

Assessment of AKI severity in an ischemia/reperfusion mouse model using T₂ mapping: Comparison with Neutrophil Gelatinase Associated Lipocalin (NGAL) and Kidney Injury Molecule-1 (KIM1)

Jan Hentschel¹, Lajos Marko², Erdmann Seeliger³, Dominik N. Müller^{2,4}, Andreas Pohlmann¹, and Thoralf Niendorf^{1,2}

¹Berlin Ultrahigh Field Facility (B.U.F.F.), Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany, ²Experimental and Clinical Research Center (ECRC), a joint cooperation between the Charité Medical Faculty and the Max-Delbrück-Center, Berlin, Germany, ³Institute for Vegetative Physiology, Charité Medical Faculty, Berlin, Germany, ⁴Department of Experimental Medicine I, Nikolaus-Fiebiger-Center for Molecular Medicine, Friedrich-Alexander-University, Erlangen-Nürnberg, Germany

Introduction: It is known that renal medullary hypoperfusion and hypoxia play a pivotal role in acute kidney injury (AKI). A widely accepted hypothesis is that AKI of various origins share one common link in the pathophysiological chain of events that ultimately leads to AKI: Imbalance between renal medullary oxygen delivery and oxygen demand. MRI is non-invasive and supports longitudinal studies. Hence it is an ideal candidate to examine renal tissue oxygenation and oedema. Alterations in renal hemodynamics and oxygenation were previously shown by MRI using a renal ischemia/reperfusion injury (I-R model) in mice [1,2]. Neutrophil gelatinase associated lipocalin (NGAL) [3] and the Kidney Injury Molecule-1 (KIM1) [4] are new markers for acute kidney injury. This study examines the correlation between MRI and NGAL/KIM1 in mouse AKI including I/R injuries of different severity (mild to severe) to elucidate the link between changes in T₂ and T₂* and kidney physiology.

Materials and Methods: Animal models. We imaged 8 male C57BL6 mice (aged 12 weeks) *in-vivo* under isoflurane anesthesia (1.8-2.2% in 100% air). Four animals underwent mild ischemia/reperfusion (17.5 min). In another four animals severe ischemia/reperfusion (30 min) was induced. For all animals the same MR protocol was used prior to onset of ischemia and 6h, 24h and 48h after ischemia/reperfusion. All procedures were performed under normothermic conditions. **MR Imaging.** Coronal T₂w images (RARE (factor 12), TR/TE = 3600ms / 16ms, FOV/matrix/res = (26.7x21)mm² / 256x202 / 0.104x0.104mm. T₂ mapping: MSME, TR/TE/FA = 1000ms / 10-70ms (7 values), FOV/ matrix /res = 26.7x42.0mm / 215x338 / 0.124x0.124mm) were acquired on a 9.4T Bruker Biospec (Ettlingen, Germany) using a four-element mouse cardiac optimized surface coil array and respiratory triggering. For T₂* mapping a multi-echo gradient echo, (TR/TE/FA = 100ms / 2.85-28.5ms (10 values) / 22°, FOV/ matrix /res = 26.7x21mm / 215x169 / 0.12x0.12mm) was applied. Two slices of 0.65mm thickness were acquired in all cases. The NGAL and KIM1 mRNA expression was assessed by quantitative real-time polymerase chain reaction (qPCR), based on post mortem biopsies. **Analysis.** T₂/T₂* maps were derived from multi-echo spin-echo and multi-echo gradient echo data using MATLAB. Statistical analysis was conducted using SPSS20. Correlations were calculated based on mean values obtained from regions of interest in the renal cortex and medulla (Fig.3).

Results: T₂*- and T₂-weighted images showed strong changes in the cortex-medulla contrast (Fig. 1 and Fig. 2). A closer examination revealed a decrease in T₂*/T₂ for the medulla. T₂ was found to be approximately 46.5±1.0 ms (mean±SEM) before I/R, 36.9±0.9 ms after 6h of I/R and 35.8±1.2ms after 48 h of I/R. In comparison an increase in T₂*/T₂ was observed for the cortex (Fig. 1 and Fig.2). Here, a T₂ of approximately 34.0±0.5 ms was observed prior to I/R. 6 h after I/R T₂ was 42.9±0.8 ms while a T₂ 46.8±0.8 ms was obtained after 48 h of I/R. Even for the mild I-R model significant T₂*/T₂ changes were observed, which were not as pronounced as those found for the severe I-R model. The analysis of the T₂-values derived from the medulla and cortex of clipped kidneys showed a strong correlation with NGAL (r = -0.82, p < 0.05 and r = 0.85, p < 0.01 respectively) as demonstrated in Figure 3. A similarly strong correlation was observed between KIM1 and T₂ deduced from the medulla (r = -0.87, p < 0.01) but not between KIM1 and T₂ deduced from the cortex (r = 0.46) of clipped kidneys (Fig.3).

Discussion and Conclusions: Our results suggest that *in-vivo* parametric MRI (i.e. T₂) correlates with the expression of NGAL and KIM1. This indicates that T₂-mapping may be useful as a non-invasive marker for the characterization of pathophysiological changes in mouse models of AKI. A recognized limitation of this pilot study is the limited number of animals used for mild and severe AKI. Hence we anticipate to extend our explorations into T₂-mapping of AKI to further underscore our preliminary conclusion that T₂ could serve as a quantitative surrogate for the severity of kidney injury.

References: [1] Pohlmann A, ISMRM 2011; [2] Oostendorp M, NRM Biomed 2011; [3] Mishra J, *Pediatr Nephrol* 2006; [4] Han WK, *Kidney Intl* 2002.

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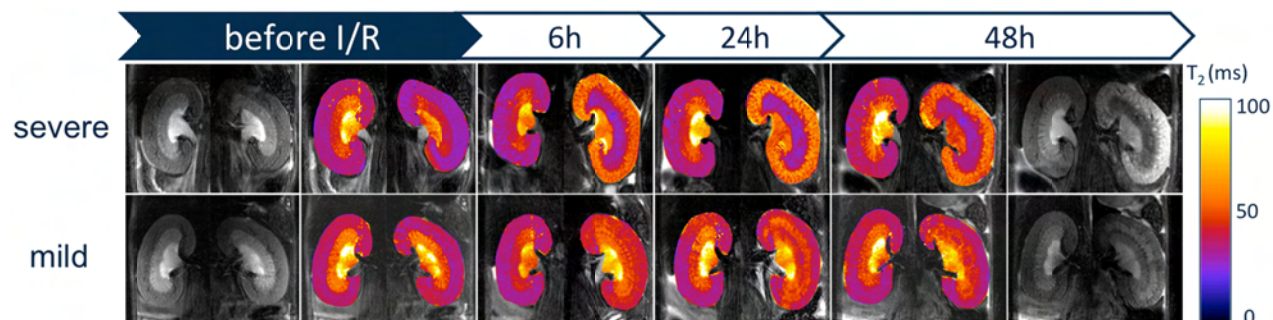


Fig.1: Changes in T₂ after I/R demonstrated in the form of color-coded T₂-maps for severe AKI (top row) and mild AKI (bottom row) of the left kidney. The color-coded maps of the kidneys were superimposed to the T₂-weighted images. The corresponding T₂-weighted anatomical images (grey) are shown for the first and last time point, i.e. before I/R and 48h after I/R. The color scale used in the T₂-maps ranged from 0 to 100ms (right).

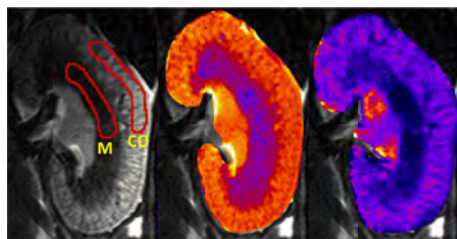


Fig. 2: T₂-weighted image of a clipped kidney showing the ROI placement (left), the corresponding T₂-map (center) and T₂*-map (right).

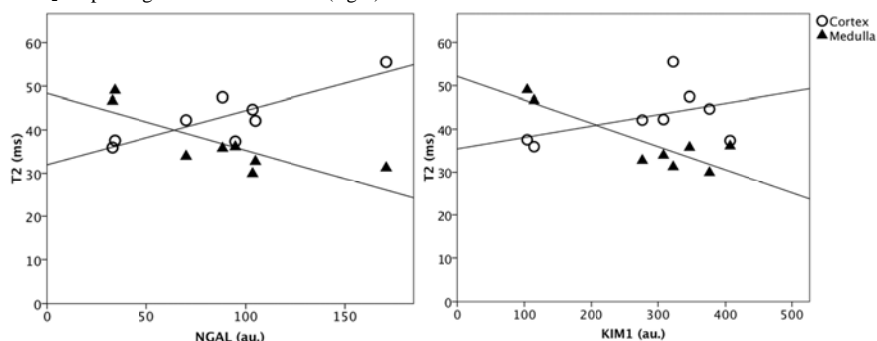


Fig. 3: T₂ of the cortex (circles) and medulla (black triangles) of the clipped kidney (after 48h) vs. NGAL (left) and vs. KIM1 (right). Black lines represent the fitted linear regression curve.