Counting glomeruli in mouse kidneys using MRI

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Introduction - The goal of this work was to measure glomerular number (thereby nephron number) in mouse kidneys using MRI. The nephron directly filters components of the blood plasma by size and charge and regulates pH and ion concentrations in the blood. Changes in the number of nephrons has been linked to many renal and systemic diseases (1, 2). It is clear that a tool to non-invasively measure nephron number would serve a useful purpose in clinical diagnostics and pre-clinical animal studies. Intravenous injection of the charged iron binding cationized ferritin (CF) nanoparticle (3) has been used to count individual nephrons with MRI (4, 5). Here we develop and validate a robust MRI technique, based on systemic injection of CF, to produce an accurate count of individual nephrons in mice using 3D MRI. With the huge library of transgenic mouse models employed in kidney research, drug discovery, and toxicity studies, such a technique may find wide application.

Methods - *Synthesis of Cationic Ferritin*: Cationic ferritin (CF) was synthesized by conjugating horse spleen ferritin (Sigma Aldrich, St Louis) to N,N-Dimethyl-1,3-propanediamine (DMPA) using 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), according to Danon et.al. (3). *In Vitro Preparation/Imaging*: Male C57BL/6 mice were given 3 bolus doses for a total of 5.75 mg/100g of CF (n=3) with 1.5 hours between injections. Another mouse was left un-injected (naive control). The mice were sacrificed via transcardial perfusion and the perfused left kidneys were imaged in glutaraldehyde on a Varian 800 MHz NMR system outfitted with a DOTY gradient probe using a 3D gradient echo sequence with TE/TR = 8/60 ms and a resolution of 34x34x54 μm. *Post-processing*: Nephrons were counted in the 3D MRI dataset using custom software written in Matlab (The Mathworks, Inc.). Maximum spatial signal gradients in the original volume were calculated first to extract any dramatic spatial changing areas in the volume. Regional minima were located in these areas and regions considered to be glomeruli were defined based on morphological thresholds. A guided watershed algorithm was used to distinguish individual glomeruli where signal overlap of multiple gloeruli might occur. *Histology*: We performed disector/fractionator sterology to confirm MRI-based counts. Serial sections of the left (imaged) kidneys were taken and the total number of nephrons is estimated based on overlapping glomeruli in serial sections identified in coupled light microscopes (6).

Results - MRI imaging of kidneys resected from mice injected with CF revealed punctate spots of signal darkening (Fig. 1). Each dark spot represents a single glomerulus and is caused by the accumulation of the superparamagnetic CF in the glomerular basement membrane. MRI of kidneys from naïve control rats shows no dark spot in their cortex. MRI-based counting of kidneys from mice injected with CF yielded 12,084 ± 1,896 glomeruli per kidney (n=3). Disector/fractionator stereology yielded an average count of 11,390 glomeruli in the imaged kidneys (n=2).

Discussion - We conclude that the MRI-based technique is capable of counting mouse nephrons in 3D. This technique yields comparable results to the disector/frationator method and has the advantage of sampling every glomerulus in the kidney. The disector/fractionator method extrapolates the total number of nephrons from a small sample population of glomeruli. It should be noted that false positives counted in the naive control kidney (~3,000) suggest that there is a systematic over-counting of nephrons when using the MRI-based method versus the disector/fractionator method. This over-counting is less profound when the algorithm is run on MRI-volumes of CF-labeled kidneys. MRI-based counts of CF-labeled glomeruli yielded ~ 6% more nephrons than stereology. These results suggest the possibility of counting nephrons in transgenic mouse models – a technique that may have a major impact on the way kidney disease research, drug discovery, and toxicology are conducted. Future work will be focused on estimating nephron endowment and function in vivo. To the best of our knowledge, this is the first report of a technique to directly count every functioning nephron in the mouse kidney.

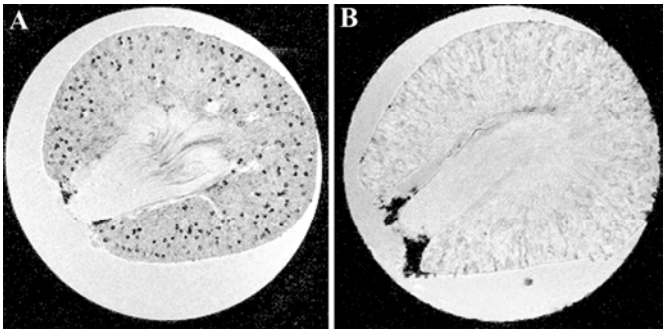

Figure 1 - MRI of CF-labeled mouse kidneys reveals punctate dark spots throughout the organ. Each spot represents a single glomerulus (A). Kidneys from naive control mice show no dark spots in the kidney (B).

References: **(1)** Brenner BM et al. Am J Hypertension 1(4), 1988. **(2)** Bertram JF et al. Cell Tissue Res. 270(1), 1992. **(3)** Danon et al. J Ultrastr Res. 38(5-6), 1972. **(4)** Beeman SC et al. AJP Renal Physiol. 300, 2011. **(5)** Heilmann, MM et al. Nephrol Dial Transpl. 27(1) 2012.