Identifying *in vivo* DCE MRI Parameters Correlated with *ex vivo* Quantitative Microvessel Architecture: A Radiohistomorphometric Approach.

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**ABSTRACT**

We introduce a novel radiohistomorphometric method for quantitative correlation and subsequent discovery of imaging markers for aggressive prostate cancer (CaP). While this approach can be employed in the context any imaging modality and disease domain, we seek to identify quantitative dynamic contrast enhanced (DCE) magnetic resonance imaging (MRI) attributes that are highly correlated with density and architecture of tumor microvessels, surrogate markers of CaP aggressiveness. This retrospective study consisted of five Gleason score matched patients who underwent 3 Tesla multiparametric MRI prior to radical prostatectomy (RP). The excised gland was sectioned and quartered with a rotary knife. For each serial section, digitized images of individual quadrants were reconstructed into pseudo whole mount sections via previously developed stitching program. The individual quadrants were stained with vascular marker CD31 and annotated for CaP by an expert pathologist. The stained microvessel regions were quantitatively characterized in terms of density and architectural arrangement via graph algorithms, yielding a series of quantitative histomorphometric features. The reconstructed pseudo whole mount histologic sections were non-linearly co-registered with DCE MRI to identify tumor extent on MRI on a voxel-by-voxel basis. Pairwise correlations between kinetic and microvessel features within CaP annotated regions on the two modalities were computed to identify highly correlated attributes. Preliminary results of the radiohistomorphometric correlation identified 8 DCE MRI kinetic features that were highly and significantly (p < 0.05) correlated with a number of microvessel parameters. Most of the identified imaging features were related to rate of washout (Rwo) and initial area under the curve (IAUC). Association of those attributes with Gleason patterns showed that the identified imaging features clustered most of the tumors with primary Gleason pattern of 3 together. These results suggest that Rwo and IAUC may be promising candidate imaging markers for identification of aggressive CaP *in vivo*.

1. INTRODUCTION

1.1 Overview

Development of diagnostic technologies has outpaced the resulting changes in clinical methodology for disease detection and characterization.\(^1\) Access to multi-scale, multi-modal data has allowed researchers to begin to identify new anomalies as well as redefine old diseases. Yet, clinical methods for diagnosis and prognosis remain largely unchanged. Such translational gaps occur as imaging modalities commonly emerge from advances in physics and/or chemistry, not necessarily from clinical need.\(^2\) Consequently, associations of acquired medical images with biological events of interest must be retrospectively studied. While it is clear that image phenotype

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harbors prognostic information, it is far from clear as to which specific imaging features are correlated with disease prognosis or how to identify or extract these features. Hence, additional studies must be performed to relate medical images with the underlying tumor biology. Improved understanding of their relationship could help set the stage for discovery of imaging biomarkers, characteristic imaging attributes associated with disease detection and/or severity. However, identification of such attributes requires long term clinical studies where imaging data is correlated with patient outcome. Such imaging based clinical trials could be very expensive as patients would have to be imaged and followed up over several years, which could significantly delay the integration of imaging technologies with clinical protocols.

1.2 Radiohistomorphometry: Correlation of radiologic and histologic attributes for imaging biomarker discovery
Interest in relating imaging phenotypes with tissue genotype to has led to the development of the field of radiogenomics for imaging biomarker identification. Radiogenomics involves correlation of quantitative imaging descriptors with gene or molecular signatures to identify prognostic imaging markers for the disease of interest. While genomics provides unique value in its ability to reveal functional changes at the molecular level, pathologic evaluation remains the clinical gold standard for disease characterization. In the context of several diseases such as breast and prostate cancer, disease phenotype on histologic specimens is used to make treatment decisions and predictions of long term patient outcome. Correlating histologic and radiologic phenotypes of a disease may allow for identification of prognostic imaging markers. However, one of the challenges in this regard is the subjective nature of histologic interpretation by pathologists. For instance, Allsbrook et al. found only a moderate agreement with an overall kappa coefficient of 0.435 between Gleason grades assigned by 41 pathologists for 38 biopsies. Furthermore, Gleason commented on intraobserver variability saying ‘I have duplicated exactly my previous histologic scores approximately 50% of the time and within ± 1 histologic score (range, 2-10) approximately 85% of the time’. In an attempt to develop consistent methods of tumor assessment, a few groups have recently begun to develop and apply advanced quantitative image analysis and feature extraction tools that model tumor appearance on digitized pathology specimens. These quantitative descriptors of tumor morphology have previously shown to be related to disease aggressiveness. We define radiohistomorphometry as the correlation of radiologic imaging markers with quantitative histomorphometric descriptors of disease aggressiveness, thereby enabling identification of radiologic biomarkers of patient outcome. In this paper, we present a radiohistomorphometric approach to identify imaging biomarkers for aggressive prostate cancer (CaP).

1.3 Need for biomarkers to determine prostate cancer aggressiveness
With advancements in CaP diagnostics, the percentage of patients with distant metastatic disease at the time of diagnosis has reduced from 20% in 1974-1985 to 5% in 1995-2000. Nonetheless, CaP incidence rate has increased significantly within the same time period, suggesting that patients with indolent disease make up a large part of CaP population. With an increasing population of overdiagnosed patients, there is growing interest in adopting active surveillance (AS) where patients are monitored for signs of disease progression without clinical intervention until required. However, success of AS strongly relies on the ability to selectively recruit patients with indolent tumors. Clinical measures, such as tumor node metastasis (TNM) stage, prostate specific antigen (PSA) level and Gleason scores, commonly obtained along the standard diagnostic pathway characterize CaP based on its appearance at a discrete time point. Such measures do not provide information about evolution of the disease to assess aggressiveness. Therefore, markers for tumor aggressiveness need to be established in order to identify patients with indolent tumors for AS.

1.4 Challenges in radiohistomorphometrics
Significant challenges must be overcome in order to (i) extract (ii) align and (iii) correlate quantitative descriptors from the two sets of images that reside at highly different resolutions. Prognostic information on histology lies in the morphology and distribution of microscopic structures. In order to quantitatively describe such attributes, accurate segmentation of histologic structures followed by appropriate feature extraction methods need to be established. A separate set of methods that capture macroscopic characteristics of tumor need to be developed for radiological feature extraction. Correlation analysis across multi-scale data obtained from radiologic and histologic features can only be meaningful when regions of interest on the two modalities are aligned. Such an alignment ensures that tumor measurements at the same locations across both modalities are being compared.
2. PREVIOUS WORK

Effectiveness of the new radiohistomorphometric method for identification of reliable imaging biomarkers correlated with disease aggressiveness and patient outcome is primarily dependent on the radiological modality under consideration as well as the quality of the surrogate markers of outcome. In this section, we briefly review clinical surrogate markers of outcome, as well as previous approaches to correlate imaging and high resolution data.

2.1 Biomarkers for CaP aggressiveness

Gleason score is a commonly used prognostic marker for CaP.\textsuperscript{9} Cellular and glandular appearance of tumor is examined to assign Gleason grades, which range from 1 to 5. As CaP is known to be highly heterogeneous, two grades reflecting the two most occurring patterns in the tumor are summed to obtain a Gleason score. Due to the qualitative nature of the scoring system, scores may be prone to interobserver variability.\textsuperscript{13} More significantly, Gleason scoring groups tumors into fixed, predefined finite number of bins, which do not directly reflect tumor aggressiveness.

Markers that capture fundamental biological characteristics provide insight into the rate of tumor progression. Cancer cells in aggressive tumors proliferate rapidly thus increasing demand for oxygen and nutrients, triggering angiogenesis and leading to increased vascular density. To monitor such biological events, tissues are stained with immunohistochemical (IHC) markers such as Ki67 and CD31/CD34 for cell proliferation and vascularity, respectively. Parameters such as tissue proliferation index and microvessel density (MVD) extracted from surgical specimens stained with these IHC markers are known to be highly correlated with disease aggressiveness.\textsuperscript{14–16} However, IHC markers constrain in vivo assessment of CaP aggressiveness to histological sections obtained via needle core biopsy, a method prone to sampling errors.

Most studies reporting correlation between MVD and outcome or a surrogate of outcome assess MVD on ex vivo specimens at hotspots, microvessel dense regions within tumor.\textsuperscript{14–16} Determination of the location and size of these hotspots is subjective due to the lack of standard, established criteria. Tretiakova et al.\textsuperscript{17} performed quantitative assessment of MVD without considering hotspots and found no correlation between MVD and Gleason scores. However, they qualitatively show differences in spatial uniformity of microvessels between benign and tumor microarray samples. Benign tissue largely shows uniform distribution of microvessels, whereas tumor tissue consists of heterogeneous microvessel arrangement.

Sustained angiogenesis, continued formation of new blood vessels, is one of the hallmarks of cancer.\textsuperscript{18} A measure of tumor angiogenic capacity may thus be indicative of tumor aggressiveness. As angiogenic capacity is not reflected in the phenotypic appearance of tumor, molecular measures such as gene expression have been explored. While in vivo assessment of angiogenic gene expression is limited by the aforementioned tissue sampling errors, ex vivo assessment generally lacks spatial resolution as molecular assays require large samples of tissue. In order to overcome the constraints of limited spatial resolution, Lenkinski et al. use a tissue printing technique to produce molecular portrait of tumor on CaP surgical specimen.\textsuperscript{19} Such methods provide a measure of spatially and temporally resolved vascular growth characteristics. On the other hand, histological measures such as MVD measure the accumulation of vascular growth over time and thus may not be able to distinguish between tumors that were once rapidly growing but have reached a plateau and the tumors that are beginning to grow. Although a local measure of angiogenesis is the ideal surrogate marker of tumor aggressiveness, accurate determination of net tumor angiogenic capacity remains challenging as it is known to be dictated by several pro and anti angiogenic factors. Therefore, expression of an isolated gene or a subset of genes may not be accurate indicator of tumor angiogenic capacity.\textsuperscript{20}

2.2 Role of radiological imaging in CaP prognosis

Multiparametric (MP) magnetic resonance imaging (MRI) has emerged as a promising tool for CaP diagnosis given its high sensitivity and specificity to detect clinically significant tumors.\textsuperscript{21} MP-MRI provides non-invasive, anatomical and functional imaging modalities, each of which convey different aspects of tissue biology. Recent studies have suggested that a combination of MP-MRI parameters may allow for identification of aggressive CaP in vivo.\textsuperscript{22–25}
Dynamic contrast enhanced (DCE) MRI is a functional imaging modality which acquires time series of T1 images capturing vascular uptake and washout of a paramagnetic contrast agent. Kinetics of dye perfusion suggest characteristics of the underlying tissue vascularity, a biological variable closely associated with tumor aggressiveness.\textsuperscript{14} Aggressive tumors, with a large network of poorly formed blood vessels, are expected to show faster dye uptake (Rup) and washout (Rwo), higher maximum uptake (MU), shorter time to peak (TTP) and increased enhancement (En) and enhancement ratio (EnR), as compared to indolent tumors.\textsuperscript{26} Therefore, we anticipate that DCE MRI kinetic features are effective imaging markers for CaP aggressiveness and will consequently be able to identify patients suited for AS.

2.3 Multi-scale data interpretation methods for imaging biomarker selection

2.3.1 Bi-variate correlation analysis

Previous studies have attempted to find DCE MRI markers using various surrogate markers of tumor aggressiveness. Lenkinski et al. qualitatively demonstrated correlation between select angiogenic molecular expression within tissue patches of RP sections and DCE MRI contrast enhancement values.\textsuperscript{19} Oto et al. presented a study comprising 73 patients who underwent RP and found a correlation of 0.453 (p=0.001) between mean vessel area fraction and \( k_{ep} \) parameter. However, tumor locations on MRI were identified manually by a radiologist on the basis of CaP annotations on whole mount RP sections.\textsuperscript{27} Similarly, Chen et al., reporting correlation of -0.75 (\( p<0.0001 \)) between washout gradient and Gleason score, localized CaP on MRI via radiologist annotations provided on the basis 12-cores TRUS biopsy.\textsuperscript{28} Such methods provide a rough estimate of tumor location, which may not be accurate on a per-voxel basis.

2.3.2 Radiogenomics (Radiomics)

Radiogenomics (or radiomics) aims to identify imaging markers correlated with known underlying biological patterns for non-invasive, \textit{in vivo} assessment of the disease. Segal et al. found that twenty eight computed tomography (CT) attributes were able to predict 78% of global gene expression profile in the context of liver cancer.\textsuperscript{29} In a related study by Gevaert et al. for non-small cell lung cancer, 114 manually segmented, qualitative and quantitative radiological features from CT and PET were found to be able to predict metagenes with 65-86% accuracy.\textsuperscript{30} Although promising, radiogenomics attempts to identify imaging markers by correlating radiological features with molecular measurements, whose clinical value remains to be explored.

Recent studies have shown that molecular profiling offers no more information than tumor morphology and IHC.\textsuperscript{31} Particularly in the context of CaP, analysis of IHC may be more informative than global gene expression profiling as it retains spatial resolution which allows us to monitor tumor heterogeneity.

Hence, we introduce a novel radiohistomorphometric approach for quantitative, rigorous, high-throughput screening of imaging markers in the context of prostate cancer aggressiveness. Unlike radiogenomics, our approach attempts to find radiological attributes that are correlated with quantitative histomorphometric characteristics. Specifically in the case of CaP, we investigate relationships between \textit{in vivo} DCE MRI kinetic parameters and \textit{ex vivo} microvessel features. Table 1 lists the various co-registration, image segmentation, feature extraction, and correlation methods employed in this work to identify imaging features associated with quantitative histomorphometric measurements of CaP aggressiveness.

### 3. METHODS

#### 3.1 Dataset Description

The patient population for this study consisted of 5 men with biopsy confirmed CaP and scheduled for radical prostatectomy (RP). \textit{In vivo} endorectal T2-weighted (T2-w) MRI and DCE MRI were acquired prior to RP with a 3-T MRI scanner. DCE MRI was imaged with temporal resolution of 16 seconds for 20 time points. \textit{Ex vivo} histological slices were divided into quadrants and stained with hematoxylin and eosin (H&E) and vascular marker, CD31. Immunohistochemistry (IHC) of formalin fixed paraffin embedded tissue was performed on a Leica Bond\textsuperscript{T M} instrument using the Bond Polymer Refine Detection System. Epitope retrieval was done for 10 minutes with Enzyme 1 solution. Antibodies against CD31 were used at a 1:25 dilution. Tumor was annotated on all digitized quadrants by expert pathologist. All the five cases included in this study had a Gleason score of 7. Primary, secondary and occasionally tertiary Gleason patterns for each section ranged from 3+3 to 4+4+5.
Table 1. List of methods comprising our radiohistomorphometric analysis to identify imaging markers for aggressive CaP

<table>
<thead>
<tr>
<th>Methodology for Radiohistomorphometric analysis</th>
<th>Section</th>
<th>Supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reconstruction of quartered radical prostatectomy (RP) fragments into pseudo whole mount RP sections</td>
<td>3.2.1</td>
<td>Figure 1</td>
</tr>
<tr>
<td>2. Histology-MRI Slice Correspondences</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>3. Histology-T2w MRI Registration</td>
<td>3.3</td>
<td>Figure 3C</td>
</tr>
<tr>
<td>4. T2w-DCE MRI Registration</td>
<td>3.3</td>
<td>Figure 3E</td>
</tr>
<tr>
<td>5. Histology-DCE MRI Registration</td>
<td>3.3</td>
<td>Figure 3G</td>
</tr>
<tr>
<td>6. Microvessel Segmentation</td>
<td>3.2.2</td>
<td>Figure 2B</td>
</tr>
<tr>
<td>7. Microvessel Feature Extraction</td>
<td>3.2.3-4</td>
<td>Figures 2C-E, Table 2</td>
</tr>
<tr>
<td>8. DCE MRI Kinetic Feature Extraction</td>
<td>3.4</td>
<td>Figure 3H, Table 3</td>
</tr>
<tr>
<td>9. Correlation Analysis</td>
<td>3.5</td>
<td>Figures 4-5</td>
</tr>
<tr>
<td>10. Evaluation of Correlated Imaging Markers against alternate surrogate markers, Gleason Patterns</td>
<td>3.5</td>
<td>Figure 6</td>
</tr>
</tbody>
</table>

Figure 1. (A) CD31 stained radical prostatectomy (RP) quadrants are first stitched into (B) hemispheres which are further combined into (C) pseudo whole mount RP section using Histostitcher.32

3.2 Tumor Microvessel Feature Extraction from *ex vivo* CD31 stained RP specimens

3.2.1 Pseudo whole mount histology reconstruction

Histological quadrants were stitched into pseudo whole mount sections using Histostitcher,32 an interactive program that assembles histological fragments into a contiguous slice as shown in Figure 1. At least three corresponding points between adjacent quadrants were manually selected. Transformations required to maximally align these points were then computed, applied and stored. The same transformations were then applied to binary images of quadrant annotations in order to obtain CaP mask for the whole slice.

3.2.2 Microvessel segmentation

Vascular structures expressing CD31 within tumor regions were segmented using Hierarchical Normalized Cuts (HNcut)33 as shown in Figure 2B. HNcut combines frequency weighted mean shift (FWMS) and normalized cuts (NCut) in a hierarchical manner at multiple resolutions to segment regions corresponding to colors defined by a user selected swatch. For this study, a global swatch was determined by sampling stain intensities from multiple cases in order to account for minor color variations. Segmentations were visually verified to ensure accuracy.

3.2.3 Microvessel Density

Although MVD is clinically determined as the number of microvessels per unit area within tumor hotspots, we use an alternate definition of MVD. We define MVD as the fraction of total microvessel area within tumor regions. In using this definition, we consider the entire tumor as opposed to hotspots due to the lack of standard criteria for hotspot identification17 thus making automated segmentation of hotspots a highly challenging task. Additionally, we leverage microvessel area instead of counts in order to minimize errors that may arise due to discontinuous segmentation of larger structure into multiple smaller structures.
3.2.4 Quantitative Histomorphometry

Spatial arrangement of microvessels was previously determined to be an important attribute that distinguishes malignant from benign prostate tissues. Benign tissues were shown to have uniform distribution of microvessels while malignant tissues consist of clusters that form vascular hotspots.\textsuperscript{17} MVD in aggressive tumors appears to increase as it is clinically calculated within such hotspots. Alternatively, we sought to obtain direct measures of tumor microvessel distribution.

Quantitative histomorphometry models histological appearance of microscopic structures with graphical representations. Previously, these graph based descriptors have been used to quantify spatial arrangement of histologic structures such as nuclei, glands for diagnostic as well as prognostic classification problems in breast and prostate cancer.\textsuperscript{3}

In this work, we extracted quantitative histomorphometric features of microvessel architecture. As depicted with representative image patches in Figure 2, tissues with similar MVD values may display highly different microvessel configurations. To account for such differences, graph based features were extracted from segmented microvessels. The centroids of segmented structures served as nodes which were connected using Delaunay, Voronoi and Minimum spanning tree graphs. A total of 50 statistical features, listed in Table 2, were then extracted from these graphical representations of microvessel distribution.\textsuperscript{34}

<table>
<thead>
<tr>
<th>Image Representation</th>
<th>Microvessel Quantitative Histomorphometric Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voronoi Graph</td>
<td>Area: Mean, Standard Deviation, Minimum-Maximum Ratio, Disorder; Perimeter: Average, Minimum-Maximum Ratio, Disorder; Chord: Standard Deviation, Average, Minimum-Maximum Ratio, Disorder</td>
</tr>
<tr>
<td>Delaunay Triangulation</td>
<td>Side Length: Minimum-Maximum Ratio, Standard Deviation, Average, Disorder; Triangle Area: Minimum-Maximum Ratio, Standard Deviation, Average, Disorder</td>
</tr>
<tr>
<td>Minimum Spanning Tree</td>
<td>Edge Length: Average, Standard Deviation, Minimum-Maximum Ratio, Disorder</td>
</tr>
<tr>
<td>Vascular Segmentation</td>
<td>Total polygon area, Average polygon area; Average, Standard deviation and Disorder of distance to 3, 5 and 7 nearest neighbors; Average, Standard deviation and Disorder of nearest neighbors in 10, 20, 30, 40 and 50 pixel radius</td>
</tr>
</tbody>
</table>

Table 2. List of 50 quantitative histomorphometric features extracted from CD31 stained histological sections to define spatial arrangement of microvessels within CaP

3.3 Histology-MRI Co-registration

**Step 1: Histology-MRI Slice Correspondence** Correspondences between T2-w MRI, H&E and CD31 stained slices were identified by expert radiologist and pathologist based on distances between slices and major anatomical landmarks.

**Step 2: Histology-T2 MRI Registration** CD31 stained sections were first registered to T2-w MRI slices since histo-T2 registration can be performed more accurately than histo-DCE registration as T2-w MRI captures far more anatomic structural detail.

**Step 3: T2-DCE MRI Registration** Inter-protocol registration between T2 and DCE can be accurately achieved due to the minimal deformations between protocols. Thus, T2 slices were registered with corresponding DCE slices, identified using DICOM header information, as an intermediate step.
Figure 2. Graphical representations of vascular structures for histomorphometric feature extraction on two representative patches with similar microvessel density but with different architecture. (A,F) Pseudo whole mount CD31 stained RP sections with CaP annotations from which (B,G) representative image patches were obtained to show microvessel segmentation performed using HNCut.\textsuperscript{33} Graphical representations of microvessel architecture are constructed using (C,H) Voronoi Diagram (D,I) Delaunay Triangulation and (E,J) Minimum Spanning Tree.

**Step 4: Histology-DCE MRI Registration** Transformation from step 3 was applied to the T2-registered histology generated in step 2 in order to obtain histology-DCE registration.

All the registration steps (Figure 3) were performed using thin plate splines (TPS). TPS is a landmark based registration method which interpolates transformation at each voxel such that it (i) maximizes the overlap between the target and template landmarks, establishing accurate spatial correspondences while (ii) minimizing the bending energy to generate smooth transformations.\textsuperscript{35} Manually selected landmarks were used to align the boundaries of prostate on the moving and target images. These landmarks were stored and subsequently applied to the corresponding histology CaP mask in order to obtain the CaP mask for MRI.

### 3.4 DCE MRI Kinetic Features

Kinetic features shown in Table 2 were extracted from signal intensity (SI)-time curves, which were generated from tumor voxels. Three different methods were used to extract tumor SI-time curves:

(i) **Per Voxel Raw SI-Time Curve** Feature values were computed from kinetic curves at each voxel. Statistical measures of the set of values were computed to obtain 18 features listed in the second row of Table 3. The motivation behind these voxel level measurements is to obtain highly localized data, which may enable us to capture tumor heterogeneity.

(ii) **Characteristic Kinetic Curve** Clinically, a small ROI of the most enhancing voxels are considered for further analysis. On the basis of this observation, Chen et al.\textsuperscript{36} compared the performance of kinetic curves generated by the most enhanced voxel within the ROI and the average of all tumor voxels. They found that kinetic curves of the most enhancing voxel within the ROI (characteristic kinetic curve) resulted in a greater classification accuracy as compared to the averaged curve in distinguishing between benign and malignant lesions. We extracted characteristic kinetic curve for each tumor from which 6 features were extracted, as listed in the third row of Table 3.

(iii) **ROI Averaged, Polynomial Fitted Kinetic Curve** Voxel level precision of CaP annotation provided by the registration allows for a higher degree of confidence in the per-voxel level analysis of tumor. Nonetheless, noise may play a significant role in such analysis. Thus, we also perform a more reproducible ROI based feature extraction where SI-time curves obtained from all tumor voxels are averaged and fitted to a third order polynomial. The polynomial fitting allowed us to interpolate between time points to obtain the IAUC at 30, 60 and 90 second time points. A detailed list of these features are provided in the fourth row of Table 3.
Figure 3. DCE MRI perfusion parameters in two Gleason score matched tumors. For each case, (A,K) quadrants were digitally stitched to construct pseudo whole mount CD31 stained radical prostatectomy (RP) specimen and annotated for cancer (red). Subsequently, (B,L) T2-w MRI slices corresponding to these RP sections were determined by a radiologist and a pathologist. In order to accurately transfer CaP annotations from histology to DCE MRI, histology MRI co-registration was performed in a multi-step manner. The steps consisted of: (C,M) Histology and T2-w MRI co-registration, (D,N) identification of DCE MRI slices that correspond to histology and T2-w MRI, (E,O) T2-DCE MRI registration followed by (H,J) histology-DCE MRI registration. (F,I) Small histological patches of the two Gleason score matched tumors with highly different microvessel densities (MVD). Histological differences in MVD is reflected in (G) DCE MRI signal intensity vs. time curves for the two tumors.
<table>
<thead>
<tr>
<th>SI-Time Curve Extraction</th>
<th>Kinetic Features</th>
<th>Notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per Voxel Raw SI-Time Curve</td>
<td>Mean, Median, Variance of Maximum Uptake, Time to Peak, Rate of Uptake, Rate of Washout, Enhancement, Enhancement Ratio</td>
<td>Avg MU, Med MU, Var MU; Avg TTP, Med TTP, Var TTP; Avg Rup, Med Rup, Var Rup; Avg Rwo, Med Rwo, Var Rwo;Avg En, Med En, Var En; Avg EnR, Med EnR, Var EnR</td>
</tr>
<tr>
<td>Characteristic Kinetic Curve</td>
<td>Maximum Uptake, Time to Peak, Rate of Uptake, Rate of Washout, Enhancement, Enhancement Ratio</td>
<td>CKC MU, CKC TTP, CKC Rup, CKC Rwo, CKC En, CKC EnR</td>
</tr>
<tr>
<td>ROI Averaged, Polynomial Fitted Kinetic Curve</td>
<td>Maximum Uptake, Time to Peak, Rate of Uptake, Rate of Washout, Enhancement, Enhancement Ratio, Area under the curve at 30, 60 and 90 seconds</td>
<td>ROI MU, ROI TTP, ROI Rup, ROI Rwo, ROI En, ROI EnR, ROI IAUC30, ROI IAUC60, ROI IAUC90</td>
</tr>
</tbody>
</table>

Table 3. List of 33 DCE MRI features extracted within CaP

Figure 4. Cluster heatmap showing correlation coefficients of DCE MRI features (horizontal axis) and microvessel histomorphometric features (vertical axis). Statistical significance analysis identified feature pairs within the black outlines to be significantly correlated with \( p < 0.05 \). 8 DCE MRI kinetic features belonging to the red subcluster were identified as candidate imaging biomarkers due to their high correlation with a number of microvessel attributes.

### 3.5 Correlation of Imaging and Vascular Markers on surgical specimens

Spearman’s rank correlation test was used to assess strengths of relationships between the extracted kinetic and microvessel features. The test seeks a general monotonic trend without assuming linearity. Unsupervised hierarchical clustering was performed on the matrix of pair-wise correlation coefficients to generate a cluster heatmap. In addition, statistical significance for each pair was determined using a student t-test where alpha was set to 0.05. Performance of the identified imaging markers was evaluated against pathologist determined Gleason patterns on a per slice basis for all five cases. Imaging marker expression was clustered hierarchically in an unsupervised manner to determine the ability of these markers to group together slices with similar Gleason patterns.
Figure 5. Correlation plots of select highly correlated parameters as determined from the heatmap. While some feature pairs such as (A) IAUC vs. Disorder in Microvessel area and (B) IAUC vs. Average Voronoi Polygon Area are linearly correlated, other feature pairs such as (C) Average rate of washout vs. average microvessel area and (D) Average enhancement vs. minimum-maximum ratio of Delaunay triangle area show nonlinear relationships. Unlike Spearman’s correlation coefficient, standard r-squared values fail to capture such relationships.

4. RESULTS AND DISCUSSION

Association heatmap of microvessel and kinetic features (Figure 4) identified 8 imaging markers that were correlated with a number of microvessel descriptors. Figure 5 shows correlation plots for select kinetic and architectural feature pairs. The nonlinear trends shown in Figures 5C and 5D demonstrate the need to consider a measure of correlation, such as Spearman’s coefficient, which captures general as opposed to linear trends.

Our results indicate that imaging features are, at most only moderately correlated with MVD. This finding may be attributable to the fact that MVD computed over the entire tumor is not a reliable indicator of the tumor microvasculature dynamics. Nonetheless, the analysis identified 8 imaging parameters that were correlated with microvessel architectural features.

Among the identified imaging markers, ROI IAUC60, ROI IAUC90 and ROI EnR were highly and significantly correlated with >90% of microvessel features. While the physiological significance of IAUC remains unclear, it has previously shown to be correlated with model based pharmacokinetic parameters, $k_{trans}$, $v_e$ and $v_p$. These parameters reflect vascular permeability, volume of extravascular extracellular space and blood plasma volume fraction, respectively. These correlations, initially identified by Walker et al., confirm the interpretation of Parker et al. in that IAUC is a reflection of ‘contrast agent kinetics determined by a combination of blood flow, blood volume, endothelial permeability, and the extracellular extravascular space volume’.

Among the remaining identified markers, Avg Rwo, Var TTP, Med Rwo, Var Rwo, ROI Rwo, most were related to rate of washout as measured from kinetic curves at both voxel and ROI level. This suggests that Rwo is a robust marker of microvessel architecture. Unlike IAUC however, Rwo markers are highly and significantly correlated with a smaller subset of microvessel features, indicated by the green subcluster (Figure 4). This subset consists
Figure 6. Association between the 8 identified imaging biomarkers and Gleason patterns of each slice in score matched cases indicate that most slices with a primary Gleason pattern of 3 are clustered together within the blue subcluster.

of a number of Voronoi graph based features, including minimum vs. maximum ratios and standard deviations of perimeter, area and chord length, that capture heterogeneity within the tumor microvessel arrangement. As tumor heterogeneity is known to be associated with CaP aggressiveness, correlation of Rwo with measures of intra-tumoral microvessel heterogeneity further strengthens our confidence that Rwo may be a promising imaging marker for aggressive CaP. In addition, our findings are consistent with previous studies that have found rate of washout to be informative in identifying as well as characterizing CaP.28, 39

Unsupervised hierarchical clustering was performed on a per slice basis to evaluate the performance of imaging markers against an independent surrogate marker of aggressiveness, Gleason pattern. Most slices with primary + secondary Gleason patterns of 3+4 and 3+3 were clustered together as shown in Figure 6. Slices with primary grades >3 however did not seem to group together. These results suggest that the identified imaging markers may be able to separate clinically significant from clinically indolent CaP.

5. CONCLUDING REMARKS

In this paper, we presented a novel radiohistomorphometric framework in the context of prostate cancer. This quantitative image analysis framework allowed us to identify promising imaging biomarkers for aggressive CaP by correlating quantitative descriptors of DCE MRI and CD31(vascular) stained histology. Challenges of extracting and relating quantitative descriptors from multi-scale data were addressed by employing automated segmentation, quantitative histomorphometric feature extraction and spatial co-registration methods.

Preliminary results from correlation analysis of 5 patients indicated that kinetic parameters, mostly related to rate of washout (Rwo) and IAUC values were highly and significantly correlated with microvessel features. These imaging markers were also able to selectively cluster most of the tumors with a primary Gleason pattern of 3. We expect to add additional data from patients with low and high Gleason scores in the near future to assess the robustness of these relationships in order to strengthen these conclusions.

Novel contributions of this work include:
1. A high-throughput radiohistomorphometric method, applied in the context of CaP, to screen for clinically relevant imaging markers.

2. Current study is the first to quantitatively attempt to identify in vivo imaging markers for CaP aggressiveness by correlating them with ex vivo measurement of vascular parameters.

3. We used image registration and high-throughput pixel level biomarker detection methods to identify and correlate imaging and vascular markers for CaP aggressiveness. Previous studies examining correlations between relatively few parameters have largely used qualitative and semi-quantitative methods to localize CaP on MRI.\cite{19, 27} In this work, post-operative histology specimens stained with IHC were registered with pre-operative DCE MRI which allowed us to identify imaging markers for aggressive CaP by correlating parameters extracted on a per voxel basis. These methods provided an unprecedented level of precision and accuracy compared to previous studies.

The presented framework allows for discovery of clinically relevant quantitative imaging biomarkers in the absence of outcome information. However, it can also be translated for further validation of the identified imaging markers by examining their relationship with outcome, when available. Radiohistomorphometrics may be able to expedite imaging biomarker development by providing a high-throughput method of screening quantitative imaging descriptors. The ability to quickly develop reliable imaging markers from new imaging technologies can have significant implications in treatment decision making and development of personalized medicine.

REFERENCES


