Original Paper

Detection of Acute Tubular Necrosis Using Blood Oxygenation Level-Dependent (BOLD) MRI

Frederic Bauer^a Jan Wald^b Felix Jan Bauer^a Lisa Maria Dahlkamp^c
Felix S. Seibert^a Nikolaos Pagonas^a Egbert Gedat^a Nina Babel^a Walter Zidek^d
Christian von Bodman^c Joachim Noldus^c Dieter Liermann^b Timm H. Westhoff^a

^aMedical Department I, University Hospital Marien Hospital Herne, Ruhr-University of Bochum, Bochum, ^bDepartment of Radiology, University Hospital Marien Hospital Herne, Ruhr-University of Bochum, Bochum, ^cDepartment of Urology, University Hospital Marien Hospital Herne, Ruhr-University of Bochum, Bochum, ^dCharité – Campus Benjamin Franklin, Dept. of Nephrology, Berlin, Germany

Key Words

Ischemia-reperfusion injury • Kidney • BOLD • MRI • Acute kidney injury

Abstract

Background/Aims: To date, there is no imaging technique to assess tubular function *in vivo*. Blood oxygen level-dependent magnetic resonance imaging (BOLD MRI) measures tissue oxygenation based on the transverse relaxation rate (R2*). The present study investigates whether BOLD MRI can assess tubular function using a tubule-specific pharmacological maneuver. **Methods:** Cross sectional study with 28 participants including 9 subjects with ATN-induced acute kidney injury (AKI), 9 healthy controls, and 10 subjects with nephron sparing tumor resection (NSS) with clamping of the renal artery serving as a model of ischemia/reperfusion (I/R)-induced subclinical ATN (median clamping time 15 min, no significant decrease of eGFR, p=0.14). BOLD MRI was performed before and 5, 7, and 10 min after intravenous administration of 40 mg furosemide. **Results:** Urinary neutrophil gelatinase-associated lipocalin was significantly higher in ATN-induced AKI and NSS subjects than in healthy controls (p=0.03 and p=0.01, respectively). Before administration of furosemide, absolute medullary R2*, cortical R2*, and medullary/cortical R2* ratio did not significantly differ between ATN-induced AKI vs. healthy controls and between NSS-I/R vs. contralateral healthy kidneys (p>0.05 each). Furosemide led to a significant decrease in the medullary and cortical R2* of healthy subjects and NSS contralateral kidneys (p<0.05 each), whereas there was no significant change of R2* in ATN-induced AKI and the NSS-I/R kidneys (p>0.05 each). **Conclusion:** BOLD-MRI is able to detect even mild tubular injury but necessitates a tubule-specific pharmacological maneuver, e.g. blocking the Na⁺-K⁺-2Cl⁻ transporter by furosemide.

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Dr. Frederic Bauer

Medizinische Klinik I, Universitätsklinik Marien Hospital Herne, Ruhr-University of Bochum
Hölkeskampring 40, 44625 Herne (Germany)
Tel. +49 2323 499-5265, E-Mail frederic.bauer@elisabethgruppe.de

Introduction

The prognosis of acute kidney injury (AKI) crucially depends on an early diagnosis of the underlying origin and immediate onset of therapy. The earlier the cause of AKI is identified, the more successful is the specific therapy [1]. Acute tubular necrosis (ATN) is one of the most frequent causes of AKI. Whereas postrenal AKI is easily detectable by ultrasound, there is no imaging technique that is able to visualize ATN so far. Since ATN is not associated with any macroscopic changes in renal architecture, neither sonography, nor computed tomography or conventional MRI is able to provide any characteristic traits of this entity.

There is a multitude of possibilities to assess glomerular function in clinical practice. Laboratory tests include creatinine or cystatin C based determination of measured or estimated glomerular filtration rate (GFR). Moreover, glomerular function can be measured by imaging techniques, e. g. a radioisotope renography. In contrast to glomerular function, there is no clinical tool to quantify tubular function. Laboratory markers like abundant urinary β_2 -mikroglobulin provide only indirect indications of tubular dysfunction. Biomarkers of acute kidney injury like kidney injury molecule 1 (KIM-1) or neutrophil gelatinase-associated lipocalin (NGAL) are released by acutely damaged tubular epithelial cells but do not reflect chronic tubular impairment in absence of ongoing tubular cell degradation [2, 3]. Due to the lack of a laboratory test to assess tubular function, an imaging technique detecting tubular dysfunction would be highly desirable.

The present study investigates whether blood oxygen level-dependent (BOLD) MRI may be a suitable technique to detect ATN. BOLD MRI is able to assess tissue oxygenation based on the transverse relaxation rate ($R2^*$). $R2^*$ serves as a measure for deoxygenated hemoglobin. $R2^*$ has recently been shown to differ between renal allografts with primary and delayed graft function [4-8]. Kidneys with early dysfunction show lower $R2^*$ values than kidneys with immediate function posttransplant. This finding may be explained by the fact that dysfunctional renal tissue consumes less oxygen than intact tissue. Hence, there is pivotal evidence that BOLD MRI can be used to provide a rather global picture of renal integrity. But is it possible to focus the global BOLD information exclusively on tubular oxygen consumption? A tubule-specific pharmacological maneuver constitutes a promising approach in this context. Furosemide induces a selective reduction of tubular oxygen consumption by inhibition of the ATP-dependent $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ -cotransporter. Our study examines the hypothesis that the furosemide-induced change of $R2^*$ in BOLD MRI serves as a marker of tubular integrity.

We enclosed patients undergoing nephron sparing partial kidney tumor resection (NSS) as a model for ischemia/reperfusion (I/R)-induced tubular damage. As we have shown before, a surgical procedure with clamping of the renal artery offers the possibility to I/R-induced tubular injury *in vivo* [9]. The contralateral kidney served as an internal control. Since ischemia time is short in the majority of cases, the resulting tubular damage is mild and usually not associated with a “clinically overt” increase of serum creatinine. The furosemide-induced $R2^*$ contrast of these kidneys was compared to subjects with established AKI due to ATN and healthy controls.

Materials and Methods

Study population

The present work constitutes a cross-sectional study at a German University Hospital and comprises a study population of 28 subjects. 9 subjects suffered from AKI due to ATN, 10 underwent NSS and 9 subjects without any renal disease served as healthy controls. Patients referred to the Department of Nephrology for AKI due to ATN were enrolled in the study. The presence of AKI – defined according to the Acute Kidney Injury Network (AKIN) criteria – was an inclusion criterion for this subgroup [10]. Prior renal transplantation and obstructive uropathy were exclusion criteria. The diagnosis of ATN was established by biopsy in 6 (66.7 %)

cases and by clinical criteria (retrospectively by two independent nephrologists) in 3 (33.3 %) cases.

NSS served as a model for mild injury-reperfusion induced tubular damage. For this purpose, 10 patients without preexisting renal disease who were referred to the Department of Urology for surgical treatment of renal tumors using NSS were enrolled. Inclusion criterion was a resection of a solid renal mass necessitating a temporary interruption of the renal arterial blood flow for bleeding control. Exclusion criteria comprised preexisting impairment of renal function with an estimated glomerular filtration rate (eGFR) < 60 mL/min according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [11]. NSS was performed either by retroperitoneoscopic open surgery, or by robotic-assisted laparoscopic surgery. Surgery related renal ischemia was induced by clamping of the arterial renal pedicle. The time of blood flow interruption was documented (clamping time). The study was approved by the local ethics committee of the Ruhr-University Bochum. All patients provided written informed consent. Laboratory documentation of tubular injury was performed by a combination of urinary NGAL and serum creatinine concentrations in order to consider time and intensity aspects of the individual tubular damage. NGAL has a higher sensitivity in the detection of mild tubular injury compared to serum creatinine and starts rising within a few hours, whereas serum creatinine concentrations usually do not increase beyond normal range before day two [2, 9].

MRI protocol

MRI imaging examinations were performed within 2 days (range 0-5.5 days) around maximum creatinine in the ATN group and 2 to 5 days after surgery in the NSS group on a Siemens Magnetom Symphony 1.5T system using body array coils. Three-plane spoiled gradient-echo localizers were performed. Axial and coronal T2WI and axial T1WI in-phase and out-of-phase were performed for a general overview, using a single-shot turbo spin-echo sequence for the T2WI images and spoiled gradient-echo sequences for the T1WI images. BOLD MRI was performed during suspended respiration using a two dimensional spoiled gradient-echo sequence with multiple echo time (TE) to obtain T2*-weighted images in the coronal plane. Eight echoes were obtained for each slice. BOLD MRI was performed before (baseline) and 5, 7, and 10 min after administration of 40 mg furosemide. Furosemide was administered intravenously and flushed with 20 ml of saline. Table 1 summarizes the BOLD MRI protocol.

*Analysis of MRI images and measurement of R2**

To determine cortical and medullary R2* values, cortical and medullary regions of interest (ROI) were traced on BOLD images. For each kidney, the slice providing optimal cortical-medullary differentiation both before and after the administration of furosemide was selected. ROI were drawn manually on the image with TE = 3 ms in areas of cortex or medulla not obscured by artefacts. This procedure was repeated on the images acquired after the administration of furosemide, using the same slice for each kidney and tracing ROI in the same areas of cortex and medulla. T2* images of the kidneys were calculated with Matlab (The Mathworks, Natick, MA, USA) by fitting an exponential-decay-function on a voxel-by-voxel-basis to the BOLD MRI data. The signal intensity as a function of echo time yields the decay parameter T2* for each voxel. T2* values and the corresponding relaxation rates R2* for kidney cortex and medulla were extracted for manually segmented ROIs for each series separately as the mean value in that region and the error given by the standard deviation of these averages. ROIs were segmented in the first image of each BOLD series, which has proton density contrast, i.e. anatomical display of tissues and negligible influence of BOLD effects due to very short echo time. This ROI was then applied to all eight echoes of the series. For each BOLD series, before and after administration of furosemide, R2* values were determined within the cortex and the medulla for each kidney.

Measurement of urinary NGAL concentrations

Urine samples were available in 7 (77.8%) of the subjects with ATN, 6 (60%) subjects undergoing NSS and 8 (88.9%) of the healthy controls. Samples were collected before the MR scan was performed. Concentrations of NGAL were assessed using an enzyme-linked immunosorbent assay (ELISA) kit (NGAL kit 037, Bioport, Gentofte, Denmark) as published previously [12]. This assay has passed a clinical validation procedure [13].

Statistical analysis

Data are presented as median and interquartile range (IQR). Intergroup differences of epidemiological and renal continuous parameters were analyzed by the Mann-Whitney U-Test. Comparison of dichotomous parameters (e.g. gender, absense/presence of concomitant diseases) was performed by Fisher's exact test. MRI derived R2* data from kidneys after NSS and the corresponding contralateral kidneys were compared by the Wilcoxon signed-rank test. In subjects with ATN and healthy controls, R2* data of both kidneys were included into further

analysis. R2* data of these groups and intergroup differences of NGAL values were analysed by the Mann-Whitney U-Test. Changes of R2* values in response to the administration of furosemide were analysed by the Friedman test. P<0.05 was regarded significant.

Table 1. MRI protocol

Parameter	BOLD
Orientation	coronal
Repetition time (ms)	140
Echo times (ms)	3, 8, 13, 18, 23, 28, 33, 38
Flip angle (°)	40
Number of slices	3
Acquired matrix (pxl)	256 x 157
Reconstructed matrix (pxl)	512 x 314
Field of view (mm)	400 x 350
Percent phase encoding (%)	87.5
Slice thickness (mm)	5
Spacing between slices (mm)	10

Results

28 subjects were enrolled in the study including 9 participants with ATN-induced AKI, 10 patients undergoing NSS and 9 healthy controls. Epidemiological data, concomitant diseases, and renal data of the subjects with AKI and those undergoing NSS are presented in Table 2. Table 3 provides tumor characteristics and surgical data of the NSS patients. Briefly, the median age of the overall study population was 55, IQR 24.3-67.5. Healthy controls had a median age of 23 years, IQR 21-28, a median BMI of 21.9 kg/m², IQR 21.5-24.2, and 3 were female (33.3 %). None of them had any history of renal or cardiovascular disease or a regular medication. In the ATN group, 1 (11.1%) suffered from AKIN stage 1, 2 (22.2%) from AKIN stage 2, and 6 (66.7%) from AKIN stage 3. ATN was diagnosed by biopsy in 6 cases (66.7%). In the remaining 3 subjects the diagnosis was established retrospectively by two independent nephrologists from clinical data. ATN was induced by contrast media in 1 patient. In 3 subjects substantial ATN was a biopsy finding associated to crescentic glomerulonephritis and in 1 subject associated to acute interstitial nephritis. In 1 patient the cause of the ATN remained unclear. Mean creatinine at baseline was 2.7 mg/dL, IQR 1.9-4.3, corresponding to an eGFR of 17 mL/min (CKD-EPI), IQR 13.5-41.5. MRI studies were performed within a median of 2 days (range 0 – 5.5 days) around maximum creatinine. Median age in the NSS group was 63.5 years, IQR 57.5-70.8. Median renal pedicle clamping time (ischemia time) was 15 min ranging from 11 to 20 min. MR imaging studies were performed after a median of 3 days (range 2 – 5 days) post-surgery. Healthy controls had a median age of 23 years, IQR 21-28.

Urine samples for assessment of NGAL concentrations were available in 7 (77.8%) patients with ATN-induced AKI, 6 (60%) NSS subjects, and 8 (88.9%) healthy controls. In the remaining 7 subjects urine could not be retrieved before the MRI studies for logistic reasons. Two NGAL values were beyond the measurement range. In these cases, the highest measured value (400 ng/mL) was used for further analysis. Urinary NGAL concentrations were significantly higher in the NSS (51.5 ng/mL, IQR 36.5-104.7) and ATN group (48.5 ng/

Table 2. Epidemiological and clinical characteristics of the study population. Numeric data are presented as median and interquartile range. ACE-I Angiotensin converting enzyme inhibitors; ARB angiotensin receptor blockers; NSAID non-steroidal anti-inflammatory drugs; NGAL Neutrophil gelatinase-associated lipocalin

	Subjects undergoing NSS (n=10)	Subjects with ATN (n=9)	P
Epidemiology			
Female	3 (30%)	5 (55.6%)	0.37
Male	7 (70%)	4 (44.4%)	
Age	63.5 (57.5-70.8)	60 (31-74.5)	0.4
Body mass index (kg/m ²)	24.5 (22-30.3)	25 (21.3-35.2)	0.84
Concomitant diseases			
Diabetes mellitus	1 (10%)	2 (22.2%)	0.58
Hypertension	5 (50%)	4 (44.4%)	1.0
Coronary heart disease	1 (10%)	0	1.0
Medication at MRI			
Diuretics	2 (20%)	2 (22.2%)	1.0
ACE-I/ARB	3 (30%)	1 (11.1%)	1.0
NSAID	0	4 (44.4%)	0.03
Renal data			
Serum creatinine on admission (mg/dL)	0.9 (0.8-1.0)	2.7 (1.9-4.3)	0.002
Serum creatinine at MRI scan (mg/dL)	1 (0.8-1.1)	4.2 (2.0-5.5)	<0.001
eGFR on admission (mL/min)	88 (68-99.3)	17 (13.5-41.5)	0.003
eGFR at MRI scan (mg/dL)	73.5 (61-87.5)	15 (9.5-36)	<0.001
Urinary NGAL at MRI scan (ng/mL)	51.5 (36.5-104.7)	48.5 (22.0-400.0)	0.72

Table 3. Tumor and surgical data of subjects undergoing nephron sparing kidney tumor resection (NSS) , Numeric data are presented as median and interquartile range

	Subjects undergoing NSS (n=10)
Tumor characteristics	
Renal cell carcinoma	8 (80%)
pT1a	4 (40%)
pT1b	4 (40%)
pT2a	0
pT2b	0
Oncocytoma	1 (10%)
Angiomyolipoma	1 (10%)
Others	0
Tumor diameter (mm)	42 (26-50)
Surgical data	
Operation time (min)	176 (142.5-197.5)
Renal artery clamping time (min)	15 (13.5-16.3)
Endoscopic surgery	0
Laparoscopic surgery	0
Roboter assisted laparoscopic	1 (10%)
Retroperitoneoscopic open surgery	9 (90%)

Fig. 1. Median and range of urinary neutrophil gelatinase-associated lipocalin (NGAL) values of subjects after ischemia/reperfusion (I/R) due to nephron sparing surgery (NSS), subjects with acute tubular necrosis (ATN) leading to acute kidney injury (AKI), and healthy controls. * $p < 0.05$ (Mann-Whitney U test).

mL, IQR 22.0-400.0) than in healthy controls (3.7 ng/mL, IQR 1.3-30.0; $p = 0.03$ and $p = 0.01$, respectively). There was no significant difference between the AKI and NSS subjects ($p = 0.72$). The NGAL findings are presented in Fig. 1. In the NSS group, serum creatinine did not differ before and after surgery (0.9 mg/dL, IQR 0.8-1, vs. 1 mg/dL, IQR 0.8-1.1, $p = 0.18$). The corresponding eGFR tended to be lower after resection of the renal mass without reaching significance (88 mL/min, IQR 68-99.3, vs. 73.5 mL/min, IQR 61-87.5, $p = 0.14$).

T2* maps were established for both kidneys of the AKI subjects, healthy controls and NSS patients. In the latter group those kidneys that had undergone clamping of the renal artery during NSS are described as "I/R" in the following, the contralateral kidneys served as internal controls. Fig. 2 presents representative T2* maps of (B) a kidney after NSS, (D) a healthy contralateral kidney and (F) a kidney with ATN-induced AKI. T2* and the corresponding R2* values were obtained in the cortex and the medulla of the kidneys. Fig. 3 provides the individual R2* values in medulla and cortex of all subjects prior to administration of furosemide. Medullary R2* values did neither differ between I/R (13.8, IQR 12.6-16.2) and healthy contralateral kidneys (14.0, IQR 13.5-17.1, Wilcoxon $p = 0.51$) nor between ATN with AKI (12.8, IQR 11.5-18.3) and healthy controls (14.7, IQR 13.3-16.0, Mann-Whitney $p = 0.32$). Accordingly, there was no significant difference in cortical R2* signals between I/R and contralateral kidneys (11.6, IQR 9.7-12.1, vs. 11.9, IQR 11.1-13.3, Wilcoxon $p = 0.11$) and between ATN with AKI and healthy controls (12.9, IQR 9.6-18.6, vs. 13.1, IQR 12.7-13.6, Mann-Whitney $p = 0.67$). Since tubular oxygen consumption is higher in the medulla than in the cortex, R2* medulla/cortex ratios were calculated. The resulting ratios were 1.3, IQR 1.1-1.6, in the I/R kidneys, 1.2, IQR 1.0-1.4, in the contralateral kidneys, 1.2, IQR 0.7-1.5, in the AKI kidneys, and 1.1, IQR 1.0-1.2, in healthy controls without any statistically significant difference between I/R and the contralateral kidney (Wilcoxon $p = 0.56$) and between AKI and healthy controls (Mann-Whitney $p = 0.86$).

Table 4 provides an overview on the effects of furosemide on R2* as a marker of deoxygenated hemoglobin. In the kidneys of healthy controls, furosemide led to a significant decrease of medullary R2* (14.7 to 10.2) and cortical R2* (13.1 to 11.9) with a median reduction of 30.7% in the medulla and 9.5% in the cortex ($p < 0.001$ each). In contrast, the diuretic had no significant effect on medullary and cortical R2* in the kidneys with ATN-induced AKI. The inhibition of the Na⁺-K⁺-2Cl⁻ cotransporter led to a significant decrease of medullary

Fig. 2. Representative assessment of T2* in (A and B) a kidney after nephron sparing surgery (NSS, ischemia-reperfusion), (C and D) a contralateral healthy control kidney and (E and F) a kidney with ATN-induced AKI. (A, C, and E) present the anatomical conditions, i.e. the first image of the BOLD series. (B, D, and F) show color coded T2* maps of the kidneys. In the anatomical images the regions of interest (ROI) are displayed for cortex (dotted red) and medulla (dotted blue).

Fig. 3. Individual baseline (A) medullary and (B) cortical $R2^*$ values and (C) medulla/cortex $R2^*$ ratio in kidneys with (ischemia/reperfusion, I/R) and without (contralateral kidney) clamping of the renal artery during nephron sparing surgery (NSS), acute tubular necrosis (ATN) induced acute kidney injury (AKI), and healthy controls. Horizontal lines indicate median $R2^*$. $R2^*$ values of I/R and the corresponding contralateral kidney were performed by the Wilcoxon matched-paired signed rank test, comparisons of AKI and healthy controls by the Mann-Whitney U-Test ($p > 0.05$ each).

($p = 0.001$) and cortical ($p = 0.03$) $R2^*$ in the healthy kidneys of subjects undergoing NSS, whereas medullary $R2^*$ values were not significantly altered in the kidneys after I/R ($p = 0.11$ and $p = 0.18$, respectively). Fig. 4 shows that the furosemide-induced change of $R2^*$ took place rapidly in healthy kidneys. $R2^*$ values were obtained 5, 7 and 10 min after administration of the diuretic. There was no further significant decrease beyond minute 5.

Discussion

To date, there is no imaging technique to assess tubular integrity *in vivo*. The definitive diagnosis of ATN can only be made by biopsy. The present study shows that BOLD-MRI is able to provide specific information on tubular (dys-)function but necessitates a functional maneuver, e.g. blocking the Na^+ - K^+ - 2Cl^- cotransporter by furosemide.

In order to assess the sensitivity of a candidate imaging technique in the detection of tubular dysfunction, we did not only enroll subjects with established ATN-induced AKI but also subjects undergoing NSS. We have previously shown that NSS with clamping of the renal artery pedicle is a helpful model to study mild acute tubular injury without a “clinically overt” increase of serum creatinine [9]. In accordance with our previous findings, NGAL values were significantly higher in the present I/R population than in healthy controls (Fig. 1). NGAL is a sensitive marker of I/R-induced acute tubular injury [12, 14, 15]. A potential decrease of eGFR after NSS is the consequence of both the resection of renal tissue and tubular I/R injury. In the

Fig. 4. Median R2* values in (A) medulla and (B) cortex of kidneys after ischemia/reperfusion (I/R) and the corresponding contralateral healthy kidney and in (C) medulla and (D) cortex of patients with ATN with AKI and healthy controls at baseline and 5, 7, and 10 min after intravenous administration of 40 mg furosemide. Data are presented as median and interquartile range. Change of R2* over time was analyzed by the Friedman test, *p<0.05, **p<0.01.

Table 4. Medullary and cortical R2* before and after the administration of furosemide, Data are presented as median and interquartile range. Changes of R2* after administration of furosemide were analyzed by the Friedman test. P<0.05 was regarded significant

	I/R-kidney	P	Contralateral kidney	P	ATN	P	Healthy Control	P
Cortical R2* at baseline	11.6 (9.7-12.1)	0.18	11.9 (11.1-13.3)	0.03	12.9 (9.6-18.6)	0.379	13.1 (12.7-13.6)	<0.001
Cortical R2* 5 min after furosemide	11.6 (9.3-12.7)		10.8 (10-11.8)		11.8 (8.3-16.5)		11.9 (11.2-13.1)	
Cortical R2* 7 min after furosemide	10.2 (8.9-11.4)		11.3 (9.7-11.9)		11.7 (8.9-17.6)		11.7 (11.2-12.9)	
Cortical R2* 10 min after furosemide	11.6 (10.2-13)		11.2 (10.7-12.2)		11.8 (9.2-14.1)		11.8 (11.1-13.3)	
Medullary R2* at baseline	13.8 (12.6-16.2)	0.11	14.0 (13.5-17.1)	0.001	12.8 (11.5-18.3)	0.299	14.7 (13.3-16.0)	<0.001
Medullary R2* 5 min after furosemide	12.1 (9.6-13.2)		10.6 (9.7-12.9)		11.8 (9.7-18.3)		10.2 (9.7-11.5)	
Medullary R2* 7 min after furosemide	12.7 (11.6-13.9)		11.1 (9.9-13)		11.2 (9.8-20.1)		9.9 (9.6-10.5)	
Medullary R2* 10 min after furosemide	11.9 (10.3-13.4)		10.3 (9.7-11.5)		12.6 (10.5-18.1)		10.1 (9.6-11.0)	

present study, eGFR tended to be lower without reaching significance after surgery. The acute tubular injury therefore remained “subclinical”. Thus, the furosemide-induced R2* contrast was able to detect even mild forms of tubular dysfunction. NSS is an attractive model for the investigation of tubular injury not only because of the precisely documented duration of unilateral ischemia but also because of the contralateral control kidney. Individual preexisting renal damage, e. g. due to hypertensive or diabetic nephropathy, occurs in both kidneys in the same manner. Therefore, the contralateral kidneys constitute a “perfectly matched control group”.

Since tubular dysfunction does not lead to any macroscopic changes of the kidney’s appearance, only an imaging technique combining an assessment of morphology with functional aspects of renal tissue constituted a promising candidate method. BOLD MRI combines morphological aspects with the information on local concentrations of deoxygenated hemoglobin. Pilot studies in renal transplant recipients showed that kidneys with delayed graft function indeed had lower R2* signals than kidneys with immediate function posttransplant [8]. In our model of mild tubular injury, however, “baseline BOLD” MRI could not detect a difference in neither medullary nor cortical R2* signaling (Fig. 3). This led to the question, whether BOLD MRI could be combined with a pharmacological maneuver that allowed to focus the rather global information of “baseline BOLD” to an exclusive assessment of tubular function and thereby to increase sensitivity for the detection of tubular dysfunction.

The extent of tubular oxygen consumption depends on tubular integrity. Due to the activity of the ATP-dependent transporters tubular cells have an exceptionally high oxygen consumption leading to a partial pressure of oxygen (pO₂) of only 10-15 mmHg even under physiological conditions. Consequently, the proportion of deoxygenated hemoglobin is high in the renal medulla. Accordingly, median medullary R2* signals were higher than cortical R2* values in the present study. In acute tubular necrosis, however, tubular cells are not able to participate in reabsorption processes. Medullary concentrations of deoxygenated hemoglobin therefore decrease resulting in lower R2* values. Intrarenal R2* signals, however, are influenced by oxygen consumption of various types of cells. To specifically examine oxygen extraction of tubular function, we administered furosemide as an inhibitor of energy-dependent electrolyte transport. If tubuli are intact, furosemide leads to a decrease of deoxygenated hemoglobin and R2*. Thus, a lacking decrease of R2* after inhibition of the Na⁺-K⁺-2Cl⁻ cotransporter was supposed to be indicative for tubular necrosis.

Indeed, R2* values of healthy controls decreased substantially after administration of furosemide. The extent of the furosemide-induced medullary R2* contrast was lower in kidneys with tubular injury. This contrast may be regarded as a marker of tubular integrity. The more intact tubules, the higher is the difference of medullary oxygen consumption and therefore the contrast of R2*. The tubules are predominantly located in the medulla. Accordingly, furosemide elicited a less pronounced – although still significant – R2* contrast in the healthy cortex than in the healthy medulla. The slight non-significant decrease of medullary R2* in kidneys with ATN-induced AKI represents the change of oxygen consumption in the remaining intact tubules. Interestingly, furosemide was not only able to detect established ATN-induced AKI but also to elicit a significant R2* contrast in kidneys with mild, subclinical I/R injury in the NSS model. The sensitivity of this approach is lower in the cortex. Whereas there was a significant R2* contrast in established AKI, there was no significant signal in mild I/R injury. As illustrated by Fig. 4 maximal R2* decrease takes place within 5 min. Therefore, sequential assessments of R2* after the application of furosemide are dispensable in future studies.

Examinations were performed using a 1.5 T MR scanner as available in many hospitals. The low field strength of 1.5 T produces less pronounced BOLD signals than that of a current generation’s 3.0 T. The fact that even this field strength was able to reveal the differences in the furosemide-induced R2* contrast in both subclinical and manifest ATN renders further support to the diagnostic potential of this approach. Several techniques including BOLD and

diffusion weighed imaging have been used before to assess renal oxygenation or perfusion. The novelty of the current approach, however, is the combination of BOLD MRI with a tubule-specific pharmacological maneuver in order to gain specific information on tubular function.

But why should we make use of a time intensive examination like this MRI approach, if we could easily determine tubular biomarkers like NGAL or KIM-1 instead? Urinary biomarkers reflect acute degradation of tubular epithelial cells. Tubular atrophy, however, is not associated with increased biomarker concentrations. In contrast, the furosemide-based BOLD approach is able to detect each kind of tubular dysfunction.

Whereas acute tubular necrosis leads to a substantial increase of these biomarkers, tubular atrophy does not. On the contrary, the furosemide-based BOLD approach reflects tubular oxygen consumption. It thereby assesses overall tubular function and is independent on whether it is acute or chronic. An analogous approach has been used to test the relevance of renal artery stenosis [16]: In normal-size kidneys downstream of high-grade renal arterial stenoses, $R2^*$ was high at baseline and fell after furosemide. Atrophic kidneys beyond totally occluded renal arteries demonstrated low levels of $R2^*$ that did not change after administration of furosemide.

The presented combination of BOLD MRI with a tubule-specific pharmacological maneuver has the limitation that it is rather laborious and time-consuming. Nevertheless, the current technique may be a helpful clinical diagnostic tool, e. g. for the differentiation of prerenal acute kidney injury and acute tubular necrosis (intrinsic acute kidney injury). Moreover, this technique could be used scientifically to investigate e.g. tubulotoxic effects of drugs.

Glomerular function can be assessed by both laboratory measurements and radiographic imaging techniques. With regard to the lack of an adequate laboratory marker, the present study searched for an imaging technique that monitors tubular (dys-)function. It shows that BOLD-MRI is a sensitive technique to detect even mild tubular injury but necessitates a functional maneuver, e.g. blocking the $Na^+-K^+-2Cl^-$ cotransporter by furosemide.

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Disclosure Statement

None declared.

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