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Tumor Vascularity in Renal Masses: Correlation of Arterial Spin-Labeled and Dynamic Contrast Enhanced MR Imaging Assessments

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Abstract

Objective—To investigate potential correlations between perfusion by arterial spin-labeled (ASL) magnetic resonance imaging (MRI) and dynamic contrast enhanced (DCE) MRI derived quantitative measures of vascularity in renal masses >2 cm and to correlate these with microvessel density (MVD) in clear cell renal cell carcinoma (ccRCC).

Methods—Informed written consent was obtained from all patients before imaging in this HIPAA-compliant, IRB-approved, prospective study. 36 consecutive patients scheduled for surgery of a known renal mass >2 cm underwent 3T ASL and DCE MRI. ASL measures (P_{ASL}) of mean, peak, and low perfusion areas within the mass were correlated to DCE-derived K^{trans} , Ke ,

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CONFLICT OF INTEREST

Ivan Dimitrov is a current employee of Phillip Healthcare. Naira Muradyan is a current employee of iCAD. However, they did not have control over the data acquisition, analysis, or interpretation of the results. Other authors declare no competing interests.

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and V_e in the same locations using a region of interest analysis. MRI data were correlated to MVD measures in the same tumor regions in ccRCC. Spearman correlation was used to evaluate the correlation between P_{ASL} and DCE-derived measurements, and MVD. $P < 0.05$ was considered statistically significant.

Results—Histopathologic diagnosis was obtained in 36 patients (25 men; mean age 58 ± 12 years). P_{ASL} correlated with K^{trans} ($\rho = 0.48$, $P = 0.0091$ for the entire tumor and $\rho = 0.43$, $P = 0.03$ for the high flow area, respectively) and K_{ep} ($\rho = 0.46$, $P = 0.01$ for the entire tumor and $\rho = 0.52$, $P = 0.008$ for the high flow area, respectively). P_{ASL} ($\rho = 0.66$, $P = 0.0002$), K^{trans} ($\rho = 0.61$, $P = 0.001$), and K_{ep} ($\rho = 0.64$, $P = 0.0006$) also correlated with MVD in high and low perfusion areas in ccRCC.

Conclusions— P_{ASL} correlate with the DCE-derived measures of vascular permeability and flow, K^{trans} and K_{ep} , in renal masses > 2 cm in size. Both measures correlate to MVD in clear cell histology.

MICROABSTRACT—Arterial spin labeling (ASL) and dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) have been proposed to quantitatively assess vascularity in renal cell carcinoma (RCC). However there are intrinsic differences between these two imaging methods, such as the relative contribution of vascular permeability and blood flow to signal intensity for DCE MRI. We found a correlation between ASL perfusion (P_{ASL}) and the DCE-derived K^{trans} , and K_{ep} parameters in renal masses > 2 cm in size and these measures correlated with microvessel density in clear cell RCC.

Keywords

kidney cancers; tumor vascularity; microvessel density; arterial spin-labeled; dynamic contrast-enhanced MRI

INTRODUCTION

Kidney cancer is among the 10 most common cancers in both men and women with more than 65,000 new cases and over 13,000 deaths in the U.S. in 2013¹. The three most common subtypes of renal cell carcinoma (RCC) are clear cell (ccRCC), papillary (pRCC), and chromophobe (chrRCC), which account for 60–70%, 15–20%, and 6–11% of all RCCs, respectively^{2,3}. Additionally, renal oncocytomas (RO) and angiomyolipomas (AML) are benign masses that are also commonly observed in clinical practice⁴. Evaluation of tumor vascularity with magnetic resonance imaging (MRI) has been proposed as a method to characterize renal masses^{5–11} and response to treatment in metastatic RCC patients^{12–14}.

Preliminary studies have shown the ability to differentiate among the most common histopathologic subtypes of RCC using multiphasic contrast-enhanced MRI protocols consisting of images acquired before and after (typically 2–3 acquisitions) the administration of a bolus of a gadolinium-based contrast agent (GBCA)^{10,11,15}. A more comprehensive quantitative analysis of tumor vascularity can be achieved using dynamic contrast enhanced (DCE) acquisitions with a higher temporal and lower spatial resolution with subsequent analysis of these imaging datasets with pharmacokinetic models such as the one proposed by Tofts et al.¹⁶. Quantitative parametric maps can be then generated such as those for the volume transfer constant (K^{trans}), the rate constant (K_{ep}), and the fractional volume of the

extravascular extracellular (V_e) and vascular (V_p) space^{16, 17}. However, direct measurement of tumor perfusion by DCE MRI is challenging because of the unknown relative contributions of both blood flow and vascular permeability to tissue enhancement¹⁸.

Arterial spin-labeled (ASL) MRI provides a means to determine tissue perfusion (in mL per minute per 100 g of tissue) without injection of contrast agents^{19–23}. With ASL, the arterial blood is used as an endogenous contrast, by virtue of magnetic labeling of the blood prior to entering the tissue of interest with radiofrequency (RF) pulses and gradient fields^{24, 25}. The tissue signal intensity difference between images acquired with and without arterial labeling is proportional to the perfusion in the tissue (i.e. blood flow). Advantages of ASL as a quantitative technique include the lack of contrast administration and the virtually negligible contribution of vascular permeability to the measurements of tissue perfusion²⁶.

Furthermore, this technique has been validated in animals using microspheres²⁷ and in the normal human brain using H_2O^{15} PET²⁸. A previous study reported the feasibility of characterizing renal masses with a pseudo-continuous arterial spin labeled (pCASL) acquisition at 1.5T⁵. However, the sensitivity of ASL for detecting low levels of perfusion (i.e. <50 mL/100g/min) in renal masses was limited⁵.

A non-invasive method to measure tumor vascularity in renal masses could be valuable for example in the longitudinal follow up of small renal masses undergoing active surveillance. However, despite the existing literature proposing both DCE-MRI and ASL approaches to measure tumor vascularity in RCC, these techniques provide different measures related to the intrinsic vascular characteristics of the tumor. Specifically, ASL offers a measure of tissue perfusion whereas DCE-derived parametric maps such as K^{trans} and K_{ep} are a product of tumor vascular permeability and blood flow. To our knowledge, the potential relationship between these measurements and their correlation with tumor histology and vascularity measurements at pathology has not been reported. The latter would be particularly relevant in ccRCC because: 1) the known relationship between the molecular alterations in the VHL-HIF pathway and tumor angiogenesis in this histologic subtype²⁹; 2) the relationship between tumor vascularity and tumor aggressiveness and potential to metastasize^{30–32}; and 3) the characteristic heterogeneity of clear cell RCC³³.

The purpose of this study was twofold: 1) To investigate potential correlations between the measurements of tumor perfusion in renal masses >2cm in size by arterial spin-labeled (ASL) MRI and the quantitative measures of vascularity derived from dynamic contrast enhanced (DCE) MRI; and 2) To correlate these measures with microvessel density (MVD) in clear cell renal cell carcinoma (ccRCC).

MATERIALS AND METHODS

Patient Population

The institutional review board approved this Health Insurance Portability and Accountability Act (HIPAA)–compliant prospective study. A written informed consent was obtained from all patients before imaging. Between January 2012 and February 2014, 36 consecutive patients (25 men, 11 women; mean age $\pm$ standard deviation, 58 $\pm$ 12 years) scheduled for surgical resection of a known renal mass participated in this study. Exclusion criteria

comprise inability to undergo an MRI examination (e.g. known unsafe indwelling device, claustrophobia), renal insufficiency (estimated glomerular filtration rate < 30 mL/min/1.73 m²), pregnancy, and renal masses smaller than 2 cm in size because of the difficulty in analyzing tumor heterogeneity with the proposed methods. All enrolled patients underwent MRI for evaluation of their renal mass prior to surgery. The mean time between the MRI and the surgery was 4 ± 3 days (range 1 – 13 days).

MR Imaging Protocol

All patients were imaged in the supine position with a commercial 3T dual-transmit MR scanner with a 16-channel SENSE-XL-Torso coil (Achieva, Philips Healthcare, Best, The Netherlands). Axial and coronal T2-weighted half-Fourier single-shot turbo spin-echo (SShTSE) images were acquired for anatomic reference with the following imaging parameters: repetition time/echo time (TR/TE) = 1115/80 ms, flip angle (FA) = 90°, number of signal averages (NSA) = 1, slice thickness = 5mm, field of view (FOV) = 402×340 mm², matrix = 284×268, bandwidth = 467 Hz/pixel. A coronal 2D pCASL acquisition was used to assess renal mass perfusion (see below). Coronal three-dimensional (3D) T1-weighted (T1W) spoiled gradient-echo (SPGR) acquisitions were then obtained with three different flip angles (10°, 5°, and 2°) and used to generate a T1 map (T1₀)³⁴. To allow the assessment of tumor vascular heterogeneity, a volumetric MRI acquisition was chosen for the DCE experiments. DCE MRI was performed using the same coronal 3D SPGR sequence before, during, and after the administration of a bolus of 0.1 mmol/kilogram body weight of gadobutrol (Gadavist; Bayer Healthcare Pharmaceuticals, Wayne, NJ) at a rate of 2 mL/sec followed by a 20 mL saline flush at 2 mL/sec. A slightly slower injection rate than previously reported was used to compensate for the lower temporal resolution of the 3D acquisition and the high flow expected in the kidney. DCE images were acquired for a total of 5 minutes 45 sec at a 5 sec temporal resolution with the following parameters: TR/TE = 3/1.53 ms, FA = 10°, NSA = 1, slice thickness = 5mm, FOV = 180×408 mm², matrix = 120×288, bandwidth = 1326 Hz/pixel. To minimize respiratory motion during DCE MRI, three consecutive dynamic phases (5 sec each) were obtained within each 15-sec breath-held acquisition period with a 15-sec period of free-breathing between consecutive acquisition periods.

ASL MR Imaging

2D ASL MR imaging was performed before the DCE MRI with a single coronal slice through the center of the renal mass in all but one patient with a posterior exophytic mass (sagittal acquisition). All ASL acquisitions were prescribed following directions of a radiologist (___ with 14-years' experience in interpretation of body MRI examinations) who selected the optimal imaging plane based on the anatomic location of the renal mass. ASL imaging was performed with pseudo-continuous labeling of the abdominal aorta, optimized with background and vascular signal suppression³⁵. The labeling plane was applied axially 8–10 cm above the center of FOV across the abdominal aorta for 1.5 sec to induce adiabatic flow inversion followed by a 1.5 sec post-labeling delay. Data were subsequently acquired using a SShTSE readout (FOV 408 mm, 176 × 176 matrix, thickness 10-mm, TR/TE of 6000/80ms). A TR of 6 s allowed complete recovery of the spin magnetization and also facilitated the timing of the respiration instructions. Data were acquired using a timed-

breathing approach where patients were instructed to hold their breath during the acquisition and to breathe in between³⁵. Patient respiratory motion was monitored with respiratory bellows, and good compliance was confirmed in all patients. Sixteen pairs of label and control were acquired. A proton density-weighted image (M_0) with the same acquisition parameters but without the labeling and the background signal suppression was also acquired at the same location for perfusion quantification. The complex k-space raw data of both ASL images and M_0 image were saved for image reconstruction.

Image Analysis

ASL perfusion maps were reconstructed offline using Matlab (The MathWorks Inc., Natick, MA). First, the perfusion difference image was generated by subtracting and averaging the complex label-control pairs in the k-space, followed by Fourier transformation including Homodyne reconstruction³⁶. Then the quantitative perfusion maps were reconstructed using the standard model for continuous labeling, assuming that the labeled protons remain within the intravascular space during image acquisition⁵.

DCE images were analyzed using a commercial software, VersaVue Enterprise (iCAD Inc., Nashua, NH), to perform voxel-by-voxel fitting with Tofts model and generate quantitative maps of K^{trans} , Kep , and Ve after motion correction. The temporal resolution of the 3D DCE MRI acquisition used in this study precluded the calculation of an accurate arterial input function (AIF). Instead, a population based arterial input function (AIF) previously reported was utilized³⁷. Similar approaches have been validated in animals with different types of gadolinium contrast agents^{38, 39} and applied in DCE MRI studies in patients with metastatic kidney cancer¹³ and other primary tumors such as breast⁴⁰ and osteosarcoma⁴¹. The T_{10} and initial area under the concentration curve for 60 sec post-contrast arrival (iAUC) were also calculated.

All images were analyzed on the open-source Digital Imaging and Communications in Medicine (DICOM) viewer (Osirix X, version 5.6, 64 bit, Bernex, Switzerland). The size of renal masses was measured on T2W images in 3 orthogonal dimensions. Regions of interest (ROIs) were drawn by the same radiologist who prescribed the imaging plane for the ASL acquisition (___), who was also blinded to the final histopathologic diagnosis. An ROI was placed to outline the periphery of the target lesions on ASL perfusion maps, avoiding the contour of the lesion to minimize partial volume effects. This ROI was used to calculate the mean perfusion level of the entire mass. To assess areas with high and low perfusion in the same tumor, two additional ROIs of approximately 1 cm² were placed within regions of the tumor that subjectively demonstrated the highest and lowest signal intensity, respectively. Low flow ROIs were selected in an area of the tumor with lower but perceptible signal on ASL; areas with no ASL signal in the mass were avoided. The mean values from these three ROIs represent blood flow levels (P_{ASL}) in milliliters per minute per 100 g of tissue (mL/min/100g).

Similar ROIs of entire tumor, and in high- and low-perfusion areas were drawn on DCE source images to match as close as possible to the selected areas on ASL perfusion maps, and then copied to K^{trans} , Kep , Ve , Vp , T_{10} and iAUC maps.

In masses exhibiting homogeneous vascularity only one representative ROI was used for correlation of ASL perfusion and DCE-derived parameters.

Histopathology Analysis

Histopathologic results after surgical resection of the tumor served as the reference standard in all cases. The final diagnosis was provided by a uropathologist (___) with more than 10 years of experience. All tumors were classified into one of the following categories: (a) low-grade ccRCC (LG ccRCC) (Fuhrman I–II), (b) high-grade ccRCC (HG ccRCC) (Fuhrman III–IV), (c) pRCC, (d) chrRCC, (e) unclassified RCC, (f) AML, or (g) RO. pRCC and chrRCCs were not assigned a Fuhrman grade (31, 32).

After radical or partial nephrectomy, the surgical specimen was positioned to match its anatomic orientation *in vivo* with the help of fiducial markers placed during the surgery. Briefly, the urologist placed sutures at two different anatomic locations in the renal mass, which were separated by 90 degrees using a clock face orientation looking at the mass intraoperatively prior to resection (e.g. 12 o'clock and 3 o'clock; 6 o'clock and 9 o'clock). The specimen was oriented by the same radiologist who planned the ASL acquisition and subsequently sliced into two halves. Fresh tissue samples were collected from one half of the tumor in the areas identified as high and low perfusion areas on ASL MRI and preserved in dry ice for future genetic analysis (data not presented). A thin slice of the other half of the tumor specimen was obtained and divided in sections that were submitted entirely for processing into formalin fixed paraffin embedded (FFPE) blocks of tissues. The edges of the tumor were inked in different colors. Photographs of the tumor were obtained prior to and after every step of this process to facilitate anatomic co-registration of the histopathologic slides with the MRI data. Tissue blocks were fixed in 10% buffered formaldehyde solution, sliced at 3 mm intervals, and embedded in paraffin. Tissue sections were sliced in 4 μm thick sections and stained using hematoxylin and eosin (H&E).

To assess tumor MVD, we performed immunostaining for CD34 and CD31 in 16 ccRCCs⁴² and slides were digitally scanned and reviewed using the Aperio systems (Leica Biosystems, Melbourne, Australia). Corresponding areas of high and low perfusion in the tumor on ASL were mapped from the gross photographs onto the H&E, and CD34 and CD31 digital slides. Quantitative measures of MVD were obtained in these areas on the CD34 and CD31 slides using the Genie (Laica, Melbourne, Australia) pattern-recognition software package and reported as number of vessels per micrometer square (μm^2). The rationale for studying these correlations in clear cell histology only have been described above. Additionally, correlation between tumor vascularity and MVD was not performed in other pathologic subtypes because of the very low perfusion levels (i.e. pRCC) and small number of tumors.

Statistical Analysis

One way ANOVA was used to compare tumor perfusion on ASL (P_{ASL}) and DCE-derived parameters (K^{trans} , K_{ep} , V_e , iAUC, and $T1_0$) among subtypes of LG ccRCC, HG ccRCC and pRCC. Other subtypes of RCC, AML and RO were excluded from this analysis due to the small number of tumors. Correlations for V_p were not included due to frequent negative pixel values for this DCE measure. Spearman correlation coefficients were used to

determine the relationship between P_{ASL} and DCE-derived parameters. For ccRCCs, ASL and DCE-derived measures were correlated to MVD on CD34 and CD31 stains and ρ correlations were interpreted as follows: <0.20 slight agreement; 0.21–0.40 fair agreement; 0.41–0.60 moderate agreement; 0.61–0.80 good agreement; and >0.80 excellent agreement. Analyses were performed with Prism (Version 6.05, GraphPad Software, Inc., La Jolla, CA) with P -values less than 0.05 considered statistically significant.

RESULTS

Thirty-six patients with 36 masses were enrolled in this study. One patient with a LG ccRCC ended the MRI examination after the ASL acquisition and was excluded from the ASL/DCE correlation analysis. The mean maximum tumor diameter was 5.3 ± 2.4 cm and did not differ among different tumor types ($P=0.22$) (Table 1).

ASL Perfusion versus Histologic Subtype

ASL acquisitions were successful in 30/36 patients. Six patients (ccRCC, $n=2$; pRCC, $n=2$; chrRCC, $n=1$; Oncocytoma, $n=1$) were not included in the analysis due to suboptimal labeling (i.e. very low perfusion signal in the uninvolved renal parenchyma without known renal disease). In three renal masses (1 LG ccRCC, 1 pRCC, and 1 chrRCC) the vascularity was uniform and thus only one representative ROI was obtained for correlation of ASL and DCE in addition to the whole tumor assessment. One clear cell papillary RCC had multiple thick septations without a solid element large enough for ROI placement; only a whole tumor ROI was used in this mass. In the other 32 masses demonstrating heterogeneous vascularity, ROI analysis was performed in both high perfusion and low perfusion areas. Table 2 summarizes the perfusion of all renal masses, which differed among histopathologic subtypes (Fig. 1–4). P_{ASL} in the high flow area was significantly lower for pRCCs than that for ccRCCs ($P=0.01$). P_{ASL} in the entire tumor for pRCCs was lower than that for ccRCCs, with a trend towards significance ($P=0.05$).

DCE MR Imaging versus Histologic Subtype

DCE-MRI acquisitions were obtained in 35/36 patients. Table 2 also summarizes DCE-derived measures in all renal masses. K^{trans} of the entire tumor in pRCCs was significantly lower than that of ccRCCs ($P=0.0009$). K^{trans} in the high perfusion region of pRCCs was significantly lower than that of all ccRCCs combined ($P=0.0004$), as well as lower than that of LG ccRCC ($P=0.01$) and HG ccRCCs ($P=0.01$).

Histopathology

Histopathologic diagnosis was obtained after radical ($n=11$) or partial nephrectomy ($n=25$) (Table 1).

Correlation between ASL and DCE-derived Measures

There was a statistical correlation between P_{ASL} and K^{trans} (Spearman correlation coefficient $\rho=0.48$, $P=0.009$), Ke ($\rho=0.46$, $P=0.01$), and iAUC ($\rho=0.37$, $P=0.04$) in the entire tumor for all tumor types (Fig. 5A – B). Significant correlations were also found between P_{ASL} and K^{trans} ($\rho=0.43$, $P=0.03$), and between P_{ASL} and Ke ($\rho=0.52$, $P=0.008$) in tumor regions

with high perfusion (Fig. 5C – D). Significant correlations were also found between P_{ASL} and K^{trans} ($\rho=0.41$, $P=0.0430$) in areas with low perfusion (Fig. 5E).

Correlation between MRI Vascularity Measures and MVD

MVD measurements were performed in 16 ccRCCs on CD34 and CD31 stains⁴². MVD data from 2 tumors were excluded due to substantial amount of hemosiderin within the ROI (one secondary to presurgical biopsy) resulting in unreliable quantification. For the other 14 ccRCCs, high- and low perfusion regions were combined together to evaluate the overall correlations between P_{ASL} and DCE-MRI with MVD. There was a statistical significant correlation between P_{ASL} and MVD measures ($\rho=0.51$, $P=0.0083$ for MVD_{CD34} ; $\rho=0.66$, $P=0.0002$ for MVD_{CD31}) (Fig. 6A – B). Statistically significant correlations were also found between K^{trans} , K_{ep} , and MVD (Fig. 6C – F).

DISCUSSION

The development of new blood vessels (i.e. angiogenesis) has been linked to the ability of clear cell renal cell carcinoma (ccRCC), the most common malignant tumor of the kidney, to grow and metastasize^{43, 44}. A quantitative, non-invasive measure of tumor vascularity could have different clinical applications including the assessment of tumor biology in patients undergoing active surveillance. Similarly, the assessment of tumor vascularity with arterial spin labeling (ASL) and dynamic contrast-enhanced (DCE) MRI approaches appear to have promise in the assessment of tumor response to anti-angiogenic therapies^{12, 14, 45}. Furthermore, renal masses with different histopathology, including benign and malignant neoplasms, can be differentiated *in vivo* based on the tumor vascularity measured using non-contrast ASL⁵ and contrast-enhanced techniques^{10, 46}. Despite these promising data, the correlation between ASL and DCE-derived assessments is unknown.

Our study provides the first correlation between quantitative ASL and DCE-derived vascular measures in renal masses. Similar correlations in healthy kidneys in a rabbit model found a close agreement between both techniques⁴⁷. DCE-derived blood flow estimates and ASL perfusion in renal cortex of healthy volunteers at 3T are also linearly correlated although blood flow is over-estimated with DCE-MRI⁴⁸. Our aim was to assess the correlation of other DCE-derived outputs of tumor vascularity (K^{trans} and K_{ep}) with ASL measures of tumor perfusion since both have been reported previously as potential biomarkers of angiogenesis in RCC^{12, 14, 45, 49}. Despite both techniques providing different vascular measures, we found that ASL perfusion exhibited moderate albeit significant correlation with DCE-derived K^{trans} ($\rho=0.48$, $P=0.009$) and K_{ep} ($\rho=0.46$, $P=0.01$). The moderate correlation may be attributed to inherent challenges in DCE MRI quantification of tumor vascularity in renal masses such as: 1) quality of the arterial input function (AIF); 2) temporal resolution; 3) respiratory motion; and 4) contribution of both perfusion and vascular permeability to tissue enhancement⁵⁰. On the contrary, ASL signal is proportional to perfusion with no contribution from vascular permeability.

As previously demonstrated by Lanzman et al.⁵, we were also able to differentiate papillary RCC from other renal masses based on their characteristic low perfusion on ASL. We did not find however a difference in the perfusion levels of oncocytoma (RO) compared to other

renal masses. Similar to Lanzman et al.⁵, we confirmed very high perfusion levels (254 ± 5 mL/min/100g) in RO although these were not statistically different than that for low grade ccRCC (270 ± 122 mL/min/100g), preventing us to differentiate these two entities.

Our ASL MRI acquisition at 3T was technically unsuccessful in 6/36 (~17%) patients. This failure rate is higher than previously reported at 1.5T using a similar pseudo-continuous ASL acquisition^{5,35}. A potential explanation is the challenge of effectively labeling the arterial spins in the upper abdominal aorta at 3T due to B_0 inhomogeneities caused by the adjacent lung parenchyma. In the authors' experience positioning the labeling plane inferiorly, away from the lung, may provide more consistent labeling efficiency although this may not be feasible for masses in the upper pole of the kidney. Further efforts should be directed toward improving the labeling efficiency at 3T.

ASL perfusion was significantly correlated with quantitative MVD on CD31 stains ($\rho=0.66$, $P=0.0002$), and this was slightly superior to that for K^{trans} ($\rho=0.61$, $P=0.0002$). ASL perfusion in LG ccRCC (164.3 ± 101.6 mL/min/100g) was higher than that for HG ccRCC (127.7 ± 57.4 mL/min/100g) and this is consistent with the previously published inverse relationship between Fuhrman grade and MVD⁴², however this difference did not reach statistical significance. The lower correlation between MRI vascularity measures and MVD on CD34 stains deserves comment.

Two distinct types of microvessels have been identified in ccRCC: undifferentiated (CD31(+)/CD34(-)) and differentiated (CD34(+))⁴². A higher level of undifferentiated vessels in ccRCC has been significantly correlated with higher tumor grades and shorter patient survival⁴². In contrast, MVD measured with both antibodies in prostate cancer correlate with recurrence after prostatectomy, although only CD34 remains as an independent predictor of PSA failure at multivariate analysis⁵¹. Regardless, CD31 reveal more vessels than CD34 in ccRCC because some blood vessels stain positively only in the former⁴². Compared to CD34, the higher correlation between ASL/DCE and CD31 in our study may be related to the inclusion of more blood vessels in the MVD count.

Both ASL and DCE techniques allow for assessment of tumor vascular heterogeneity. Tumor heterogeneity is an intrinsic characteristic of ccRCC and it is intimately related to its underlying molecular biology³³. The combined measures of tumor perfusion in high- and low-perfusion areas correlated to CD31 MVD measures. This study offers an assessment of tumor vascular heterogeneity *in vivo* with pathologic correlation and provides the rationale for pursuing correlations between tumor imaging characteristics and molecular/genetic heterogeneity.

Our study has several limitations. First, our DCE MRI protocol had only a modest temporal resolution of 5 sec and utilized a population-based AIF³⁷. This compromise was chosen because of the ability to perform this type of acquisitions in most modern MRI scanners, the simplicity of the image postprocessing algorithm, and the ability to assess tumor heterogeneity within the entire renal mass with the volumetric coverage. We were interested in assessing an MRI protocol that could potentially be implemented in clinical practice. Higher resolution, slightly lower resolution volumetric acquisitions have been recently

encouraged by the National Cancer Institute (NCI) recommendations on the use of DCE MRI in oncology (<http://imaging.cancer.gov/programsandresources/reportsandpublications/reportsandpresentations/dynamiccontrastenhancedmrimeetingreport>). Moreover, although intuitively V_p measures (i.e. tumor fractional plasma volume), should correlate to MVD calculations, we did not include this parameter in our analysis. We encountered frequent negative pixel values on V_p calculations likely due to AIF estimate inaccuracies and/or errors in exact contrast arrival detection for each voxel. While use of a population-based AIF a population-based AIF for DCE MRI studies is supported by the Experimental Cancer Medicine Centres Imaging Network Steering Committee of the European Society of Radiology⁵², further refinements in the acquisition protocol and kinetic modeling may help address these issues. Second, although every attempt was made to co-register MRI acquisitions with tissue specimens with the use of fiducial markers, this process is far from perfect and can lead to erroneous correlation between MRI and histologic measures. Third, we used a 2D ASL acquisition, which allows for the assessment of different regions of tumor vascularity only in one plane. A volumetric acquisition would be necessary to assess heterogeneity in the entire tumor. Moreover, the MVD measures at histopathology are also challenging due to tumor heterogeneity making this measure an imperfect reference standard. Furthermore, our ASL acquisition failed in 17% of our patients likely due to inconsistent performance of the labeling strategy. Further technical refinements are necessary to improve the robustness of this method at 3T.

CONCLUSION

ASL perfusion quantifications showed significant correlation with DCE-derived measures of tumor vascularity such as K^{trans} and K_{ep} . This correlation was however moderate, likely due to intrinsic differences in the vascular measures provided by these techniques. Both ASL and DCE-derived analysis correlate with histopathologic MVD measures supporting the use of these techniques for the non-invasive assessment of tumor vascularity *in vivo* in renal masses > 2cm in size.

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Clinical Practice Points

Quantitative arterial spin labeled (ASL) magnetic resonance imaging (MRI) and dynamic contrast-enhanced MRI at 3T can be used to non-invasively measure tumor vascularity in renal masses. The correlation of these vascular measures with microvessel density at histopathology supports the use of these MRI techniques as non-invasive biomarkers of tumor angiogenesis. These techniques may facilitate a new role of MRI as a surrogate of tumor biology in longitudinal follow up of patients undergoing active surveillance. Further studies are needed to assess this potential new application of MRI.

Figure 1. Patient with low grade clear cell RCC in lower pole of the right kidney

Coronal T2-weighted single-shot turbo spin-echo image (A) shows a heterogeneous renal mass (white circle) with hypointense components in its periphery. Coronal ASL perfusion map (B), K^{trans} map (C), and Kep map (D) show heterogeneous vascularity in the mass. White, black, and yellow circles denote regions of interest for measures in the entire tumor, high perfusion area, and low perfusion area, respectively. ASL perfusion (P_{ASL}) measures in the entire tumor, high flow area, and low flow area were 91 mL/min/100g, 290 mL/min/100g, and 35 mL/min/100g, respectively. K^{trans} measures in the entire tumor, high flow area, and low flow area were 1.0 min⁻¹, 6.2 min⁻¹, and 0.08 min⁻¹, respectively. Kep measures in the entire tumor, high flow area, and low flow area were 1.8 min⁻¹, 9.6 min⁻¹, and 0.3 min⁻¹, respectively. (E) Gross photograph of the tumor specimen correlating to the same anatomic location as MRI images. Representative photomicrographs of CD31 immunohistochemistry (ICH) slides in anatomically co-registered areas of high (F) and low (G) tumor perfusion. Microvessel density (MVD) measures obtained from areas with high (MVD= 0.00072 μm^{-2}) and low perfusion (MVD= 0.00023 μm^{-2}) confirmed differences in tumor vascularity correlating to MRI findings.

Figure 2.

Representative coronal images for a patient with high grade clear cell RCC in upper pole of the right kidney. Coronal T2-weighted single-shot turbo spin-echo image (A) shows a relatively homogeneous mass (white circle) isointense to the renal parenchyma. ASL perfusion map (B), K^{trans} map (C), and Kep map (D) show heterogeneous vascularity in the mass. White, black, and yellow circles denote regions of interest for measures in the entire tumor, high perfusion area, and low perfusion area, respectively. ASL perfusion (P_{ASL}) measures in the entire tumor, high flow area, and low flow area were 135 mL/min/100g, 312 mL/min/100g and 58 mL/min/100g, respectively. K^{trans} measures in the entire tumor, high

flow area, and low flow area were 0.8 min^{-1} , 1.5 min^{-1} , and 0.5 min^{-1} , respectively. *Kep* measures in the entire tumor, high flow area, and low flow area were 3.7 min^{-1} , 6.0 min^{-1} , and 2.1 min^{-1} . (E) Gross photograph of the tumor specimen correlating to the same anatomic location as MRI images. Representative photomicrographs of CD31 immunohistochemistry (ICH) slides in anatomically co-registered areas of high (F) and low (G) tumor perfusion. Microvessel density (MVD) measures obtained from areas with high (MVD= $0.00076 \mu\text{m}^{-2}$) and low perfusion (G) (MVD= $0.00028 \mu\text{m}^{-2}$) confirmed however differences in tumor vascularity correlating to MRI findings.

Figure 3.

MRI in a patient with papillary RCC in the interpolar region of right kidney. Coronal T2-weighted single-shot turbo spin-echo image (A) shows a renal mass (white circle) with heterogeneous low signal intensity relative to renal parenchyma. ASL perfusion map (B) shows low perfusion levels ($P_{ASL} = 68.5$ mL/min/100g) in the mass. K^{trans} (C) and Kep (D) maps at the same level demonstrate low values for these DCE-derived vascular measures ($K^{trans} = 0.3$ min $^{-1}$; $Kep = 0.8$ min $^{-1}$).

Figure 4.

MRI in a patient with oncocytoma in the upper pole of right kidney. Coronal T2-weighted single-shot turbo spin-echo image (**A**) shows a renal mass (white circle) with heterogeneous signal intensity, predominantly similar to that of the renal parenchyma with a central hyperintensity. ASL perfusion map (**B**) shows high perfusion levels ($P_{ASL} = 197$ mL/min/100g) in the mass, similar to that of the renal cortex. K^{trans} (**C**) and K_{ep} (**D**) maps at the same level demonstrate high values for these DCE-derived vascular measures ($K^{trans} = 0.9$ min⁻¹; $K_{ep} = 3.4$ min⁻¹) peripherally with central low values. At histopathology, a central scar was noted corresponding with the central hypo-perfused area.

Figure 5.

Scatterplots show relationship between ASL Perfusion measurements (P_{ASL}) and K^{trans} , Kep in the entire tumor (A – B), in the high perfusion areas (C – D), and relationship between P_{ASL} and K^{trans} in the low perfusion areas (E). P_{ASL} was significantly correlated with K^{trans} and Kep in the entire tumor, and in the high perfusion areas, and with K^{trans} in the low perfusion areas. ρ is Spearman correlation coefficient and P indicates statistical significance.

Figure 6.

Scatterplots show relationship between ASL Perfusion measurements (P_{ASr}) and MVD based on (A) CD34, and (B) CD31, and relationship between DCE-derived measurements and MVD (C–H). P_{ASr} , DCE-derived measurements and MVD measurements reflect values for high- and low-perfusion regions combined. P_{ASr} were significantly correlated with MVD. K^{trans} and Kep had a significant correlation with MVD measured on CD34 (C and E) and CD31 (D and F). Ve (G) and $iAUC$ (H) were also significantly correlated with MVD

measured on CD31. ρ is Spearman correlation coefficient and P indicates statistical significance.

Table 1

Patients characteristics.

Characteristic	No. of Patients	%	
Age (years)	58±12		
Gender			
M	25	69.4	
F	11	30.6	
T Stage			
I	25	69.4	
II	2	5.6	
III	5	13.9	
Grade			
I	0	0	
II	15	41.7	
III	6	16.7	
IV	1	2.8	
Treatment			
RPN	22	61.1	
LRN	8	22.2	
OPN	3	8.3	
ORN	3	8.3	
Histology			Tumor Size (cm)
Clear Cell RCC	22	61.1	
Low grade (Fuhrman 1–2)	15	41.7	5.0±1.8
High grade (Fuhrman 3–4)	7	19.4	6.0±2.6
Papillary RCC	6	16.7	5.3±2.2
Chromophobe RCC	3	8.3	7.5±3.4
Unclassified RCC	1	2.8	3.1
Oncocytoma	3	8.3	3.5±1.9
Angiomyolipoma	1	2.8	4.3

Data in cells represent mean values ± standard deviation. RPN= Robotic partial nephrectomy. LRN= Laparoscopic radical nephrectomy. OPN= open partial nephrectomy. ORN= open radical nephrectomy

Table 2
Average, High and Low Perfusion Values for Different Subtypes of Renal Masses.

	Low Grade ccRCC	High Grade ccRCC	pRCC	chrRCC	mixed RCC	RO	AML
ASL perfusion (mL/min/100g)							
Entire tumor	164± 102	128± 57	82± 36	138± 68	54	142± 79	151
High Perfusion	270± 122	196± 105	80± 9 §	221± 6		254± 5	309
Low Perfusion	178± 189	137± 86	56± 15	94		138± 148	61
K^{trans} (min ⁻¹)							
Entire tumor	0.9±0.7	1.13± 1	0.20± 0.1 +	0.8± 0.7	0.6	1.14± 0.6	1.2
High Perfusion	1.97± 1.7	1.65± 0.8	0.31± 0.1 ++	1.9± 2.1		1.91± 0.6	2.0
Low Perfusion	0.33± 0.3	0.67± 0.6	0.12± 0.1 +++	0.4± 0.2		0.35±	0.7
Kep (min ⁻¹)							
Entire tumor	1.95± 1.3	2.82± 1.9	0.62± 0.2 *	3.7± 1.7	4.0	2.46± 1.3	2.5
High Perfusion	3.67± 2.4	3.66± 1.4	0.91± 0.4 **	4.6± 3.3		3.71± 1.3	3.4
Low Perfusion	1.36± 1.1	2.20± 1.5	0.60± 0.3	2.7± 0.4		0.75± 0.4	2.6
Ve							
Entire tumor	0.4±0.1	0.4±0.1	0.3±0.1	0.2±0.1	0.1	0.5±0.2	0.5
High Perfusion	0.5±0.2	0.5±0.1	0.4±0.1	0.3±0.2		0.6±0.3	0.6
Low Perfusion	0.3±0.2	0.3±0.1	0.2±0.1	0.1±0.1		0.6±0.2	0.3

Data in cells represent mean values ± standard deviation. RO= Renal Oncocytoma. AML= Angiomyolipoma. Light orange colored cells represent values reaching statistical significance ($P<0.05$) as follows:

§ high perfusion in pRCC lower than for all ccRCCs combined ($P=0.0105$), and low grade ccRCC ($P=0.0258$);

+ K^{trans} in the entire tumor for pRCC lower than for all ccRCCs combined ($P=0.0009$), low grade ccRCC ($P=0.0181$), and high grade ccRCC ($P=0.0141$);

++ K^{trans} in the high perfusion area for pRCC lower than for all ccRCCs combined ($P=0.0004$), low grade ccRCC ($P=0.0131$), and high grade ccRCC ($P=0.0198$);

+++ K^{trans} in the low perfusion area for pRCC lower than for all ccRCCs combined ($P=0.0340$) and high grade ccRCC ($P=0.0379$);

* Kep in the entire tumor in pRCC lower than for all ccRCCs combined ($P=0.0057$) and high grade ccRCC ($P=0.0197$);

** Kep in the high perfusion area for pRCC lower than for all ccRCCs combined ($P=0.0008$), low grade ccRCC ($P=0.0201$), and high grade ccRCC ($P=0.0175$).