Arterial Spin Labeling Perfusion Measurements Reflect Histologic Microvessel Density in an Experimental Model of Tumor Response and Eventual Resistance to Antiangiogenic Therapy

X. Wang[1], L. Zhang[2], M. P. Collins[3], B. Bahamon[3], S. Signoretti[3], M. B. Atkins[2], D. C. Alsop[1], and R. S. Bhatt[2]

[1]Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, United States, [2]Division of Hematology-Oncology and Cancer Biology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [3]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States

Background

Histologic assessment with intratumoral microvessel density (MVD) technique has been regarded as the reference standard for measuring tumor angiogenesis[1]. However, histologic evaluation might not be an appropriate technique in clinical practice because of its invasive nature and sampling error vulnerability[2]. Arterial spin labeling (ASL) MRI has recently demonstrated sensitivity to antiangiogenic therapy in tumors and may serve as a noninvasive and precise surrogate for vascular changes associated with antiangiogenic therapy [3].

Objectives

There have been limited studies relating ASL-MRI to tumor angiogenesis and none, to our knowledge, demonstrating the relationship to MVD during the course of antiangiogenic therapy. Thus, the purpose of our study was to investigate the correlation of ASL-MRI tumor perfusion measurement with MVD in renal cell carcinoma (RCC) mouse model, to provide support for the use of ASL-MRI to assess tumor angiogenic status and the response to antiangiogenic therapy.

Methods

We established two human RCC xenografts in nude mice with implantation of 786-O cells or A498 cells in the right flank of mice. ASL-MRI was performed in tumors between 12mm and 20mm in size using a 3.0 T whole-body clinical MRI scanner with a 3 cm diameter receive-only custom-built surface coil. The ASL imaging protocol and analysis have been previously described[4]. Briefly, a single slice ASL imaging was acquired with a single-short fast spin echo sequence by using a background-suppressed flow-sensitive alternating inversion-recovery strategy at the center of tumor. A TR of 5000 ms, a TE of 60 ms, slice thickness 2 mm, FOV 8 × 8 cm, and matrix 128 × 128 were used. Twenty-four label and control pairs were acquired and averaged for the ASL acquisition. A reference proton density image was acquired by turning off all background suppression and labeling pulses in the ASL preparation. In addition, to examine the reproducibility of ASL technique, ASL imaging was performed twice in six tumors within 48 hours. Tumors were dissected and prepared for MVD analysis after MRI. Histologic sections obtained in planes corresponding to the slice of ASL image were stained with H&E and CD34 immunohistochemical (IHC) staining. Quantification of average MVD was assessed using a modified microvessel analysis algorithm.

Results

ASL MRI and CD34 IHC of 27 tumors derived from RCC xenografts were conducted to measure tumor blood flow and MVD. Of these tumors, 15 tumors were from mice that were treated with aVEGFR tyrosine kinase inhibitor. The average overall tumor perfusion was 67.9 ± 28.2 ml/100g/min (n=27); MVD was 159.9 ± 87.7 vessels/mm^2. There was a very good correlation (r=0.92, Fig1) between the tumor perfusion of ASL measurement and MVD in the first 11 tumors compared when plotted the data with a regression analysis. The average tumor perfusion was significantly lower in the tumors derived from mice treated with anti-angiogenic therapy as compared with that of untreated tumors (49.3 ± 15.0 ml/100g/min vs 102.6 ± 6.0 ml/100g/min, p<0.001). Both correlation plots (interclass correlation coefficient r=0.98, p=0.0005) and Bland–Altman analysis show excellent agreement for the interscan of ASL measurement (Fig2). Figure 3 are a representative proton density image (Fig3A), ASL image (Fig3B), and a CD34 section (Fig3C), which show good correlation between tumor perfusion measued with ASL and MVD.

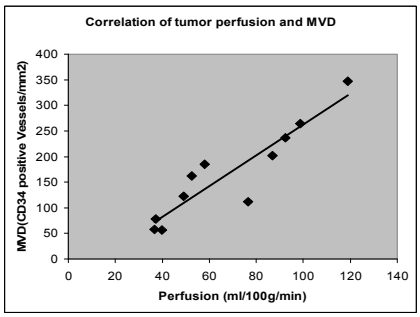

Figure1

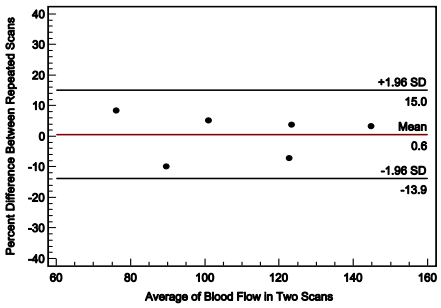

Figure2

Conclusions

ASL-MRI may serve as an accurate and noninvasive method for mornitoring tumor angiogenesis and response to treatment.

References

1. Weidner N, Semple JP. N Engl J Med 1991; 324:1-8.
2. Schor AM. Histochem J 1998; 30:849-56.
3. Bazelaire CD,Alsop DC, et al. Clin Cancer Res 2008; 14(17): 5548-54
4. Schor-Bardach R, Alsop DC, et al. Radiology 2009; 251(3): 731–42

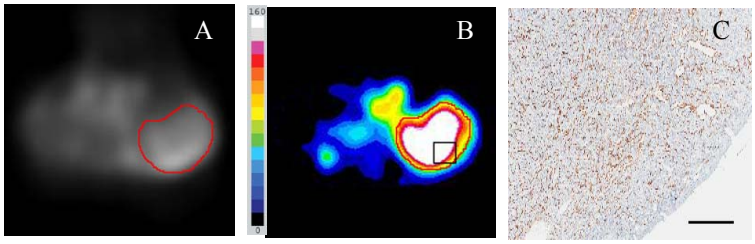

Figure3. Representative proton density (A), and ASL (B) images, the color bar indicates the value of perfusion ranging from a high of160ml/100g/min (white gray) to very low (dark blue). A CD34 IHC stained section from the square box in the tumor defined in B to detect microvessels (brown stain, scale bar=1mm) (C). The tumor is highlighted with a red line in A and B.