)</td>
</tr>
<tr>
<td>Papillary</td>
<td>8 (22%)</td>
</tr>
<tr>
<td>Lobulated</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>NA*</td>
<td>8 (22%)</td>
</tr>
</tbody>
</table>

* Not reported
were 81% (29/36), 63% (5/8) and 77% (34/44) for observer 1, and 72% (26/36), 63% (5/8), and 70% (31/44) for observer 2. The kappa score for the evaluation of interobserver agreement was 0.63 (95% CI: 0.38-0.88) for the detection with T2w-MRI alone.

With the addition of DCE-MRI data, thirty-three malignant tumors were identified by both observers (Figures 2, 3 and 4). Six negative cases were confirmed by observer 1 and five by observer 2. The sensitivity, specificity, and accuracy were 92% (33/36), 75% (6/8), and 89% (39/44) for observer 1, and 92% (33/36), 63% (5/8), and 86% (38/44) for observer 2. The kappa score increased to 0.78 (95% CI: 0.55-1.00) with additional DCE-MRI data.

Compared to T2w-MRI alone, DCE-MRI significantly (P < 0.01) improved the sensitivity and accuracy of bladder cancer localization by observer 2. DCE-MRI also increased the sensitivity and accuracy of the bladder cancer localization by observer 1, however, not significant (P=0.1 for sensitivity and P=0.06 for accuracy).

In terms of the number of cases found with malignancy, the sensitivity, specificity and accuracy of bladder cancer detection with T2w-MRI alone were 86% (24/28), 63% (5/8) and 81% (29/36) for observer 1 and 75% (21/28), 63% (5/8) and 72% (26/36) for observer 2. Adding DCE-MRI data, these values were 93% (26/28), 75% (6/8), and 89% (32/36) for observer 1, and 93% (26/28), 63% (5/8), and 86% (31/38) for observer 2.
Figure 3.2: MR images of a 64 year-old male patient. A, Axial T2w Image; B, Amp+k_{ep} map. Patients were treated with 4 cycles of neoadjuvant chemotherapy. Tumor location (indicated by orange arrows and enclosed in the white contours) is at the anterior and dome aspect of the bladder wall. Tumor stage: T3b; size: 38 mm. Both T2w image and pharmacokinetic map accurately visualizes the malignant tumor. The malignancy can be identified with continuous color pixels on the color DCE map.

Figure 3.3: MR images of a 63 year-old female patient. A, Axial T2w Image; B, Amp+k_{ep} map. Patients were treated with 4 cycles of neoadjuvant chemotherapy. Tumor location (indicated by orange arrows and enclosed in the white contours) is at the right bladder wall. Tumor stage: T2; size: 24 mm. The malignant tumor is identified with an irregular margin and slightly slower signal intensity than normal bladder wall on the T2w image. The continuous color pixels on the color Amp and k_{ep} map further confirms the tumor location.
Figure 3.4: MR images of a 68 year-old male patient. A, Axial T2w Image; B, Amp+k_{ep} map. Patients were treated with 4 cycles of neoadjuvant chemotherapy. Tumor location (indicated by an orange arrow and enclosed in the white contour) is located at the left and posterior aspect of the bladder wall. Tumor stage: T2; size: 9 mm. The malignant tumor with a smooth margin cannot be visualized on the T2w image. The malignancy can be identified with continuous color pixels that show strong signal enhancement. It is noteworthy that the malignancy was both within the bladder wall thickening and less than 10 mm.

3.3.3 Detection of malignant tumors treated with neoadjuvant chemotherapy

Out of twenty-three patients treated with neoadjuvant chemotherapy, seventeen were found positive for malignancy with a total of 20 tumors identified by pathology, and six were found negative for malignancy. The sensitivity, specificity, and accuracy of the detection with T2w-MRI alone were 70% (14/20), 50% (3/6) and 65% (17/26) for observer 1, and 55% (11/20), 50% (3/6), and 54% (14/26) for observer 2. Adding DCE-MRI data produced the sensitivity, specificity, and accuracy of 90% (18/20), 67% (4/6), and 85% (22/26) for observer 1, and
85% (17/20), 50% (3/6), and 77% (20/26) for observer 2. McNemar test showed that DCE-MRI significantly (P < 0.02) improved the sensitivity and accuracy of bladder cancer detection with T2w-MRI alone for observer 2. For observer 1, the differences were not found significant (P=0.1 for sensitivity, and P=0.06 for accuracy).

### 3.3.4 Detection of sub-centimeter malignant tumors

Out of thirty-six malignant tumors, there were seven (19%) tumors less than 10 mm (1 cm) in the largest measurable diameter. The two radiologists were able to localize four (57%, 95% CI: 18%-90%) of these seven sub-centimeter tumors on T2w images. With the addition of pharmacokinetic DCE-MRI maps, there were a total of six (86%, 95% CI: 42%-100%) sub-centimeter tumors identified by the two observers (Figure 4). DCE-MRI increased 50% of the number of identified sub-centimeter tumors, however, not significant (P=0.32) in this small population.

### 3.3.5 Detection of malignant tumors within bladder wall thickening

There were eleven out of thirty-six (28%) malignant tumors found within the bladder wall thickening. These eleven tumors were found in seven patients (six treated with chemotherapy). The two readers identified six (55%, 95% CI: 23%-83%) malignant tumors on T2w MR images in those patients (Figure 3). The identification of a malignant tumor on T2w MR images was confirmed with the irregular margin and different signal intensity of the malignant tumor within the bladder wall thickening. The other five tumors were isointense and had a smooth
margin within the bladder wall, thus, not identified on T2w images. With the dynamic characteristics of signal enhancement of the contrast agent, four of these five T2w-missed tumors were delineated on the pharmacokinetic maps Amp and \( k_{ep} \). Therefore, a total of ten (91%, 95% CI: 59%-100%) malignant tumors were distinguished against the benign bladder wall thickening on DCE maps (Figures 3 and 4). DCE-MRI significantly improved the delineation of malignant tumors within the benign bladder wall thickening (\( P<0.05 \)).

Of eleven malignant tumors within the bladder wall, three were less than 10 mm and could not be characterized on T2w MR images. These three sub-centimeter tumors were distinguished from the benign bladder wall thickening on pharmacokinetic parameter maps.

### 3.4 Discussion

Patient management of bladder cancer depends on the accuracy of the cancer diagnosis. The current standard for bladder cancer diagnosis is cystoscopic imaging. Due to its invasiveness, cystoscopy cannot be performed on some patients due to intense discomfort, bleeding, or local implications such as infections or mechanical obstructions (3). Furthermore, cystoscopy is limited in providing information on the perivesical invasion of bladder malignancies and identifying flat tumors or tumors behind the bladder wall (3). Current imaging modalities such as CT and ultrasound have been brought forward to provide additional tools to improve bladder cancer diagnosis (4, 5, 18-22). However, CT
has not overcome its limitations such as understaging of advanced tumors and interobserver variability (4, 5, 22). In addition to CT, PET/CT also has been evaluated to improve those limitations: catheter-assisted 18F-FDG-PET/CT imaging using standardized bladder flushing and filling presented with a sensitivity 63% (95% CI: 0.36-0.84) (18); C-acetate PET/CT revealed a sensitivity of 80% (8 out of 10 malignant tumors) (21). While multidetector CT with multiplanar reformatted imaging and virtual cystoscopy had a high sensitivity of 94% in comparison with conventional cystoscopy, it was shown in the study that conventional cystoscopy had discrepancies with histopathology (3). Ultrasound, as an alternative methodology for bladder imaging, was limited in detecting bladder tumors on the bladder floor and fundus (19, 20). Ultrasound was reported to have a sensitivity of 66% with micro-bubble contrast enhancement vs 61% without contrast (20). Therefore, accurate diagnosis of bladder cancer is still an unmet clinical need in cystoscopy and bladder imaging including CT and ultrasound. MRI has been shown to be the most accurate technique to assess the depth of tumor infiltration (6), and useful in evaluating the chemotherapy in bladder cancer (7) to resolve this unmet clinical need. Therefore, it is essential to conduct a multi-modal MRI study that uses histopathological examination of cystectomy bladder specimens as a reference standard and systematically assesses the capabilities of conventional and functional MRI in the detection, staging, and assessment of therapeutic response of bladder cancer in which detection with MRI must be firstly evaluated. Compared to histopathological findings as a reference standard, our study found a sensitivity of 81% (observer
1) and 72% (observer 2) with conventional 3T MRI (T2w-MRI) and an improved sensitivity of 92% (both observers) with the additional use of DCE-MRI in the detection of bladder cancer.

High field 3T MRI has been shown to be superior to lower field MRI (such as 1.5T) in the spatial resolution, signal-to-noise ratio (10), the differentiation of cancer from normal tissues (11), and the delineation of the depth of tumor invasion (9) without compensation for longer scan time. However, there has been only one study that used 3T DCE-MRI in bladder cancer diagnosis (T and N staging of invasive bladder cancer)(12). This study showed the improvement of DCE-MRI in T and N staging of bladder cancer and suggested that a standardized protocol needs to be developed. Furthermore, there has been no study that evaluates the value of 3T MRI in bladder cancer detection. Our study evaluated the capability of 3T MRI in the detection of bladder cancer and demonstrated that 3T high-resolution conventional T2w-MRI can help detect small bladder tumors and 3T DCE-MRI can better visualize sub-centimeter tumors and tumors within the bladder wall thickening via the characteristics of dynamic contrast enhancement in malignant tissues to improve the sensitivity and accuracy of the detection of bladder cancer, especially bladder cancer in patients treated with chemotherapy.

Contrast-enhanced MRI has been shown to have good reproducibility and high accuracy in the differentiation of superficial from invasive bladder cancer (8). Our study showed a similar result in the reproducibility of DCE-MRI. Adding DCE-MRI
increased the interobserver agreement (Kappa score = 0.78), suggesting that a combined reading further improves the robustness of the radiological read of bladder cancer assessment.

Pelvic imaging at 3T has been traditionally limited due to field inhomogeneities (13) which were overcome in this study to achieve consistently good image quality by combination of an advanced 32-channel coil with the utilization of multitransmit. Our prior pilot work (unpublished) had already demonstrated that the consistent application of multitransmit acquisition obtained after prior B1 mapping with a less-than-one-minute scan of the target volume enables a consistent substantial improvement of image quality. Thus, we have used in this prospective study the multitransmit acquisition approach which also has become our standard clinical procedure for 3T imaging.

One of the common limitations of previous studies on bladder cancer diagnosis is that the tumor size was large (23, 24). Most of the bladder tumors in the study by Abou-El-Ghar were large and false negative findings were tumors of sub-centimeter size. All bladder tumors in the study by El-Assmy were from 20 to 80 mm. Our study included tumors that were from sub-centimeter to multiple centimeters in size. All tumors above 20 mm were detected by both T2w-MRI and DCE-MRI using 3T multitransmit. There were four (57%) sub-centimeter tumors visualized on T2w images and six (86%) delineated on DCE maps. The results have shown that the detectability of both 3T conventional (T2w) and
functional (DCE) MRI of sub-centimeter tumors can be achieved with high spatial resolution and RF field homogeneity.

The presence of the bladder wall thickening has been reported to lower the accuracy of bladder cancer diagnosis in cystoscopy (25, 26), CT (4, 22), and lower field (1.5T) MRI (8, 27, 28). The inflammatory and fibrous changes after neoadjuvant chemotherapy were also reported to have a negative impact on the accuracy of bladder cancer staging (27). The cohort in our study included patients who only had TURBT and who both had TURBT and were treated with chemotherapy. Using T2w-MRI alone, the bladder cancer detection in all thirty-six patients had the sensitivity, specificity, and accuracy of 81%, 63% and 77% for observer 1, and 72%, 63%, and 70% for observer 2; these values for only twenty-three patients treated with chemotherapy were 70%, 50% and 65% for observer 1, and 55%, 50%, and 54% for observer 2. These results also showed that the reactive and inflammatory changes induced by chemotherapy had a strong negative influence on the detection of bladder cancer. Our 3T DCE-MRI significantly improved the detection of malignant tumors within bladder wall thickenings from 55% to 91%. This significant improvement of DCE-MRI in the differentiation of bladder malignancy from benign wall thickenings helped reduce the negative impact of reactive changes after neoadjuvant chemotherapy to increase the sensitivity and accuracy of the detection with T2w-MRI alone in all patients. Accompanied by good interobserver agreement, the detection of
bladder cancer had a sensitivity of 92% with the addition of DCE-MRI in both observers for this heterogenous patient population.

The most common pharmacokinetic models used to assess DCE-MRI are the linear two-compartment model proposed by Tofts (29) and the one by Brix (14). Due to its limitation (15), Brix’s model has recently been less often applied to process DCE-MRI than Tofts’s model. The modification to Brix’s model proposed by Yang (15) solved the limitation to acquire pharmacokinetic parameters that have comparative tissue specificity to the pharmacokinetic parameters derived from the Tofts’s model. Compared to fast injection, slow injection in our study allowed a better observation of wash-in phase which is characterized by pharmacokinetic parameter $k_{ep}$. Moreover, the IV injection of the contrast agent at a slow rate was given in a more controlled way with the modified Brix’s model than a fast rate (30). Our DCE-MRI data was processed with the modified Brix’s model. The results demonstrate the reliability of the model for the characterization and assessment of bladder cancer.

Our study had several limitations: (1) the number of negative cases (N=8) was small. This small number may have been the factor that did not allow demonstrating the significant improvement of DCE-MRI in the specificity. A large number of cases will be included in future studies to both solidify our findings and demonstrate more diagnostic value of DCE-MRI; (2) the correlation between radiological read and pathological examination was in some cases challenging due to the lack of the 3D matching between pathological findings and radiological
localization. This limitation can be resolved in future studies with an approach to the 3D matching and specimen MR imaging; (3) Not all patient’s bladders were fully distended. The research protocol comprised of conventional imaging and different functional imaging modalities required a long scan time (1 hour). Therefore, a fully distended bladder caused the interruption of MRI exams. The future study will be performed with a more refined protocol to reduce the scan time, which allows maintaining a fully-distended bladder.

In conclusion, multitransmit 3T DCE-MRI improves the reproducibility and the characterization of bladder cancer, especially small malignant tumors and those within the bladder wall thickening. 3T MRI with DCE appears to be a promising substantially more accurate approach to improving bladder cancer imaging beyond the current limitations of cystoscopy and CT.
4 K-means clustering of pharmacokinetic parameters for prediction of chemotherapeutic response

4.1 Introduction

The standard treatment for muscle-invasive bladder cancer is radical cystectomy and pelvic lymph node dissection with the consideration of neoadjuvant chemotherapy (58). However, not all bladder cancer patients benefit from neoadjuvant chemotherapy. While the treatment has been reported to reduce tumor burden before radical cystectomy to improve the prognosis for responders (59), it can cause an unnecessary delay of the definitive cystectomy and, in some cases, lead to tumor progression during the treatment for non-responders (60). Thus, the ability to predict early the chemotherapeutic response in bladder cancer is essential to ensure the best clinical outcome. Currently, there is no effective tool to manage this clinical challenge.

Two current approaches that have been studied to predict the therapeutic response of bladder cancer are gene expression profiling and bladder MR imaging (40, 42, 60-63). Gene expression analyzes the complexity in molecular activities to identify a gene biomarker for the prediction of therapeutic response.
Even though several gene markers have been found promising, gene expression profiling lacks the analysis of in vivo factors and their findings still need extensive validation in multi-center clinical trials (60). MRI of bladder with the ability to non-invasively perform functional imaging such as diffusion-weighted MRI (DWI) and dynamic contrast-enhanced MRI (DCE-MRI) can reveal the pathophysiological characteristics such as high cellularity and angiogenesis in cancer tissues to guide the assessment of therapeutic response in bladder cancer (40, 42, 63). It has been shown that DWI and DCE-MRI are useful in assessing therapeutic response in bladder cancer (40, 42, 63), while other image modalities including CT and Ultrasound have not demonstrated any value in this aspect of bladder cancer management.

Dynamic Contrast-Enhanced MRI (DCE-MRI) with the ability to characterize the microvascular heterogeneity of cancerous tissues via pharmacokinetic parameters can provide quantitative assessment of therapeutic response in different types of cancers (40, 64-67). In bladder cancer, previous studies showed that DCE-MRI revealed the correlation between pharmacokinetic parameters and therapeutic response at mid-cycle and post-therapy, and suggested that the analysis of DCE-MRI pharmacokinetic parameters can be an important tool to assess and predict therapeutic response in bladder cancer (40)(68).

K-means clustering, one of the most commonly used data mining methods, has been shown to be useful in the analysis of the pathological characteristics of
cancer tissues and more powerful than other clustering methods in the
differentiation of malignancies versus benign tissues and in the assessment of
therapeutic response and patient prognosis (69-72).

Current generation 3T MRI using multi-transmit technology can take advantage
of higher spatial resolution and signal-to-noise ratio, leading to better delineation
of the tumor invasion than lower field MRI (47, 48). In addition, 3T has a longer
longitudinal relaxation time T1 compared to 1.5T, thus, can provide a higher
signal contrast in contrast-enhanced MRI and DCE-MRI (73). Simultaneously, 3T
MRI with multi-transmit technology can overcome the dielectric effects at high
field, improve image quality, and reduce local SAR (74).

The aim of this study is to apply k-means clustering of DCE-MRI pharmacokinetic
parameters in the analysis of micro-circulation and perfusion characteristics of
bladder tumors to predict early chemotherapeutic response in bladder cancer.

4.2 Materials and Methods

4.2.1 Patient inclusion

Patients included in the analysis of this DCE-MRI study had to meet the following
inclusion criteria: (1) patients had clinical diagnosis of invasive bladder cancer;
(2) patients completed at least two cycles of chemotherapy and baseline and
mid-cycle MRIs; (3) bladder distension was at least shown moderate (appeared
to be filled at two thirds of fully-distended volume) on baseline and mid-cycle MR
images via visual inspection by the radiologists. Eighteen patients had only 1
Figure 4.1: Study flow chart. Of the twenty-five patients that had TURBT, all tumor specimens were examined by pathology. All had a baseline MRI and an MRI after 2 cycles of neoadjuvant chemotherapy. Three patients were then directed to radical cystectomy due to their tumor progression or their specific co-morbidity during chemotherapy. The other twenty-two patients completed chemotherapy before radical cystectomy. The bladder tumors in two patients were unresectable. Therefore, only twenty-three cystectomy bladder specimens were examined by pathology.

MRI exam; three patients had poor bladder distension in their baseline or mid-cycle MRI; and four patients had incomplete baseline or mid-cycle DCE-MRI data. These twenty-five patients were excluded in this DCE-MRI study.
As a result, twenty-five patients (twenty-two males and three females, age: range, 38-86 years; median, 66) were included in this DCE-MRI study. All patients had transurethral resection of bladder tumor (TURBT)/biopsy prior to their first MRI exam. The pathological examination of TURBT tumor specimens confirmed invasive bladder cancer in the patients. The pathological stage of TURBT tumor was used as baseline stage.

The study flow chart is described in Figure 4.1. After baseline MRI, all twenty-five patients were treated with two cycles (twenty-one days per cycle) of neoadjuvant cisplatin-based chemotherapy and had a mid-cycle MRI. Three patients were subsequently directed to cystectomy due to their tumor progression or specific co-morbidity. The other twenty-two patients completed the other two cycles of chemotherapy and had their last (post-chemotherapy) MRI. Two patients had a bladder tumor that was unresectable; twenty patients had radical cystectomy. All surgical bladder specimens were examined by pathology. The pathological stage of cystectomy bladder specimen was used as post-chemotherapy stage.

4.2.2 Response criteria

Axial T2w-MR images of the first and last MRI exams were used to estimate the tumor volume change after chemotherapy. The tumor volume change, baseline tumor stage (found in the TURBT specimen), and post-chemotherapy tumor stage (found in the cystectomy bladder specimen) were combined to define a responder or a non-responder.
A bladder patient was defined as responder if the patient had one of the following criteria:

(i) No malignancy was found by pathology in the cystectomy bladder specimen (pathological complete response).

(ii) Baseline stage was less than post-chemotherapy stage and there was no tumor volume increase measured on T2w MR images (downstaging).

(iii) There was tumor volume reduction at 50% or more after chemotherapy (significant volume reduction).

A non-responder was defined when none of these criteria was met.

4.2.3 K-means clustering algorithm

K-means clustering is a clustering method that partitions a group of \( N \) data points \((\mu_1, \mu_2, ..., \mu_N)\) into \( k \) subgroups (\( k \) clusters) of data points which each have a centroid (center of cluster) \( c_i (i = 1, 2, ..., k) \) (75).

K-means clustering was performed on Microsoft Office Excel (Version 2010) with the aid of Solver Add-in for optimization by using a method proposed by Aravind (76). K-means clustering algorithm was performed with a given number of clusters \( (k) \) and by an iteration process as follow:

1. Choose \( k \) centroids of \( k \) clusters: \( c_i (i = 1, 2, ..., k) \)
2. Calculate the measure $Intra_k$ that is the mean intra-cluster distance:

$$Intra_k = \frac{1}{N} \sum_{j=1}^{k} \sum_{i=1}^{N_j} \| \mu_i - c_j \|$$

where $c_j$ is the centroid of cluster $j$, $N_j$ is the number of data points in cluster $j$, and $\mu_i$ is a data point in cluster $j$.

3. Use Solver Add-in to minimize the measure: $Intra_k$. This step was an iteration process step 1, 2, and 3.

4. Once the minimization of $Intra_k$ was done, a new set of $k$ centroids were determined.

The two voxel-wise pharmacokinetic parameters used for k-means clustering were the amplitude of signal enhancement ($Amp$) and the AIF-adjusted exchange rate of the contrast agent between EES and the plasma space ($k_{ep}$).

K-means clustering was performed on the data that includes all twenty-five Amp and $k_{ep}$ datasets of the twenty-five baseline MRI scans to determine the optimal number of clusters ($k_o$) and the corresponding centroids.

The value of $k_o$ was selected among a series of $k$ from 2 to 10 based on a cluster validation index (validity) that was chosen between two validation indices $V_1$ and $V_2$ (69):

$$V_1 = \frac{Intra_k}{Inter_k}$$

$$V_2 = Intra_k + \frac{k}{Inter_k}$$
where \( \text{Inter}_k = \min_{j \neq l} \| c_j - c_l \| \)

Each data point has a coordinate of \( k_{ep} \) and Amp on cluster plots (see Figures 4 and 5). The validity was a function of the mean intra-cluster distance measure \( (\text{Intra}_{\text{k}}) \), which characterizes for the intra-cluster scattering, and inter-cluster minimum distance \( (\text{Inter}_{\text{k}}) \), which characterizes for the inter-cluster separation. The optimal k-means clustering provides the smallest \( \text{Intra}_k \), i.e. the clusters are the least scattered or the most compact, and the largest \( \text{Inter}_k \), i.e. the clusters are the most well-separated while the number of clusters \( (k) \) is still small. Therefore, \( k_0 \) was selected when \( V_1 \) or \( V_2 \) is minimized.

In brief, the determination of \( k_0 \) and the centroids by k-means clustering were implemented in the following steps:

(i) Input \( k \) of 2.

(ii) Perform k-means clustering to find \( k \) centroids.

(iii) Calculate \( V_1 \) and \( V_2 \).

(iv) Repeat (i) to (iii) for \( k \)'s from 3 to 10.

(v) Evaluate which validity \( V_1 \) or \( V_2 \) should be used for the data in the study

(vi) Select \( k_0 \) that corresponds to the minimum of the selected validity.

With the determined \( k_0 \) and the \( k_0 \) centroids, the volume fractions of \( k_0 \) clusters were subsequently calculated for each bladder tumor. The fraction volumes of \( k_0 \) clusters at baseline and their changes from baseline to mid-cycle were correlated with tumor responsiveness.
4.2.4 Statistical Analysis

Descriptive statistics (i.e. mean, standard deviation, and 95% confidence interval) were provided for each cluster at baseline and the change of cluster volume fraction for responders and non-responders, respectively. The differences between responders and non-responders in the change of cluster volume fractions were evaluated by two sample t-test. The Holm-Bonferroni method was used to adjust for multiplicity. P<0.05 was considered to be statistically significant.

ROC curve analysis was used to evaluate the robustness of the three cluster volume fraction changes in the assessment of bladder cancer response to chemotherapy. Area under the ROC curve (AUC) was estimated for each cluster. The cut-off values of the three cluster volume fraction changes were determined to classify responders and non-responders. Each cutoff value was selected to be corresponding with the point that was closest to the ROC curve top left corner.

4.3 Results

4.3.1 Defined responders and non-responders

Out of twenty-five patients, six were found as pathological complete response; five had post-chemotherapy tumor stage smaller than baseline tumor stage and no tumor volume increase measured on T2w MR images; and eight had a significant (greater than 70%) volume reduction from baseline to post-chemotherapy MRI exams. These nineteen cases were defined as responders. Of the remaining six cases, four were found with no tumor downstaging and had
volume progression or volume reduction less than 50%; two did not have radical
cystectomy due to their unresectable bladder tumor, but, were measured with a
volume increase and a volume reduction less than 50% from baseline to post-
chemotherapy on axial T2w MR images. These six cases were defined as non-
responders. Table 4.1 summarizes the number of defined responders and non-
responders.

4.3.2 Determination of optimal $k$ and centroids

Figure 4.2 shows the dependence of $V_1$ and $V_2$ on the number of cluster $k$. Both
$Intra_k$ and $Inter_k$ decreases with the number of clusters $k$. The minimum $V_2$ is
obtained with $k$ of 2. Due to the presence of the factor $k$, $V_2$ drastically increased
with the values of $k$. In other words, $V_2$ is more dependent of factor $k$ than of
$Intra_k$ and $Inter_k$. However, the latter two quantities are the most important
measures for the outcomes of k-means clustering. Meanwhile, $V_1$ showed its
reliability and stability over the values of $k$ and is minimized at $k$ of 3 which is a
desired small value. Hence, $k$ of 3 was selected as the optimal number of
clusters.

The three corresponding centroids were determined at ($k_{ep}$ (min$^{-1}$), Amp (a.u.)) of
(0.4, 1.7), (0.5, 4.0), and (2.1, 2.2). DCE-MRI pharmacokinetic parameters of
bladder tumor voxels was partitioned by k-means clustering in three clusters:
cluster 1 with low $k_{ep}$ and low Amp, cluster 2 with low $k_{ep}$ and high Amp, and
cluster 3 with high $k_{ep}$ and medium Amp. The signal enhancement characteristics
of the three clusters are illustrated in Figure 4.3.
Figure 4.2: Validity indexes $V_1$ and $V_2$ versus the number of clusters $k$. Both $\text{Intra}_k$ and $\text{Inter}_k$ decreases with the number of clusters. The upper plot shows the dependence of $V_1$ on $k$; the lower plot shows the dependence of $V_2$ on $k$. While $V_1$ slightly changes with $k$ and reaches minimum at $k$ of 3, $V_2$ is minimum at $k$ of 2 and drastically increases with $k$.

Figure 4.3: Signal enhancement characteristics of the three clusters. Cluster 1 with low $k_{ep}$ and low Amp has a flat and shallow curve. Cluster 2 with low $k_{ep}$ and high Amp has a high curve. Cluster 3 with high $k_{ep}$ and medium Amp has a steep-sloped curve. $k_{ep}$ and Amp values above each graph are the centroids.
4.3.3 Responders versus non-responders

At baseline, the volume fractions of cluster 1, 2, and 3 were 52±13(%), 31±11(%), and 17±13(%) for responders, and 51±20(%), 40±15(%), and 10±8(%) for non-responders, respectively. There was no significant difference found between responders and non-responders in the three cluster volume fractions.

From baseline to mid-cycle, k-means clustering showed that there was heterogeneity of chemotherapeutic response, which is shown by volume reduction, among the three clusters in the group of responders. On color cluster maps, it was seen that cluster 1 and cluster 3 had a larger volume reduction than cluster 2, and/or that the voxels of cluster 1 and cluster 3 were replaced with the voxels of cluster 2 in responders at the mid-cycle time point (Figure 4.4). Quantitatively, this resulted in the difference of the volume fraction change among the three clusters. The cluster 2 volume fractions of eighteen responders increased while the cluster 1 volume fractions of fourteen responders and the cluster 3 volume fraction of fifteen responders decreased (Figure 4.6).

On the contrary, non-responsive tumors showed a larger volume increase in cluster 1 and cluster 3 than in cluster 2, and/or a replacement of the voxels of cluster 1 and cluster 3 for the voxels of cluster 2 on color cluster maps (Figure 4.5) at mid-cycle. Compared to responders, non-responders showed an opposite trend in the cluster volume fraction changes. Cluster 2 volume fractions of all non-responders decreased while cluster 1 volume fractions of all non-responders and cluster 3 volume fractions of five non-responders increased (Figure 4.6).
Figure 4.4: The change of cluster volume fractions from baseline to mid-cycle in a responder. The upper figures show the color cluster maps that overlay on an axial bladder image at baseline (Image A) and mid-cycle (Image B). The lower plots demonstrate the change of cluster volume fractions of an entire responsive bladder tumor from baseline (left) to mid-cycle (right).
Figure 4.5: The change of cluster volume fractions from baseline to mid-cycle in a non-responders. The upper figures show the color cluster maps that overlay on an axial bladder image at baseline (Image A) and mid-cycle (Image B). The lower plots demonstrate the change of cluster volume fractions of an entire non-responsive bladder tumor from baseline (left) to mid-cycle (right).
4.3.4 Cluster volume fraction change as a response predictor

Area-under-the-curve (AUC) values determined from ROC curve analysis were 0.82, 0.96, and 0.84 for cluster 1, cluster 2, and cluster 3, respectively (Figure 4.7). The selected cutoff values were 0%, 2%, and 1% for cluster 1, 2, and 3, respectively (Figures 4.6 and 4.7). The sensitivities, specificities, and accuracies given by these cutoff values were shown in Table 4.3. Among the three clusters, cluster 2 volume fraction change presented with the highest sensitivity, specificity, and accuracy of 89%, 100%, and 92% (Figures 4.6 and 4.7, and Table 4.3).

The 95% Confidence Intervals for the volume fraction changes of cluster 1, 2, and 3 were respectively (-15%, 1%) for responders and (0%, 22%) for non-responders, (9%, 24%) for responders and (-39 %, 4%) for non-responders, and (-15%, -4%) for responders and (-8%, 22%) for non-responders (Table 4.2).
Figure 4.6: The change of cluster volume fractions from baseline to mid-cycle in responders versus non-responders. The upper left graph shows the average value of volume fraction change of the three clusters for responder group vs. non-responders group. The other three graphs illustrate the volume fraction change of cluster 1 (upper right), cluster 2 (lower left), and cluster 3 (lower right) for all nineteen responders (blue triangles) and six non-responders (red triangles). The dot lines represent the cutoff values -0.1% for Cluster 1, 2.4% for Cluster 2, and 0.9% for Cluster 3.
Figure 4.7: ROC curve analysis of the volume fraction changes for predicting chemotherapeutic response in bladder cancer. Upper left plot is ROC curve for cluster 1; upper right plot is ROC curve for cluster 2; lower left plot is ROC curve for cluster 3; lower right plot is the comparison of the three ROC curves. The cutoff values of the volume fraction changes for cluster 1, 2, and 3 were selected with the points marked with 0.80, 0.80, and 0.67, respectively. It is shown on the ROC curves for comparison that cluster 2 volume change is the best parameter for predicting chemotherapeutic response.
Table 4.1: Responders and non-responders

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathological complete response</td>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Downstaging and no volume increase</td>
<td>5&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>More than 50% volume reduction</td>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>No downstaging and a volume increase or no significant volume reduction</td>
<td>4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No cystectomy and a volume increase or no significant volume reduction</td>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Responder, <sup>b</sup> three of them had more than 50% volume reduction, <sup>c</sup> Non-responder

Table 4.2: Volume fraction changes of cluster 1, 2, and 3 in responders vs. non-responders.

<table>
<thead>
<tr>
<th>Volume fraction change</th>
<th>95% CI for responders</th>
<th>95% CI for non-responders</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>(-15%, 1%)</td>
<td>(0%, 22%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>(9%, 24%)</td>
<td>(-39%, 4%)</td>
<td>≤ 0.001</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>(-15%, -4%)</td>
<td>(-8%, 22%)</td>
<td>≤ 0.02</td>
</tr>
</tbody>
</table>

Table 4.3: Using ROC curve analysis, the cutoff values of volume fraction changes of clusters 1, 2, and 3 were determined to predict chemotherapeutic response in bladder cancer.

<table>
<thead>
<tr>
<th>Volume fraction change</th>
<th>Cutoff value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>0%</td>
<td>74%</td>
<td>83%</td>
<td>76%</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>2%</td>
<td>89%</td>
<td>100%</td>
<td>92%</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>1%</td>
<td>84%</td>
<td>83%</td>
<td>84%</td>
</tr>
</tbody>
</table>


4.4 Discussion

Neoadjuvant chemotherapy has been established as a standard treatment before definitive radical cystectomy for muscle-invasive bladder cancer to reduce the tumor burden (down-staging or treatment of micro-metastatic disease) (58-60). About half of bladder malignant tumors that are resistant to chemotherapy may progress during the time of the treatment, causing the avoidable complication during cystectomy (58-60). Thus, it is essential to early predict the final chemotherapeutic response of bladder cancer in order to direct resistant cases to early cystectomy (58-60). Currently, there are no tools to meet this critical need in both clinical tests and bladder imaging including Computed Tomography (CT) and Ultrasound (US). Extensive research is being performed to seek a biomarker that can effectively classify chemo-resistant and chemo-sensitive bladder tumors (40, 42, 60-63). By analyzing the complexity in molecular activities, gene expression has found several potential gene biomarkers for the prediction of therapeutic response (60-62). For example, gene expression profiling has found DNA damage as an indicator of chemo-resistance and several potential gene markers. However, gene expression profiling lacks the analysis of in vivo factors and the findings still need further validation in multi-center clinical trials (60). The other approach that has been evaluated is MRI of bladder cancer. With the ability to non-invasively perform functional imaging such as diffusion-weighted MRI (DWI) and dynamic contrast-enhanced MRI (DCE-MRI), MRI can reveal the in vivo pathological characteristics such as high cellularity and angiogenesis in cancerous tissues to extract the information on therapeutic response in bladder
cancer (40, 42, 63). Quantitative assessment of tumor water diffusion showed the relationship of apparent diffusion coefficient (ADC) with pathological complete response of bladder cancer. Even though performed at low field 0.5 Tesla MRI, the study by Dobson et al (40) found that DCE-MRI had high negative predictive value and high sensitivity in distinguishing the residual or recurrent tumors from radiation change in bladder cancer patients. The findings of these studies have demonstrated that the detailed analysis of the pathological profile of cancer tissues via functional MRI parameters can be an effective tool for the assessment of therapeutic response in bladder cancer. Our study has also shown that k-means clustering-based analysis of pharmacokinetic parameters partitioned each bladder tumor in three clusters that have different characteristics in responsiveness to chemotherapy. These different characteristics in the three clusters helped identify responders and non-responders at the mid-cycle time point of chemotherapy.

DCE-MRI pharmacokinetic parameters characterize for the micro-vasculature and the leakage of micro-vessels in cancer tissues and have been shown to be potential to early predict therapeutic response in different types of cancers (64-67, 69, 71). Early changes in DCE-MRI pharmacokinetic parameter $K^{trans}$ was reported to be the best parameter (with AUC of 0.93 from ROC analysis) at predicting final clinical and pathological response to neoadjuvant chemotherapy in primary breast cancer (64). The increase of $K^{trans}$ and $k_{ep}$ was the indication of good response of oral cancer to chemoradiotherapy (65). Pharmacokinetic parameters $K^{trans}$, $v_e$ and $k_{ep}$ from high field 3T DCE-MRI had a potential to
predict tumor response to therapy in colorectal (66) and cervical cancers (67). In bladder cancer, previous studies showed that DCE-MRI revealed the correlation between pharmacokinetic parameters and therapeutic response at mid-cycle and post-therapy, and suggested that the analysis of DCE-MRI pharmacokinetic parameters can be an important tool to assess and predict therapeutic response in bladder cancer (40)(77). Our 3T DCE-MRI study has found that the volume fraction change of cluster 2 was the best predictor of bladder cancer response to chemotherapy at the mid-cycle time point with the AUC value of 0.96 from ROC analysis.

K-means clustering is one of the most effective data mining tools and has been more widely used to characterize the heterogeneity within a malignancy and to understand the pathogenic pattern of malignant tissues (69-72). It was shown that k-means clustering of ADC values was better than pure mean ADC at characterizing neck malignancies and distinguishing malignant from benign tissues (72). Baudelet (70) showed that k-means-based cluster analysis could display the heterogeneity of tumor hemodynamic response. K-means clustering of DCE-MRI pharmacokinetic parameters can take the advantages of both k-means clustering method and DCE-MRI to partition the whole tumor voxels based on microvascular characteristics to extract useful diagnostic information (69, 71). Comparing k-means clustering and three-time-points methods in the analysis of malignant intra-tumoral kinetic curves in DCE-MRI, the study by Lee et al (71) indicated that optimal k-means clustering was better at partitioning intra-tumoral kinetic patterns than the other technique. In our study, k-means
clustering of bladder tumoral pharmacokinetic parameters partitioned bladder
tumors in three clusters whose volume fraction changes were different between
responders and non-responders. Cluster 2 volume fraction in the group of
responders increased while that of non-responders decreased after two cycles of
chemotherapy. The increase of cluster 2 volume fractions in responders was
partly attributed to the inflammatory or reactive changes in responsive tumors.
The volume fractions of cluster 1 and cluster 3 in responders decreased while
those in non-responders increased. The increase of cluster 3 (high $k_{ep}$ and
medium $Amp$) volume fraction in non-responders was attributed to the growth of
malignant tissues while the increase of cluster 1 volume fraction (low $Amp$ and
low $k_{ep}$) was attributed to the presence of necrotic tissues.

Brix’s linear two-compartment pharmacokinetic model (56) has been a commonly
used pharmacokinetic model to analyze DCE-MRI data. However, this model has
recently been applied less often to process DCE-MRI data due to its limitation in
explaining the biexponential arterial input function (45). The modification to Brix’s
model proposed by Yang (45) solved the limitation to acquire pharmacokinetic
parameters that have comparative tissue specificity to the pharmacokinetic
parameters derived from the Tofts’s model, the other widely used linear two-
compartment pharmacokinetic model (55). In this study, the modified Brix’s
model was used to quantify the micro-vascular characteristics of tumor tissues
through the dynamic signal enhancement by MR contrast agent. The slow
injection of contrast agent used in our study not only allowed the observation of
wash-in phase which is characterized by pharmacokinetic parameter $k_{ep}$, but also was given in a more controlled way with the modified Brix’s model (57). The results have demonstrated that pharmacokinetic parameters derived from the modified Brix’s model were useful in the assessment of microcirculation change of bladder cancer induced by chemotherapy.

One of the foremost problems in k-means clustering is the determination of $k$, the number of clusters. Andersen et al (69) used the validation index $V_2$ to determine the optimal number of clusters in k-means clustering of two Tofts’s pharmacokinetic parameters of cervical cancers and found that $k$ of 3 was the optimal number of clusters and that one of the three clusters had the volume fraction associated with primary tumor control. Making comparison of the two validation indices $V_1$ and $V_2$, our study found that $V_2$ is not reliable in k-means clustering of the two modified Brix’s pharmacokinetic parameters in bladder cancer because the strong influence of $k$ on $V_2$ outweighed the influence of the two more important measures that characterize for the intra-cluster compactness and the inter-cluster separation. Using the validation index $V_1$, the optimal $k$ of 3 was also determined in the study. The changes of the three cluster volume fractions from baseline to mid-cycle MRIs trended in the opposite directions and were significantly different between responders versus non-responders.

Our study had two limitations. The number of non-responders was small (N=6). This limitation is difficult to control since it naturally depends on the patients’ response to chemotherapy. However, a larger population will generally increase
the number of non-responders. The other limitation was that we did not evaluate
the effect of noise filter on the voxel-wise Amp and $k_{ep}$ datasets. This limitation
can be solved in the future studies by applying different noise-filtering methods to
find out whether any filter methods help better analyze voxel-wise Amp and $k_{ep}$
parameters.

In conclusion, while assessing therapeutic response based on MRI volume
change is not reliable, this proposed mathematical analysis of pharmacokinetic
parameters demonstrates its robustness in characterizing the complex changes
of microcirculation at mid-cycle MRI to enable early prediction of
chemotherapeutic response in bladder cancer. These promising findings already
have led to an ongoing prospective validation study to use this analytical
approach for the assessment of neoadjuvant chemotherapeutic response in
bladder cancer.
5 Diagnostic Value of Relative Apparent Diffusion Coefficient in T Staging of Bladder Cancer

5.1 Introduction
Local staging (T staging) of bladder cancer is essential for the determination of treatment plan and the assessment of patient outcome. Cystoscopy remains the standard tool for the detection and staging of bladder cancer. Its disadvantages include the invasive procedure, the limitation in diagnosing flat lesions, and the lack of the assessment of extravesical invasion of bladder tumors (8, 58).

Computed Tomography (CT), the most widely used imaging modality for bladder cancer, requires ionized radiation dose, lacks soft tissue contrast to visualize the depth of tumor invasion (12) and has low interobserver agreement in bladder cancer staging (15). Hence, non-invasive and accurate staging of bladder cancer is still an unmet clinical need in bladder cancer patient management.

Magnetic Resonance Imaging (MRI) without any risk of ionized radiations is able to non-invasively perform multiplanar and functional imaging with high soft tissue contrast to improve bladder cancer diagnosis. Diffusion-weighted MRI with the ability to quantify the water diffusion in body tissues via the Apparent Diffusion Coefficient (ADC) has been evaluated to improve the diagnosis of bladder cancer
Quantitative assessment with DWI has been shown to be useful in the diagnosis of bladder cancer including the differentiation of malignant versus benign tissues, determination of tumor stage and grade, and the assessment of chemotherapeutic response in bladder cancer.

The measurement of ADC is influenced by numerous factors that include the MRI sequence parameters, especially b-values, and the post-processing methodology. Even though they used the same type of DWI sequence (Echo planar imaging-EPI), the reported ADCs varied in the published studies. ADC was calculated by using two different b-values that were b-value of 0 and a non-zero b-value. Since these studies used different non-zero b-values, they obtained different ADCs. Therefore, a more universal approach to quantifying ADC needs to be developed to reduce the impact of b-value difference and the calculation methodology on ADC. To this end, our study proposes to determine ADC from curve-fitting of signal intensities with four b-values and to use a relative tumor ADC that is determined in relation to the ADC of the urine-filled bladder volume instead of using the absolute tumor ADC.

The goal of this study is to demonstrate the feasibility and to explore the reliability of the relative ADC for T staging of bladder cancer.
5.2 Materials and Methods

5.2.1 Patients

Inclusion criteria for patient data are: (1) The patients completed their MRI exams; (2) There was no image distortion in DWI data; (3) the visual inspection by radiologists evaluated that the bladder distention volume was moderate and enabled diagnosis; (4) patients had radical cystectomy and pathological staging of cystectomy bladder specimens was available.

One patient did not complete MRI exams with DWI due to their morbidity. Seven patients did not have radical cystectomy. Seven patients had low bladder distension or image distortion in DWI acquisition. Seventeen patients had no bladder tumor seen on DWI images. These thirty-two patients were excluded from this DWI-focused study.

Therefore, eighteen patients could be included in this study. All patients had transurethral resection of bladder tumor (TURBT) prior to their first MRI exam. The pathological examination of the TURBT bladder specimens confirmed the invasive bladder cancer in all patients. The flow chart of DWI study was described in Figure 1. Eight patients had radical cystectomy after their baseline MRI. Three patients had a baseline MRI and a mid-cycle MRI after two cycles of chemotherapy, followed by the cystectomy. The other seven patients had all three MRIs and completed four cycles of chemotherapy, followed by the cystectomy. The pathological examination of cystectomy bladder specimens
5.1 Study flow chart. All patients had a baseline MRI. Eight patients did not have chemotherapy and had cystectomy after their baseline MRI. Three patients were sent to cystectomy after two cycles of chemotherapy. The other seven patients completed chemotherapy before cystectomy. Pathological staging of cystectomy bladder specimens was used as reference standard.

staged the bladder tumors. Pathological tumor staging was used as a reference standard.

5.2.2 Concept of relative ADC

The measured (calculated) ADC of the same region of interest (ROI) can vary with the method and parameters, specifically b-values, used for the calculation. These calculated ADC values are composed of actual component (Actual ADC) and fluctuated component (ADC fluctuated by the method or b-values).

Mathematically, it can be described as follow:

\[
\text{Calculated ADC} = \text{Actual ADC} + \text{Fluctuated ADC} \quad [1]
\]
Because the fluctuating factors are similar in different ROIs of the DWI data (with the same calculation method and the same b-value), calculated ADC was hypothesized to be similar in different ROIs. Thus, if a relative ADC was used, the fluctuated component can be more or less canceled out. We propose to use the tumor ADC that is relative to urine ADC as follow:

\[
\text{\( ADC_{\text{relative}} = ADC_{\text{urine}} - ADC_{\text{tumor}} \)} \quad [2]
\]

Calculated \( ADC_{\text{urine}} = \text{Actual } ADC_{\text{urine}} + \text{Fluctuated } ADC_{\text{urine}} \) \quad [3]

Calculated \( ADC_{\text{tumor}} = \text{Actual } ADC_{\text{tumor}} + \text{Fluctuated } ADC_{\text{tumor}} \) \quad [4]

We hypothesize that \( \text{Fluctuated } ADC_{\text{urine}} \approx \text{Fluctuated } ADC_{\text{tumor}} \). Therefore, there is no fluctuated component in the calculated relative ADC as follow:

\[
\text{Calculated } ADC_{\text{relative}} = \text{Actual } ADC_{\text{urine}} - \text{Actual } ADC_{\text{tumor}} \quad [5]
\]

With this definition, \( ADC_{\text{relative}} \) is inversely proportional to \( ADC_{\text{tumor}} \). Therefore, large \( ADC_{\text{tumor}} \) will lead to small \( ADC_{\text{relative}} \) and vice versa.

To test our hypothesis, we compare the standard deviation of \( ADC_{\text{relative}} \) with 3 different b-values and that of \( ADC_{\text{tumor}} \) with 3 different b-values to find out if the former is significantly smaller than the latter:

\[
\delta_{\text{tumor}} = \text{Standard deviation} (ADC_{333}^{tumor}, ADC_{667}^{tumor}, ADC_{1000}^{tumor}) \quad [6]
\]

\[
\delta_{\text{relative}} = \text{Standard deviation} (ADC_{333}^{relative}, ADC_{667}^{relative}, ADC_{1000}^{relative}) \quad [7]
\]
5.2.3 Data and statistical analysis

A radiologist (more than 10 years of experience) determined tumor ROIs on ADC color maps. The ROIs were required to cover the whole tumor regions. ROI was also placed on bladder urine for each patient. $ADC_{urine}^{u1000}, ADC_{urine}^{u667}, ADC_{urine}^{u333}$, and $ADC_{urine}^{urine}$ were recorded.

Calculation of $\delta_{tumor}$ and $\delta_{relative}$ for each tumor was done in the following steps:

(i) $\delta_{tumor}$ was calculated by using Eq. [6] and recorded $ADC_{tumor}^{tumor}, ADC_{tumor}^{667},$ and $ADC_{tumor}^{1000}$

(ii) $ADC_{urine}^{rel}, ADC_{urine}^{rel},$ and $ADC_{urine}^{rel}$ were calculated using Eq. [2] and recorded $ADC_{urine}^{u1000}, ADC_{urine}^{u667},$ and $ADC_{urine}^{u333}$

(iii) $\delta_{relative}$ was calculated using Eq. [7]

The two standard deviations were compared using student t-test. P<0.05 was considered to be statistically significant.

For each tumor, instead of using one of the ADC values ($ADC_{tumor}^{tumor}, ADC_{tumor}^{667},$ and $ADC_{tumor}^{1000}$), we used $ADC_{cf}^{tumor}$ to determine $ADC_{cf}^{rel}$ using Eq. [1]; and the determined $ADC_{cf}^{rel}$ was correlated to the pathologically confirmed tumor stage. P<0.05 from student t-test was considered to be statistically significant.
5.3 Results

5.3.1 Relative tumor ADC versus absolute tumor ADC

The standard deviation of absolute tumor ADC, $\delta_{tumor}$, was found from $0.1 \times 10^{-3}$ (mm$^2$/s) to $1.2 \times 10^{-3}$ (mm$^2$/s) with a median of $0.4 \times 10^{-3}$ (mm$^2$/s) and an average of $0.4 \times 10^{-3}$ (mm$^2$/s) in the eighteen patients. The standard deviation of relative tumor ADC, $\delta_{relative}$, was found from $0.0 \times 10^{-3}$ (mm$^2$/s) to $1.1 \times 10^{-3}$ (mm$^2$/s) with a median of $0.3 \times 10^{-3}$ (mm$^2$/s) and an average of $0.2 \times 10^{-3}$ (mm$^2$/s). Student t-test showed that $\delta_{relative}$ is significantly smaller than $\delta_{tumor}$ ($P<0.05$).

5.3.2 Pathological findings

Pathological examination of eighteen patients found two with stage Tis, one with stage T1, five with stage T2, six with stage T3, and four with stage T4. There were three patient with non-muscle-invasive tumors (stage Tis, Ta, or T1) and fifteen patients with muscle-invasive tumors (stage T2, T3, T4). Eight patients were found with an organ-confined tumor, and ten were found with a non-organ-confined tumor. All tumors were found in high grade. Histologic type of tumor cells was urothelial carcinoma in twelve tumors, a mixture of small (98%) and glandular (2%) cells in one, neuroendocrine cells in one, and urothelial carcinoma with other type (squamous cells or myxoid stroma) differentiation in four. Tumor size ranged from 8 to 100 mm with a median of 33 mm (average ± standard deviation = 41 ± 28 mm).
Figure 5.2: A male patient, age of 65 years old. The left image is a color-coded ADC map. The right graph is the signal intensity curve with 4 different b-values. Tumor location (marked by black ROI contour) is trigone. Tumor stage is Tis.

\[
\text{ADC}_{333} = 1.2 \times 10^{-3} \text{ (mm}^2/\text{s)}; \quad \text{ADC}_{667} = 1.4 \times 10^{-3} \text{ (mm}^2/\text{s)}; \quad \text{ADC}_{1000} = 1.3 \times 10^{-3} \text{ (mm}^2/\text{s)} \\
\text{ADC}_{\text{cf}} = 1.3 \times 10^{-3} \text{ (mm}^2/\text{s)}; \quad \text{ADC}_{\text{urine}} = 2.6 \times 10^{-3} \text{ (mm}^2/\text{s)}; \quad \text{ADC}_{\text{relative}} = 1.3 \times 10^{-3} \text{ (mm}^2/\text{s)}
\]
Figure 5.3: A male patient, age of 59 years old. The left image is a color-coded ADC map. The right graph is the signal intensity curve with 4 different b-values. Tumor location (marked by black ROI contour) is left lateral wall. Tumor stage is T2.

\[
\begin{align*}
\text{ADC}_{333} &= 1.8 \times 10^{-3} \text{ (mm}^2/\text{s)}; \\
\text{ADC}_{667} &= 1.6 \times 10^{-3} \text{ (mm}^2/\text{s)}; \\
\text{ADC}_{1000} &= 1.3 \times 10^{-3} \text{ (mm}^2/\text{s)}; \\
\text{ADC}_{cl} &= 1.3 \times 10^{-3} \text{ (mm}^2/\text{s)}; \\
\text{ADC}_{urine} &= 2.7 \times 10^{-3} \text{ (mm}^2/\text{s)}; \\
\text{ADC}_{\text{relative}} &= 1.4 \times 10^{-3} \text{ (mm}^2/\text{s)}
\end{align*}
\]
Figure 5.4: A male patient, age of 55 years old. The left image is a color-coded ADC map. The right graph is the signal intensity curve with 4 different b-values. Tumor location (marked by black ROI contour) is left wall and trigone. Tumor stage is T3.

\[
\text{ADC}_{333} = 1.7 \times 10^{-3} \text{ (mm}^2/\text{s}) ; \quad \text{ADC}_{667} = 1.5 \times 10^{-3} \text{ (mm}^2/\text{s}) ; \quad \text{ADC}_{1000} = 1.2 \times 10^{-3} \text{ (mm}^2/\text{s}) \\
\text{ADC}_{\text{cf}} = 1.3 \times 10^{-3} \text{ (mm}^2/\text{s}) ; \quad \text{ADC}_{\text{urine}} = 4.5 \times 10^{-3} \text{ (mm}^2/\text{s}) ; \quad \text{ADC}_{\text{relative}} = 3.2 \times 10^{-3} \text{ (mm}^2/\text{s})
\]
Figure 5.5: A female patient, age of 83 years old. The left image is a color-coded ADC map. The right graph is the signal intensity curve with 4 different b-values. Tumor location (marked by black ROI contour) is posterior. Tumor stage is T4a, invasion of the uterus (indicated by the white arrow and oval).

$\text{ADC}_{333} = 1.9 \times 10^{-3} \, (\text{mm}^2/\text{s})$; $\text{ADC}_{667} = 1.9 \times 10^{-3} \, (\text{mm}^2/\text{s})$; $\text{ADC}_{1000} = 1.5 \times 10^{-3} \, (\text{mm}^2/\text{s})$

$\text{ADC}_{\text{urine}} = 1.7 \times 10^{-3} \, (\text{mm}^2/\text{s})$; $\text{ADC}_{\text{relative}} = 7.6 \times 10^{-3} \, (\text{mm}^2/\text{s})$; $\text{ADC}_{\text{relative}} = 5.9 \times 10^{-3} \, (\text{mm}^2/\text{s})$
Figure 5.6: Average relative ADC of tumor stage T1 or lower, T2, T3, and T4.

Figure 5.7: Boxplot of relative ADC of organ-confined tumors vs. non-organ-confined tumors.
5.3.3 Correlation of relative ADC with tumor stage

The absolute ADCs (average ± standard deviation) for the groups of Tis-Ta-T1 stage, T2 stage, T3 stage, T4 stage were \((2.5 \pm 0.9) \times 10^{-3}\), \((1.9 \pm 0.9) \times 10^{-3}\), \((1.6 \pm 0.5) \times 10^{-3}\), \((2.0 \pm 0.6) \times 10^{-3}\) (mm²/s), respectively. There was no statistical significance found in the absolute ADCs of different stages.

The relative ADC values (average ± standard deviation) for group Tis-Ta-T1, T2, T3, T4 were \((1.3 \pm 0.3) \times 10^{-3}\), \((1.9 \pm 0.4) \times 10^{-3}\), \((1.8 \pm 0.9) \times 10^{-3}\), \((3.7 \pm 1.5) \times 10^{-3}\) (mm²/s), respectively (Figure 6). The relative ADC of group Tis-Ta-T1 was found to be significantly lower than that of group T2 (P<0.03), group T3 (P<0.01) and group T4 (P<0.03). The relative ADC of group T2 was also significantly smaller than that of group T3 (P<0.04) and T4 (P<0.05). There was no significant difference found between ADC of T3 vs T4 stages, and T1 vs T3 stages. Figures 2, 3, 4, and 5 show the color map and signal intensity curve for each stage group (Tis to T1 – Figure 5.2, T2- Figure 5.3, T3- Figure 5.4, and T4 – Figure 5.5). Figure 5.6 presents the average relative ADC of each stage group.

There was a significant difference (P<0.001) in relative ADC between non-muscle-invasive tumors (stage Tis, Ta, or T1) and muscle-invasive tumors (stage T2, T3, or T4). There was also a significant difference (P<0.01) in relative ADC between organ-confined tumors (T2 or lower stage) and non-organ-confined tumors (T3 or higher stage) (Figure 5.7).
5.4 Discussion

Quantification of tissue Apparent Diffusion Coefficient (ADC) recently has been more commonly used to assess the pathophysiological characteristics of bladder cancer. ADC was shown to be able to differentiate malignant tissues from normal bladder wall or benign tissues (27, 78, 79). In tumor grading, ADC values were reported to be significantly lower in high grade than in low grade (24, 27). In addition, Takeuchi et al (80) found that ADC in G3 grade (severe degree of anaplasia) was significantly lower than that in G2 (mediate degree of anaplasia) and G1 (the least degree of anaplasia) grades. Rosenkrantz et al (82) reported that ADC in cases with metastatic disease was significantly lower than that in cases without metastatic disease. In local staging of bladder cancer, ADC was found to be significantly higher in non-muscle-invasive tumors than in muscle-invasive tumors (28). Our study has also found that relative ADC in non-muscle-invasive tumors was significantly lower than that in muscle-invasive tumors. This implies that non-muscle-invasive tumor ADC was significantly higher than muscle-invasive tumor ADC. Furthermore, relative ADC helped differentiate T1 or lower from T2 and T4 stage, and T2 from T4 stage. It also helped distinguish organ-confined from non-organ-confined tumors. Therefore, ADC is useful not only for the determination of treatment approach but also for making decision during cystectomy.

In previous studies, different b-values were used to quantify ADC. Rosenkrantz et al (82) used b-values of 0, 400, 800 s/mm² and found a malignant tumor ADC range of (0.85-1.84)x10⁻³ mm²/s. Avcu used b-values of 0, 500, 1000 s/mm² and
obtained a tumor ADC average of \((1.1 \pm 0.26 \text{ [standard deviation]}) \times 10^{-3} \text{ mm}^2/\text{s}\).

These two studies used different non-zero b-values, but, did not specify how ADC was calculated using these b-values. Daggulli et al (28) used b-values of 0, 100, 600, 1000 s/mm² and acquired malignant tumor ADC of \((1.4\pm0.1) \times 10^{-3} \text{ mm}^2/\text{s}\), \((1.1\pm0.1) \times 10^{-3} \text{ mm}^2/\text{s}\), \((0.9\pm0.1) \times 10^{-3} \text{ mm}^2/\text{s}\), respectively. Other studies used only one non-zero b-value of 800 s/mm² (78, 79) or 1000 s/mm² (24, 28, 80). Even though patient cohorts played an important role in ADC difference among studies, methodological difference must have mainly contributed to the ADC difference. It was clearly shown in the study by Daggulli et al (28) in which different ADCs were obtained with different b-values in the same patient. Our study also indicates that ADC changed with b-values. Previous studies measured ADC by using b-value of 0 and a non-zero b-value. This method was largely affected by the selected non-zero b-value. Our study proposes to use curve-fitting with b-value of zero and three different non-zero b-values to acquire ADC to alleviate the bias of using only one non-zero b-value among different studies.

A limitation in our study is that the number of patients is small (N=18). However, it is still able to statistically demonstrate its potential. This justifies a larger prospective study to validate this approach.

In conclusion, the ADC derived from curve-fitting and in reference to the individual bladder urine has a potential to serve as a biomarker in local staging of bladder cancer and can help stratify treatment planning including decision-making during cystectomy.
6 Summary

6.1 Major findings

6.1.1 Diagnostic value of DCE-MRI in bladder cancer diagnosis

With the ability to quantify the microcirculation characteristics within bladder tumors, DCE-MRI was shown to be able to improve the detection of bladder cancer and early predict its chemotherapeutic response.

In bladder cancer detection, the addition of combined pharmacokinetic parameter (Amp and $k_{ep}$) maps to conventional T2w MR images helped identify sub-centimeter malignant tumors. The pharmacokinetic parameter maps also helped differentiate malignant tumors from benign bladder wall thickenings including the reactive and inflammatory changes induced by neoadjuvant chemotherapy. With these additional valuable visualizations, DCE-MRI overall significantly improved the sensitivity and accuracy of bladder cancer detection.

In the assessment of chemotherapeutic response in bladder cancer, DCE-MRI quantitatively revealed the heterogeneity of the micro-vascular density, characterized by pharmacokinetic parameter Amp, and the micro-vessel leakage, characterized by pharmacokinetic parameter $k_{ep}$, within bladder tumors. It was shown by these pharmacokinetic parameters that this heterogeneity leaded to
the heterogeneous chemotherapeutic response within bladder tumors. K-means clustering of Amp and $k_{ep}$ could partition a bladder tumor in three clusters that had different characteristics of microcirculation, thus, of chemotherapeutic response. The volume fraction changes of the three clusters within a tumor can be potential biomarkers to classify chemotherapy-responsive and chemotherapy-resistant bladder tumors at the mid-cycle time point of chemotherapy.

6.1.2 Diagnostic of DWI in bladder cancer diagnosis

With the capability of quantifying the cellularity within tumor tissues, DWI could assist in local staging of bladder cancer. The Apparent Diffusion Coefficient (ADC) relative to bladder urine ADC helped differentiate muscle-invasive from non-muscle-invasive tumors, and organ-confined from non-organ-confined tumors. On stage-by-stage basis, relative ADC was able to identify non-muscle-invasive-invasive tumors (in Tis, Ta, or T1 stage) and muscle-invasive tumors without fat invasion (in T2 stage). Therefore, DWI with relative ADC can aid in the determination of treatment strategy and the surgeon’s decision-making.

6.2 Limitations

6.2.1 The number of included patients

In the bladder cancer detection, there were thirty six patients included in the data analysis, of which twenty-eight were pathologically confirmed with malignant tumors and eight were negative for malignancy. The small number (N=8) of negative cases may be the reason that the increase of specificity by DCE-MRI
was found to be not statistically significant while the improvement of both sensitivity and accuracy by DCE-MRI were statistically significant.

Twenty-five patients were included in the chemotherapeutic response assessment. Based on the response criteria, nineteen patients were defined as responders; and six were defined as non-responders. The number of defined responders (N=6) was small.

Only the MRI data of eighteen patients were included in the tumor staging. The number of non-muscle-invasive tumors was small (N=3) for the assessment of muscle invasiveness of the cancer. On stage-by-stage basis, the numbers of tumors in different stages were small (N=3 for T1 or lower, N=5 for T2, N=6 for T3, and N=4 for T4).

6.2.2 Noise filtering

The study did not apply a noise filtering method to the assessment of pharmacokinetic parameters. There may have been the negative impact of unfiltered noise on the robustness of these pharmacokinetic parameters when they were used to assess the tumor microcirculation.
7 Prospective Studies

7.1 Comprehensive data assessment

More patient data will be included in the future studies to not only solidify the above summarized findings but also to enable a comprehensive assessment of conventional, DCE-MRI, and DWI datasets. When the number of patients whose three datasets are all assessable is large enough, the comprehensive assessment will be performed to evaluate: (1) the additional value of DWI compared to DCE-MRI in the diagnosis (detection, staging, and chemotherapeutic response assessment) of bladder cancer; (2) The usefulness of the combination of all three sequences in the bladder cancer diagnosis; (3) the robustness of the combination of pharmacokinetic parameters and ADC to assess the tumor stage and chemotherapeutic response in bladder cancer. The first two assessments will be based on the radiological interpretation of MRI data; and the third is based on the quantitative assessment.

7.2 Application of noise filtering methods

Several noise filtering methods will be applied to evaluate: (1) whether noise filtering significantly improves the robustness of pharmacokinetic parameter-
based assessment; (2) what filtering method is the most efficient in pharmacokinetic parameters.

7.3 Further application of relative ADC

The increased patient population will allow solidifying the findings of this study that showed the reliability of relative ADC in assessing bladder cancer. A new methodology in which relative ADC will take place of absolute ADC in characterizing bladder cancer will be developed. This methodology will: (1) generate the map of relative ADC that may help visualize bladder cancer for the detection and the local staging; (2) acquire voxel-wise relative ADC to add to the quantitative assessment of bladder cancer.

In summary, we develop a new imaging algorithm to improve the detection and characterization of bladder cancer and its changes during chemotherapy. We validate the use of multi-transmit to enable a consistent high-quality imaging and establish a comprehensive MRI approach combining high-resolution conventional imaging for morphology, DCE-MRI to map the microcirculation, and DWI to further characterize the biophysical properties of bladder cancer in vivo and during chemotherapy.
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