Measuring human glomerular morphology and pathology with MRI

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INTRODUCTION: ~90,000 patients and $40 billion will be lost to chronic kidney disease (CKD) this year in the US alone[1]. CKD develops when there are too few functional nephrons to maintain homeostasis. Therefore nephron number (N_{glom}) and size (V_{glom}) are correlated with risk for chronic cardiovascular and kidney disease and may predict renal allograft viability. Unfortunately, there are no techniques to assess total glomerular number and volume in intact human kidneys. This work demonstrates the use of cationized ferritin (CF)[2-4] as a glomerulus-specific magnetic resonance imaging (MRI) contrast agent to measure glomerular number, volume, and spatial distribution and to detect arterial and glomerular damage in human kidneys.

METHODS: CF was synthesized according to Danon, et al.[2] Four human kidneys (unsuitable for transplant) were obtained at autopsy though a donor network (The International Institute for the Advancement of Medicine, Edison, N.J.) after Institutional Review Board approval and informed consent. Kidneys were perfused via the renal artery with 120 ml of phosphate buffered saline (PBS), 300 mg of CF in PBS per kg kidney weight (**Table 1**), 120 ml of PBS to remove any unbound CF, and 10% neutral buffered formalin. One kidney received no CF (as a control), but received the same number of PBS and formalin perfusions. Biopsies (~1 mm^3) were taken from the cortex from each kidney and prepared for immunofluorescence (IF) and transmission electron (TEM) microscopy. Fixed kidney were imaged using a T_2^*-weighted 3D gradient echo pulse sequence at 7T (resolution = 117x117x117 μm^3) and 3T (resolution = 270x270x540 μm^3). The apparent glomerular number (aN_{glom}) and the individual volumes of all glomeruli (aV_{glom}) were calculated from the 7T MR images using a custom algorithm written for MATLAB (The Mathworks, Natick, MA). We performed image texture analysis on the 7T data to assess changes in the pattern of glomerular labeling in the MR images of CF-labeled kidneys. The MRI-based measurements of aN_{glom} and aV_{glom} were validated using the disector/fractionator stereological method[5] and histological sections were examined by an expert renal pathologist.

RESULTS: CF labeling and MRI revealed the distribution of glomeruli throughout the kidney, visible as punctate dark spots in the images (**Fig. 1A**). Each dark spot in the cortex is ~50-80% darker than the surrounding cortex. These dark spots were not present in the un-injected control kidney (**Fig. 1D**). The specific binding of CF to the glomerulus and tubule was confirmed with IF (**Fig. 1B,E**) and TEM **(Fig. 1C,F)**. The custom algorithm was able identify (**Fig. 1H**), count, and measure the size of all glomeruli in the 7T images of the CF-inoculated kidneys. MRI- and stereology-based measurements of glomerular number and volume are detailed in **Table 1**. The algorithm counted $0.057x10^6$ glomeruli in the unlabeled kidney, indicating a ~6% false positive rate. Kidney were also scanned using low resolution 3T MRI and, while individual glomeruli were not visible at these lower resolutions, the average signal magnitude in the cortex of CF-labeled kidneys was ~20% lower than in the medulla. Minimal difference between signal magnitude in the cortex and medulla (<2%) was seen in the control kidney (images not shown). Histopathological analysis (**Table 1**) revealed that kidney CF3 had relatively healthy glomeruli, CF2 had regions of severe glomerular and arterial sclerosis, and that kidney CF1 had mild glomerular sclerosis. Large regions of the cortex of the CF2 kidney lacked CF-labeled glomeruli (**Fig. 2A**). These regions corresponded with regions of severe glomerular and arterial sclerosis, defined by histopathology on the same regions (**Fig. 2C**). Spatial spectral analyses of line profiles drawn through CF-labeled glomeruli of each kidney (**Table 1)** suggest that sclerotic glomeruli are represented by lower frequency spatial spectral peaks at k = 0.8 mm^{-1} and healthy glomeruli by higher frequency spatial spectral peaks at k = 1.2-1.5 mm^{-1}. A representative spectral analyses of CF1 is shown in **Figure 3**.

	Age	Gen	Race	Cause of death	Initial Cr (mg/dl)	Initial GFR (ml/min)*	Peak Cr (mg/dl)	Last Cr (mg/dl)	Pathology report	Stereology			MRI		
										N_{glom} ($x10^6$)	V_{glom} ($x10^{-3}$ mm^3)	Kidney weight (g)	aN_{glom} ($x10^6$)	median aV_{glom} ($x10^{-3}$ mm^3)	CF-related spectral peaks (mm^{-1})
CF1	68	m	C	cardiac arrest	1.6	43	2.9	2.5	mild glom sclerosis	1.13	5.01	167	1.27	4.8	0.8, 1.2
CF2	45	f	AA	hyprtn stroke	1.1	65	2.7	2.7	moderate to severe glom/arterial sclerosis	0.74	4.68	110	0.92	3.2	0.8
CF3	37	f	C	cardiac arrest	1.9	30	6.05	6.05	minimal sclerosis	1.46	2.82	186	1.52	3.2	1.2 - 1.5
ctrl	55	f	C	stroke	1.6	45	1.8	1.2	-	-	-	-	0.057	1.6	-

Table 1. Clinical data and MRI/stereology estimates. Cr = creatinine, N_{glom} = glomerular number, and V_{glom} = glomerular volume. *MSRD used to calculate GFR.

DISCUSSION: The ability to clinically measure glomerular morphology and spatial distribution has the potential to directly improve patient care and clinical outcomes. Such a technique could be used to assess the viability of donor kidneys and early detection and regular monitoring of kidney disease would enable early education and therapy. We have shown that glomeruli may be visualized, counted, and measured in viable human kidneys using MRI. To the best of our knowledge, this is the first technique to count and measure the volume of every glomerulus in the human kidney and to identify large regions of arteriolar and glomerular sclerosis. This study is thus a first step toward characterizing human kidney glomeruli in vivo.

Refs: *(1) USRDS, 2013, (2) Danon, et al. J Ultra Struct R, 1972, (3)Bennett, et al. Magn Reson Med, 2008 (4) Beeman, et al. Am J Physiol Renal Physiol, 2011, (5) Cullen-McEwen, et al. Methods in Mol Biol, 2012.*

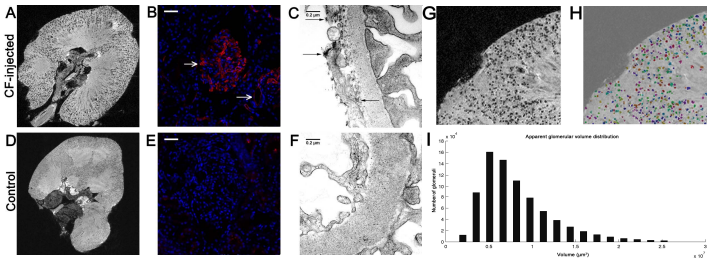

Figure 1 (above). CF specifically labels glomeruli in perfused human donor kidneys, making them visible with 7T MRI (A). Immunofluorescence confirmed the accumulation of CF (red) in glomeruli (B, upper arrow) and leakage of CF into tubules of CF-perfused kidneys (B, lower arrow). Transmission electron microscopy confirmed the accumulation of CF in the glomerular basement membrane and the endothelial glycocalyx (arrows) (C). Zoomed panels show that individual glomeruli are resolved with MRI (G) and that individual glomeruli may be identified using the image processing algorithm (H). This method allows for histograms of glomerular volumes for entire kidneys - a parameter which was previously un-measurable (I). White bars = 50 µm. Black bars = 0.2 µm.

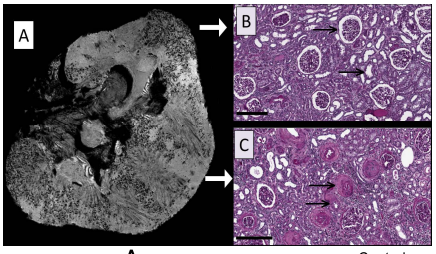

Figure 2 (left). The CF2 kidney had large regions of cortex lacking CF-labeled glomeruli (A). Histopthology revealed that glomeruli and arteries in these regions were severely sclerosed (C) which prevented perfusion of glomeruli in these regions.

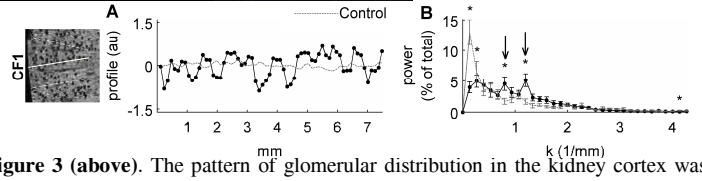

Figure 3 (above). The pattern of glomerular distribution in the kidney cortex was quantified using line signal profiles (A) and spatial spectral analysis (B). Grey traces are data from the naive control, stars represent a statistically significant difference between CF-labeled and the naive control kidneys ($\alpha = 0.04$), and arrows denote peaks of interest. The analysis shown here is from the CF1 kidney.