Am J Physiol Renal Physiol 308: F1444–F1451, 2015.
First published April 29, 2015; doi:10.1152/ajprenal.00064.2015.

Functional MRI detects perfusion impairment in renal allografts with delayed graft function

Katja Hueper,[1] Faikah Gueler,[2] Jan Hinrich Bräsen,[3] Marcel Gutberlet,[1] Mi-Sun Jang,[2] Frank Lehner,[4] Nicolas Richter,[4] Nils Hanke,[2] Matti Peperhove,[1] Petros Martirosian,[5] Susanne Tewes,[1] Van Dai Vo Chieu,[1] Anika Großhennig,[6] Hermann Haller,[2] Frank Wacker,[1] Wilfried Gwinner,[2] and Dagmar Hartung[1]

[1]*Institute for Diagnostic and Interventional Radiology, Hannover Medical School, Hannover, Germany;* [2]*Department of Nephrology, Hannover Medical School, Hannover, Germany;* [3]*Institute for Pathology, Hannover Medical School, Hannover, Germany;* [4]*Department of General, Abdominal and Transplant Surgery, Hannover Medical School, Hannover, Germany;* [5]*Section on Experimental Radiology, University of Tübingen, Tübingen, Germany; and* [6]*Institute for Biostatistics, Hannover Medical School, Hannover, Germany*

Submitted 18 February 2015; accepted in final form 28 April 2015

Hueper K, Gueler F, Bräsen JH, Gutberlet M, Jang M, Lehner F, Richter N, Hanke N, Peperhove M, Martirosian P, Tewes S, Vo Chieu VD, Großhennig A, Haller H, Wacker F, Gwinner W, Hartung D. Functional MRI detects perfusion impairment in renal allografts with delayed graft function. *Am J Physiol Renal Physiol* 308: F1444–F1451, 2015. First published April 29, 2015; doi:10.1152/ajprenal.00064.2015.—Delayed graft function (DGF) after kidney transplantation is not uncommon, and it is associated with long-term allograft impairment. Our aim was to compare renal perfusion changes measured with noninvasive functional MRI in patients early after kidney transplantation to renal function and allograft histology in biopsy samples. Forty-six patients underwent MRI 4–11 days after transplantation. Contrast-free MRI renal perfusion images were acquired using an arterial spin labeling technique. Renal function was assessed by estimated glomerular filtration rate (eGFR), and renal biopsies were performed when indicated within 5 days of MRI. Twenty-six of 46 patients had DGF. Of these, nine patients had acute rejection (including borderline), and eight had other changes (e.g., tubular injury or glomerulosclerosis). Renal perfusion was significantly lower in the DGF group compared with the group with good allograft function (231 ± 15 vs. 331 ± 15 $ml \cdot min^{-1} \cdot 100\ g^{-1}$, $P < 0.001$). Living donor allografts exhibited significantly higher perfusion values compared with deceased donor allografts ($P < 0.001$). Renal perfusion significantly correlated with eGFR ($r = 0.64$, $P < 0.001$), resistance index ($r = -0.57$, $P < 0.001$), and cold ischemia time ($r = -0.48$, $P < 0.01$). Furthermore, renal perfusion impairment early after transplantation predicted inferior renal outcome and graft loss. In conclusion, noninvasive functional MRI detects renal perfusion impairment early after kidney transplantation in patients with DGF.

MRI; renal perfusion; kidney transplantation; delayed graft function

DELAYED GRAFT FUNCTION (DGF) is a significant diagnostic and clinical challenge in the early postoperative period after kidney transplantation. It occurs in ~20–25% of patients in Europe and the United States after deceased donor transplantation and is defined as failure of an adequate serum creatinine (S-creatinine) decrease to occur or need for dialysis during the first week after transplantation (28, 29). In other countries where there are increased cold ischemia times, the DGF rate is as high as 70%. It has been shown previously that prolonged cold ischemia and increasing donor age contribute to DGF (5, 24). DGF is associated with an increased risk of acute allograft rejection, impaired long-term allograft function, and graft loss (24, 35). Therefore, early detection of DGF is urgently needed if we hope to initiate appropriate therapy and limit irreversible allograft damage.

Methods for evaluating renal allograft function have changed little in the last decade; we mainly rely on repetitive measurements of S-creatinine or cystatin C and renal ultrasound with measurement of the resistance index. Kidney biopsy is recommended in patients with DGF to identify the reason for graft dysfunction and to distinguish acute kidney injury (AKI) from rejection episodes to deliver antirejection treatment. Biopsies are invasive, however. They are stressful for the patient and associated with complications (e.g., bleeding, arteriovenous fistula, femoral vein thrombosis) in up to 10–15% of cases (9, 27). Furthermore, as only small tissue samples are obtained, biopsies may not always allow us to detect renal allograft pathology; i.e., there are sampling errors (31). The resistance index, which reflects intrarenal vascular resistance and compliance, is measured by Doppler ultrasound. It has been reported to be increased in patients with renal allograft dysfunction early after transplantation (4, 6) and to predict allograft survival (4, 15, 25), but results vary from operator to operator, and they are influenced by extrarenal factors such as age, atherosclerosis, and arterial stiffness (16, 23).

Arterial spin labeling (ASL) is a noninvasive functional MRI technique which allows renal perfusion to be quantified without administration of a contrast agent by using water protons of the arterial blood as an endogenous tracer (8, 20, 21). ASL perfusion values have been shown to correlate with single-photon emission-computed tomographic renal perfusion measurements (8), renal plasma flow measured by PAH clearance (26), and renal perfusion measured by dynamic contrast-enhanced MRI (34) in native kidneys.

Since renal perfusion impairment is a hallmark of the pathogenesis of DGF (24), ASL may be valuable for assessment of patients with allograft dysfunction in the early posttransplantation period. The purpose of this study was to compare renal perfusion changes in patients early after kidney transplantation detected by functional contrast-free MRI to renal function,

Address for reprint requests and other correspondence: K. Hueper, Institute for Diagnostic and Interventional Radiology, Hannover Medical School, Carl-Neuberg-Str.1, 30625 Hannover, Germany (e-mail: hueper.katja@mh-hannover.de).

allograft histology in biopsy samples, and renal outcome 1 yr after transplantation.

MATERIALS AND METHODS

Patients. The prospective study was approved by the local institutional review board, and written informed consent was obtained from all participants. Between July 2012 and April 2014, 46 selected adult kidney transplant recipients were included in the study. Patient characteristics, medical histories, and transplantation details were recorded. The clinical follow-up period was 6 mo in all subjects recruited and 12 mo in 39/46 patients. One patient was lost to follow-up, and six patients have not yet reached the 12-mo follow-up point. DGF was defined as a failure of S-creatinine to decrease by at least 10% daily on 3 consecutive days or need for dialysis during the first week after transplantation.

MRI protocol and analysis. MRI was performed in patients 4–11 days after kidney transplantation using a 1.5-T magnet (MAGNETOM Avanto, Siemens Healthcare). T2-weighted turbo spin echo sequences were acquired in axial and coronal planes to assess renal morphology. For renal perfusion measurement, a flow-alternating inversion recovery (FAIR) true FISP ASL technique was used as described previously (18, 21). In brief, images were acquired in an oblique sagittal orientation to avoid covering the aorta or the pelvic arteries. Images were obtained after global and slice-selective inversion pulses using the following parameters: TR/TE = 4.6/2.3 ms, TI = 1,200 ms, flip angle = 70°, averages = 30, FOV = 340 × 340 mm^2, matrix = 128 × 128, slice thickness = 5 mm, and acquisition time = 4.5 min. In addition, a proton density true FISP image without inversion was acquired to determine the equilibrium magnetization. Motion artifacts were compensated by registration of individual MRI images, and parameter maps of renal perfusion were calculated with Matlab (version 7.11.0.584, MathWorks, Natick, MA) according to

$$f = \frac{\lambda}{2TI}\frac{\Delta M(TI)}{M_0}\exp\left(\frac{TI}{T1}\right)$$

as described previously, where ΔM is the signal difference between FAIR images with global and slice-selective inversion, M_0 is the equilibrium magnetization per unit mass, and λ is the blood-tissue water partition coefficient, which was set to 80 ml/100 g (18, 21). Three regions of interest were placed manually on perfusion maps into the renal cortex in the top, middle, and bottom third of the kidney by one author who was blinded to clinical, laboratory, and histological outcome data, and mean perfusion was calculated. Visible perfusion defects of renal parenchyma were identified and were excluded from the analysis. To evaluate intraobserver variability, a second reader, who was blinded to the results of the first reader and clinical data, analyzed renal perfusion in 20 randomly selected patients.

Clinical data. S-creatinine was determined daily during the first 10 days after kidney transplantation as well as 6 wk and 3, 6, and 12 mo thereafter. Glomerular filtration rate (eGFR) was calculated based on S-creatinine according to the MDRD formula (19). Urine output, blood pressure, and immunosuppressive medication including serum levels of calcineurin inhibitors (cyclosporine or tacrolimus) were determined on the day of MRI.

Doppler ultrasound. Ultrasound of the renal transplant was performed by a nephrologist or transplant surgeon within the first and second week after transplantation as described previously (25). The renal resistance index (RI) was measured in two or three proximal segmental arteries and was calculated from peak systolic velocity (V_{max}) and minimal diastolic velocity (V_{min}) according to the formula

$$\text{RI} = 1 - \frac{V_{min}}{V_{max}}.$$

The RI in the early postoperative period was available in 38/46 patients.

Histology. Renal allograft biopsies within 5 days of MRI were considered for the analysis. Indication biopsies were performed in 17/26 of DGF patients 7.8 ± 0.5 days after transplantation In 2/20 patients who had initial graft function but slower S-creatinine reduction than expected, biopsies were performed in the second week after transplantation. Biopsy specimens were stained with hematoxylin and eosin and periodic acid Schiff. Sirius red staining was used to visualize collagen fibers. For quantitation, the total stained area of each biopsy specimen after exclusion of major vessels and glomeruli was determined using image J software (National Institutes of Health). Biopsy specimens were analyzed by a nephropathologist and classified according to Banff criteria (30). AKI was diagnosed by tubular necrosis, vacuolization, lysis or loss of the brush border, and tubular epithelial cell flattening. AKI score was defined as follows: 0 = no AKI; 1 = <10%; 2 = 11–25%; 3 = 26–50%; and 4 = >51% affected tubuli.

Statistical analysis. Statistical analysis was performed with SPSS 22.0 (IBM, Armonk, NY). As no deviations from normal distribution of renal perfusion values were detected by the Kolmogorov-Smirnov test (P = 0.2), parametric statistical tests were used for analysis. Differences in renal perfusion between patients with and without DGF were assessed by unpaired *t*-tests. In addition, subgroup analyses were performed to evaluate renal perfusion differences between *1*) patients with living and with deceased donor transplantation, *2*) DGF patients with and without biopsy-proven acute allograft rejection, and *3*) DGF patients with persistent severe impairment of renal function (eGFR <30 ml/min) or graft loss and DGF patients with eGFR ≥30 ml/min at 12-mo follow-up by using one-way ANOVA. Adjustment for multiple comparisons was performed with the Sidak method. The intraobserver variability of renal perfusion quantification was evaluated using the coefficient of variation. For comparison of clinical and laboratory parameters between patients with normal graft function and with DGF, unpaired *t*-tests and Fisher's exact test were used. The correlations of renal perfusion with various clinical parameters were determined by Pearson's coefficient of correlation.

Furthermore, receiver operating characteristic curve analyses were performed to determine the diagnostic performance of renal perfusion measurement by MRI for *1*) detection of DGF, *2*) prediction of the need for >2 hemodialysis sessions during the first and second week after transplantation, and *3*) prediction of graft loss and severe impairment of renal allograft function (eGFR <30 ml/min) in DGF patients at 12 mo follow-up. In addition, Youden-selected thresholds were determined and sensitivities and specificities at threshold are given.

Values are given as means and SE. This is an exploratory study, thus two-sided P values <0.05 are considered statistically significant.

RESULTS

Transplantation details and clinical data. In total, 20 patients with functional grafts and 26 patients with DGF were examined. As selected patients were included into the study, the proportion of patients with DGF was higher than it is on average at our center (on average 26% DGF patients). Patient characteristics are summarized in Table 1. All patients received triple immunosuppressive therapy with cyclosporine or tacrolimus in addition to mycophenolate mofetil (MMF) and prednisolone. Basiliximab was applied as induction therapy. DGF patients were significantly older (58.9 ± 2.1 vs. 48.8 ± 3.8 yr, $P < 0.05$) and had more coronary and peripheral artery occlusive disease ($P < 0.05$). Donor S-creatinine was significantly lower in patients with initial graft function (67.7 ± 4.9 vs. 116.0 ± 19.8 μmol/l, $P < 0.05$, Table 1). Living donor transplantation was performed in 6/26 DGF patients and in 9/20 patients with initial graft function. No differences between recipients with initial graft function and DGF in the number of human leukocyte antigen mismatches, cold isch-

Table 1. *Patient characteristics, transplantation details, and clinical data*

	Initial Graft Function (n = 20)	Delayed Graft Function (n = 26)	P Value
Recipient data			
Age, yr	48.8 ± 3.8	58.9 ± 2.1	P = 0.026
Sex female	9/20 (45.0%)	7/26 (26.9%)	ns, P = 0.23
Height, cm	172 ± 3	174 ± 2	ns, P = 0.51
Weight, kg	68.1 ± 3.4	83.7 ± 3.6	P = 0.003
Hypertension	19/20 (95.0%)	23/26 (88.5%)	ns, P = 0.62
Diabetes mellitus	1/20 (5.0%)	5/26 (19.2%)	ns, P = 0.21
Coronary artery disease	2/20 (10.0%)	10/26 (38.5%)	P = 0.043
Peripheral artery occlusive disease	0/20 (0%)	6/26 (23.1%)	P = 0.029
Donor data			
Age, yr	55.5 ± 2.9	60.2 ± 2.8	ns, P = 0.25
Sex female	14/20 (70.0%)	12/24 (50.0%)	ns, P = 0.23
Height, cm	171 ± 1	173 ± 2	ns, P = 0.29
Weight, kg	83.0 ± 4.7	80.1 ± 3.2	ns, P = 0.60
S-creatinine, μmol/l	67.7 ± 4.9	116.0 ± 19.8	P = 0.025
Living donors	9/20 (45%)	6/26 (23.1%)	ns, P = 0.20
Transplantation details			
HLA mismatchs, no. of antigens	2.6 ± 0.5	2.9 ± 0.3	ns, P = 0.65
Cold ischemia time deceased donor transplantation, h	12.2 ± 1.1	13.3 ± 1.1	ns, P = 0.55
Cold ischemia time living donor transplantation, h	2.6 ± 0.2	2.4 ± 0.1	ns, P = 0.45
Immunosuppressive regime with cyclosporin	8/20 (40.0%)	15/26 (57.7%)	ns, P = 0.37
Cyclosporin A level, μg/l	125 ± 8	129 ± 10	ns, P = 0.77
Immunosuppressive regime with tacrolimus	12/20 (60%)	11/26 (42.3%)	ns, P = 0.37
Tacrolimus level, μg/l	8.9 ± 0.6	10.4 ± 1.7	ns, P = 0.43
Clinical data posttransplantation			
Systolic blood pressure, mmHg	143 ± 6	141 ± 5	ns, P = 0.71
Diastolic blood pressure, mmHg	81 ± 3	78 ± 2	ns, P = 0.45
Urine output, ml/24 h	3,330 ± 314	2,288 ± 248	P = 0.011
Hemodialysis	0/20 (0%)	16/26 (61.5%)	P < 0.001
No. of hemodialysis sessions per patient	0	1.62 ± 0.4	P < 0.001
Graft loss	0/20 (0%)	3/26 (11.5%)	ns, P = 0.25

Values are means ± SE. HLA, human leukocyte antigen. Levels of immunosuppressive drugs, blood pressure, and urine output represent values on the day of MRI. The number of hemodialysis sessions is given for the first and second weeks. Resistance index was measured 1–2 wk after transplantation.

emia time, the immunosuppressive regime, and levels of immunosuppressive drugs were observed.

Renal function measured by eGFR 7 days after transplantation was reduced in all patients with DGF compared with patients with initial graft function (14.5 ± 1.7 vs. 42.5 ± 16 ml/min, $P < 0.001$). Consequently, many patients in the DGF group required hemodialysis after transplantation whereas in the group with initial graft function no dialysis was necessary (16/26 vs. 0/20, $P < 0.001$).

Allograft histology after clinically indicated biopsies. Indicated biopsies were performed in 17 patients in the DGF group within 5 days of the MRI examination. Histological analysis in DGF patients revealed five patients with borderline rejection according to the Banff classification, three with acute T cell-mediated rejections (Banff Ib, IIa, and III), and one with an antibody-mediated rejection. Eight patients in the DGF group had other changes such as tubular damage or glomerulosclerosis. One patient had severe thrombotic microangiopathy 6 days after transplantation in combination with signs of T cell-mediated rejection, and removal of the kidney was necessary. Two patients with initial graft function had biopsies 8 and 10 days after kidney transplantation because their S-creatinine decreased more slowly than expected. One of these patients had borderline changes and was treated with steroid pulse therapy, and one had mild acute tubular injury. Mean AKI scores were 0 in the group with initial graft function and 0.9 ± 0.3 in the group with DGF.

Renal perfusion measured by functional MRI. Intraobserver variability of renal perfusion quantification was low, with a coefficient of variation of 2.4%. Renal perfusion in allografts from DGF patients was significantly lower than in allografts with initial renal function (231 ± 15 vs. 331 ± 15 $ml \cdot min^{-1} \cdot 100\ g^{-1}$, $P < 0.001$). Representative perfusion maps can be seen in Fig. 1. Living donor grafts exhibited higher perfusion values compared with deceased donor grafts (339 ± 19 vs. 243 ± 14 $ml \cdot min^{-1} \cdot 100\ g^{-1}$, $P < 0.001$), and renal perfusion significantly correlated with cold ischemia time ($r = -0.48$, $P < 0.01$). Furthermore, slightly lower perfusion values were observed in patients with acute renal allograft rejection at histology vs. patients with DGF but without signs of an acute allograft rejection (202 ± 35 vs. 246 ± 14 $ml \cdot min^{-1} \cdot 100\ g^{-1}$, not significant, $P = 0.14$, Fig. 1*D*).

Renal perfusion measured by MRI was positively correlated with eGFR on the day of MRI ($r = 0.64$, $P < 0.001$, Fig. 2*A*) and was negatively correlated with RI ($r = -0.57$ $P < 0.001$, Fig. 2*B*). In addition, renal perfusion significantly correlated with the number of dialysis sessions required in the first and second week after transplantation ($r = -0.63$, $P < 0.001$), with cold ischemia time ($r = -0.48$, $P < 0.01$, Fig. 2*C*), and recipient age ($r = -0.42$, $P < 0.01$). Serum levels of cyclosporine or tacrolimus, number of human leukocyte antigen mismatches, blood pressure, and recipient height and weight did not correlate significantly with renal perfusion.

The best diagnostic performance to detect patients with DGF was achieved by renal perfusion below a Youden-selected threshold of 278 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ with a sensitivity and specificity of 81% (95% CI [61%;93%]) and 75% (95% CI [51%;91%]), respectively (Youden index at threshold 56%,

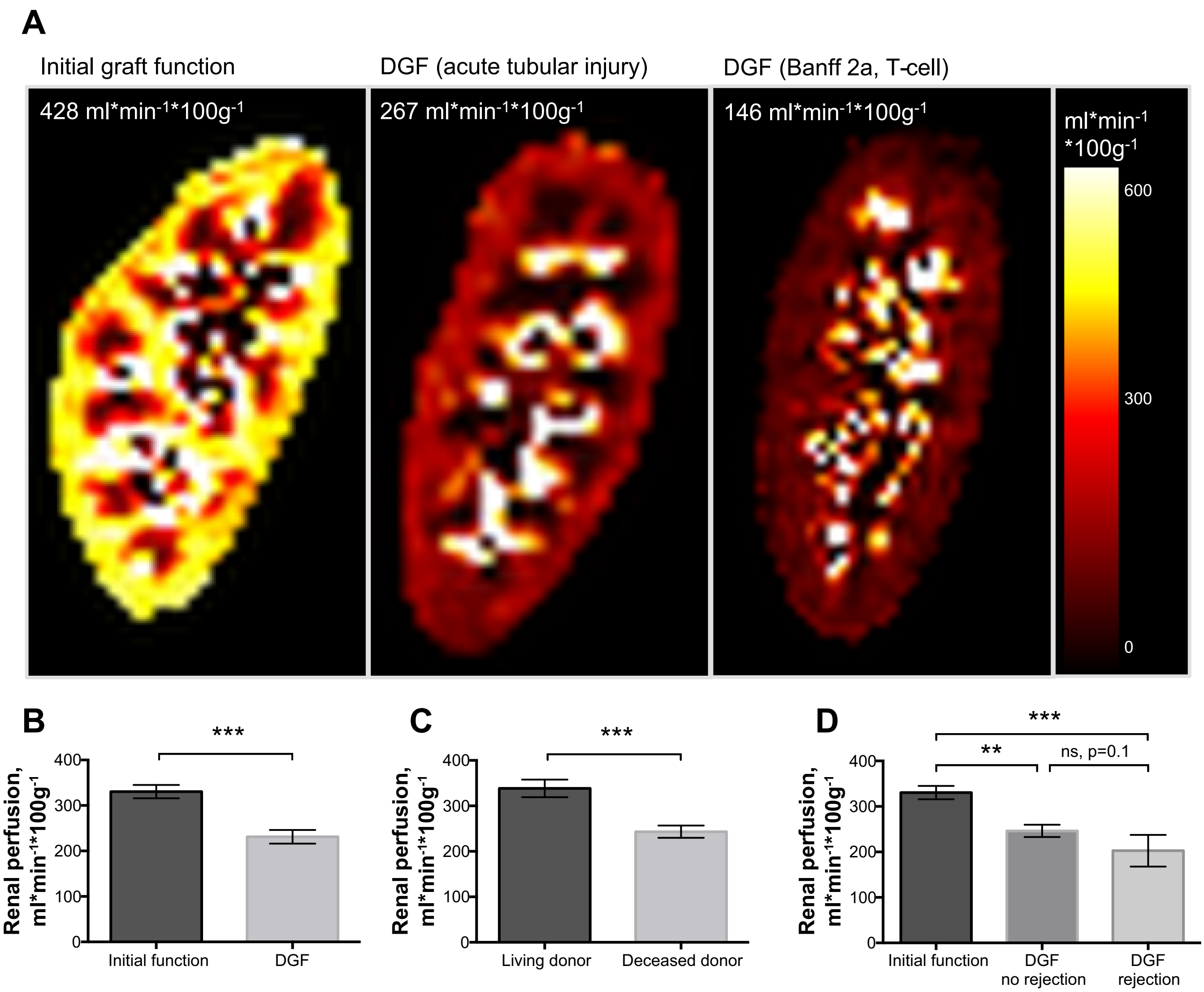

Fig. 1. Renal allograft perfusion. Parameter maps of renal allograft perfusion measured by functional MRI in patients with initial graft function, with delayed graft function (DGF) and acute tubular injury, and with DGF with T cell-mediated rejection (Banff 2a, *A*). Mean renal perfusion values and SE in patients with initial graft function and DGF (*B*), living and deceased donor transplants (*C*), and in the subgroups of DGF patients with and without acute rejection (*D*) are shown. Significant differences are indicated after adjustment for multiple comparisons with the Sidak method: $^{**}P < 0.01$, $^{***}P < 0.001$.

Fig. 2*D*; CI, confidence interval). The sensitivity of renal perfusion measurement to identify patients who required more than two hemodialysis sessions was 100% (95% CI [54%; 100%]), and the specificity was 80% (95% CI [64%;91%]) at a threshold of 236 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ (Fig. 2*E*).

Renal perfusion and renal outcome after 1 yr. After 12 mo, DGF patients were reevaluated and divided into two groups based on their renal function measured by eGFR: one group improved over time and reached eGFR levels ≥30 ml/min; the other group continued to have impaired renal function 12 mo after transplantaton with eGFR <30 ml/min (Fig. 3*A*). In addition, three patients did not reach the 12-mo time point due to allograft nephrectomy because of severe thrombotic microangiopathy (TMA) and mycotic allograft nephritis 9 days and 6 wk after transplantation, respectively. One patient with transplant glomerulosclerosis, severe tubular atrophy, and graft fibrosis died before the scheduled nephrectomy due to cerebral infarction followed by sepsis 5 mo after transplantation.

Note that DGF patients with better renal function after 12 mo (eGFR ≥30 ml/min) had significantly higher renal perfusion early after transplantation (259 ± 16 $ml \cdot min^{-1} \cdot 100\ g^{-1}$) than DGF patients with persistent severe impairment of renal function eGFR <30 ml/min or graft loss (163 ± 22 $ml \cdot min^{-1} \cdot 100\ g^{-1}$, $P < 0.01$, Fig. 3*B*). Renal allograft perfusion impairment below 186 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ in DGF patients predicted graft loss or severe impairment of renal function (eGFR <30 ml/min) 1 yr after transplantation, with a sensitivity of 75% (95% CI [35%;97%]) and a specificity of 92% (95% CI [62%;100%]) (Fig. 3*C*).

For comparison, renal fibrosis quantified by the sirius red-positive tubulointerstitial area in indicated biopsy specimens early after transplantation was highest in patients with graft loss or persistent severe reduction of eGFR <30 ml/min after 1 yr compared with patients without DGF or better renal function (28.1 ± 3.4 vs. 10.3 ± 6 vs. 20.3 ± 2.9%, not significant, Fig. 4).

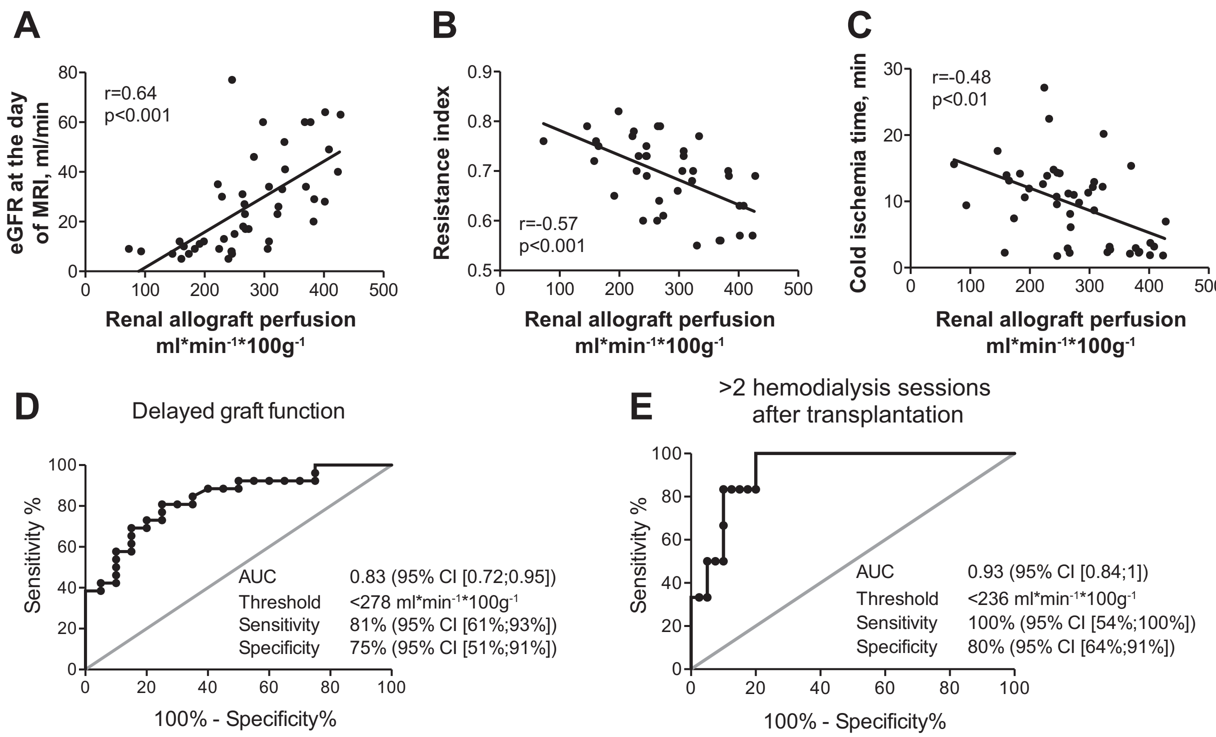

Fig. 2. Correlations of renal allograft perfusion and receiver operating characteristic (ROC) curve analyses. Depicted are the significant positive correlation of renal perfusion with estimated glomerular filtration rate (eGFR) at the day of MRI (*A*), and the negative correlations of renal perfusion with the resistance index (*B*) and with cold ischemia time (*C*). ROC curves show the value of renal perfusion measured by functional MRI to predict DGF (*D*) and the need for more than 2 hemodialysis sessions after transplantation (*E*). Area under the curve (AUC) and sensitivities and specificities at the Youden selected thresholds are given.

DISCUSSION

We noninvasively quantified renal allograft perfusion using functional MRI early after kidney transplantation and demonstrated that renal perfusion is significantly impaired in patients with DGF compared with patients with adequate initial graft function. Renal perfusion significantly correlated with renal function, RI, and cold ischemia time, and was predictive of DGF and the need for dialysis early after transplantation. In addition, renal allograft perfusion impairment early after transplantation was less severe in DGF patients with better outcome (eGFR $\geq$30 ml/min) than in DGF patients with persistent severe impairment of renal function (eGFR $<$30 ml/min) or graft loss 1 yr after transplantation.

Currently, kidney allograft imaging and follow-up of renal function impairment is mainly done by Doppler ultrasound with measurement of the RI. RI measurement is easy and inexpensive to perform and can be done repetitively without stressing the patient. However, recently the value of RI for assessment of renal allograft pathology has been questioned (23). In a longitudinal follow-up study of 321 renal allograft recipients who had repetitive Doppler ultrasound investigations along with protocol biopsies at 3, 12, and 24 mo after kidney transplantation, an elevated RI was not associated with allograft histology and the need for dialysis. The strongest determinant for elevated RI in these transplanted patients was the recipient's age. The authors concluded that the RI, which is strongly dependent on aortic pulse pressure and aortic stiffness, is related to recipient hemodynamics rather than allograft pathology (23). Nonetheless, discriminating different reasons for initial nonfunction or DGF is still a major challenge in kidney transplantation, and further effort is needed to develop more advanced noninvasive imaging techniques.

In the last several years, functional MRI techniques in addition to ASL such as blood oxygen level dependency (BOLD) imaging or diffusion-weighted imaging have been identified as promising noninvasive techniques to evaluate renal allograft function and pathology (2, 11, 17, 18, 32). Nonetheless, the functional MRI techniques and in particular perfusion imaging by ASL, are not routinely available and not yet accepted as standard imaging tests for examination of renal allograft pathology. Therefore, studies with clearly defined patient populations and a detailed comparison of MRI parameters with clinical data and the standard diagnostic tests such as creatinine/eGFR, ultrasound, and renal biopsy are of importance to establish noninvasive functional MRI as a routine technique for evaluation of graft perfusion and to identify groups of patients, who will benefit from the new diagnostic imaging technique. In this study, we evaluate renal perfusion changes by ASL for the first time in a clearly defined group of patients in the first and second week after kidney transplantation compared with the presence of DGF, renal function, renal histology after indicated biopsies, and outcome at 12 mo. This is of great clinical importance as DGF has a high prevalence and portends a high risk for complications such as acute rejection, impaired long-term allograft function, and graft loss (10).

Consistent with current literature, DGF patients in this study had typical risk factors to develop DGF such as a higher percentage of deceased donor kidney grafts, higher donor S-creatinine levels, a higher percentage of coronary artery and

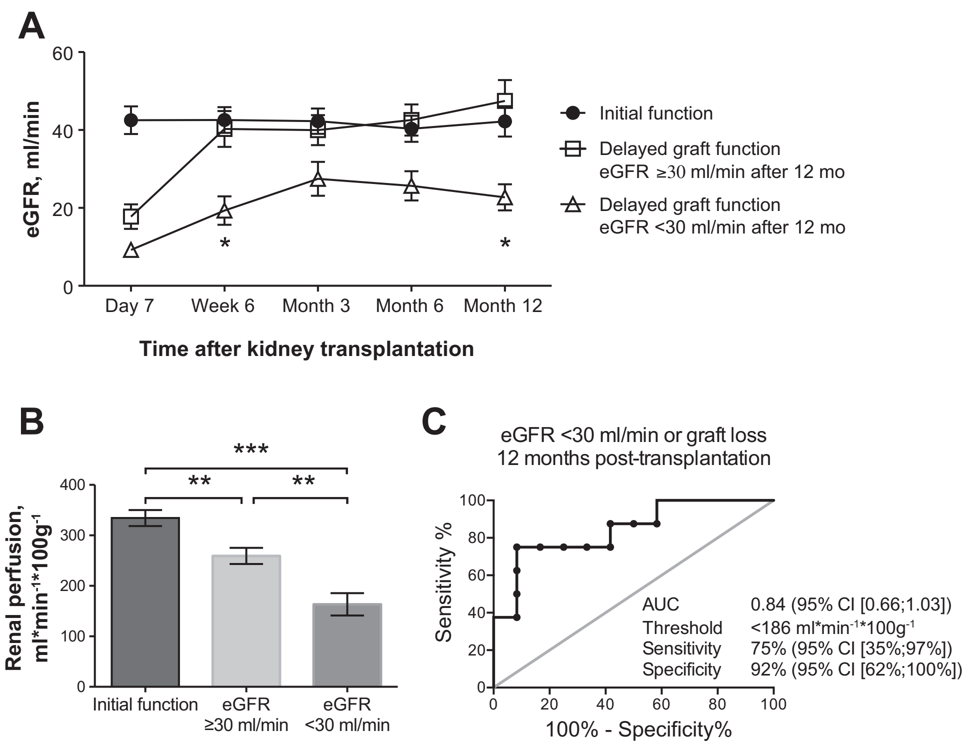

Fig. 3. Renal outcome until 1 yr after transplantation compared with renal allograft perfusion early after transplantation. *A*: courses of eGFR (MDRD formula) in patients with initial allograft function (●) as well as in the subgroups of DGF patients with better renal function with eGFR ≥30 ml/min 1 yr after transplantation (□) and with persistent severe impairment of renal function with eGFR (<30 ml/min) or graft loss (△) are depicted. Significant differences between the subgroups of DGF patients are shown. *B*: differences of renal perfusion early after transplantation in the 3 groups are depicted. *C*: ROC curve analysis demonstrates the predictive value of early renal perfusion measurement for renal outcome 1 yr after transplantation. AUC and sensitivities and specificities at the Youden selected threshold are given. Significant differences are indicated after adjustment for multiple comparisons with the Sidak method: $*P < 0.05$. $**P < 0.01$. $***P < 0.001$.

peripheral artery occlusive diseases of the recipient, and higher recipient body weight (24, 28). Renal perfusion was significantly reduced in DGF patients, well correlated with renal function, and early perfusion impairment predicted the development of DGF and the need for dialysis early after transplantation. As there is an overlap of the perfusion values between patients with and without DGF, in an individual patient it is not possible to predict the presence of DGF by perfusion measurement. Nonetheless, renal perfusion measurement may help to risk stratify the patients and to evaluate the cause of renal dysfunction early after transplantation. In addition, renal perfusion negatively correlated with the RI. The RI measured serially in the early period after kidney transplantation has been shown to be a valuable, noninvasive, widely available, and

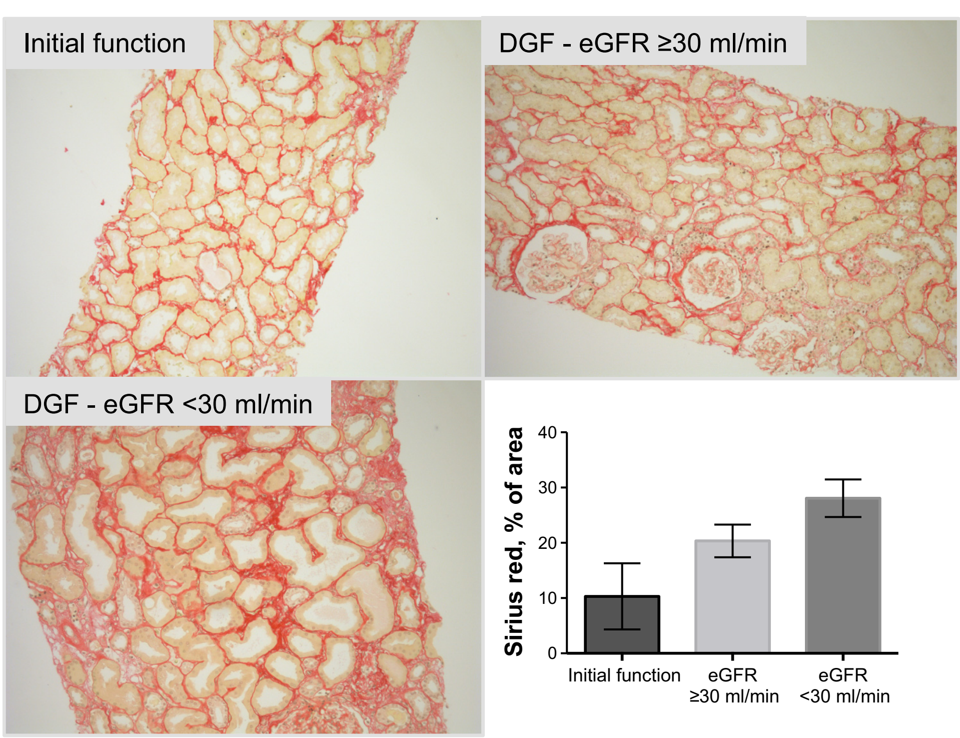

Fig. 4. Renal allograft fibrosis. Sirius red stains to visualize collagen fibers are shown in representative biopsy specimens for the group with initial graft function, DGF with better (eGFR ≥30 ml/min), and with inferior outcome (eGFR <30 ml/min) 1 yr after transplantation. In addition, mean sirius red-positive tubulointerstitial area after exclusion of large vessels and glomeruli in the groups is shown. In the DGF group with inferior outcome, collagen expression was higher than in the groups with initial graft function and the group of DGF patients with better outcome.

cost-effective marker for determining renal graft function; an increased RI early after transplantation is associated with impaired renal function and inferior renal outcome (1, 4, 15). However, the RI is strongly user dependent with high interobserver variation and is limited due to patient-related factors such as tachycardia, tachypnea, recipient arteriosclerosis, and arteriovenous fistulas due to prior renal biopsies. Furthermore, RI is measured in the segmental arteries and direct quantification of renal perfusion in the tissue on a regional basis, as it is performed with functional MRI, is not possible. Thus, in difficult situations such as in patients with an unclear impairment of graft function, in specific pathologies such as thrombotic microangiopathy or in the case of inconclusive ultrasound and biopsy results (e.g., sampling error), renal perfusion measurement may be advantageous in elucidating renal pathology. By MRI, focal perfusion defects can be visualized and distinguished from general impairment of renal perfusion. Furthermore, in patients with a high risk of bleeding complications due to anticoagulant therapy, functional MRI may characterize graft pathology and facilitate therapy decisions while reducing the need for renal biopsies. Allografts from living donors exhibited significantly better renal perfusion than those from deceased donors in the early postoperative phase. These findings are compatible with the fact that DGF is closely related to ischemia-reperfusion injury of the transplant, which is characterized by inflammation, tissue edema, and renal perfusion impairment (3). As cold ischemia time was substantially shorter for living vs. deceased donor allografts, presumably less ischemia-reperfusion injury occurred, thus contributing to better graft perfusion in this group. Consequently, an inverse correlation between cold ischemia time and renal perfusion was observed. The close relationship of renal perfusion impairment to ischemia time has previously been demonstrated in a mouse model of ischemia-reperfusion injury (14). Consistent with our results, Heusch et al. (12) recently found significantly lower renal perfusion by functional MRI in patients with severely reduced renal allograft function (eGFR $\leq$30 ml/min) than in patients with eGFR $>$30 ml/min. These authors also found that there was a close correlation between renal perfusion and eGFR in patients with variable time intervals between MRI and kidney transplantation (3 days to 11 yr) (12). Eisenberger et al. (7) examined 15 renal allograft recipients 5–19 days after transplantation by diffusion-weighted imaging. They demonstrated significantly reduced perfusion fraction of diffusion in patients with allograft dysfunction and a significant correlation of the perfusion fraction with eGFR. As the perfusion fraction of diffusion has been shown to be closely related with renal perfusion measured by ASL (13), their results are consistent with our findings in allografts early after transplantation.

When renal perfusion between the subgroups of DGF patients with and without rejection were compared, we observed a trend toward lower perfusion values in patients with an acute rejection (including borderline changes), but this was not statistically significant. Perhaps this was due to the limited number of patients with acute rejection, and the fact that mostly mild changes were observed at a very early time point after kidney transplantation. This may be associated with only a small additional decrease in renal perfusion compared with patients with DGF alone. Renal perfusion in both DGF groups, with and without acute rejection, was significantly lower than in grafts that were initially functional. Using a rat model of renal transplant rejection, Wang et al. (33) found strongly reduced kidney perfusion in animals with acute rejection compared with animals that had isogenic transplants and had grafts that were initially functional. However, in animal studies severe rejection was induced with dense inflammatory infiltrates. Consequently, the animals cannot be compared with humans whose early signs of rejection include limited leukocyte infiltration.

One patient in our study had biopsy-proven TMA, which was characterized by strong impairment of renal perfusion (87 $ml \cdot min^{-1} \cdot 100\ g^{-1}$). This finding is of particular interest because TMA is a severe complication after kidney transplantation (22), which is often difficult to diagnose even with renal allograft biopsies. Thus ASL might be helpful in identifying patients with TMA and estimating the severity of renal perfusion impairment. This hypothesis needs further investigation.

In DGF patients with persistent severe impairment of renal function (eGFR $<$30 ml/min) or graft loss 1 yr posttransplantation, early perfusion impairment was significantly stronger than in DGF patients with better renal outcome (eGFR $\geq$30 ml/min) after 1 yr. Furthermore, reduced renal perfusion ($<$186 $ml \cdot min^{-1} \cdot 100\ g^{-1}$) predicted inferior renal outcome with a sensitivity of 75% and a specificity of 92% in the group of DGF patients, thus showing the predictive value of MRI perfusion measurement in the early posttransplantation period. Of note, allograft histology revealed a higher degree of renal fibrosis early after transplantation in allografts with an inferior outcome.

Our study has limitations. First, the number of patients is relatively small and the follow-up period may be too short to give detailed insights into the prognostic value of early renal perfusion measurement by ASL. Second, MRI results could only be correlated with histological findings in 19 patients with impaired renal function, since not all patients required biopsies. Third, reliable quantification of renal perfusion was possible in the renal cortex, but not in the renal medulla as the signal-to-noise ratio was not sufficient to measure perfusion changes in the medulla, where the perfusion is substantially lower compared with the cortex. Improvement of ASL to allow quantification of small perfusion changes in the medulla is desirable and of clinical importance, as pathophysiological effects of renal ischemia-reperfusion injury are most pronounced in this renal compartment.

In conclusion, renal perfusion measured by functional MRI is reduced in patients with DGF, correlates well with renal function, and is predictive of renal allograft function 1 yr after transplantation. Thus noninvasive perfusion quantitation with functional MRI may help us detect renal damage in selected transplant recipients soon after their surgeries.

ACKNOWLEDGMENTS

We thank Michael J. Brownstein for help in editing the manuscript.

GRANTS

This work was supported by the German Federal Ministry of Education and Research, IFB Tx Hannover Medical School (reference no. 01EO0802).

DISCLOSURES

K. Hueper reports grants from Deutsche Forschungsgemeinschaft (DFG), Rebirth-Cluster of Excellence and research collaboration with Siemens Health-

care, outside the submitted work. F. Gueler reports grants from Federal Ministry of Education and Research (BMBF) and DFG, outside the submitted work. M. Gutberlet reports grants from Rebirth-Cluster of Excellence, German Center for Lung Research (DZL), and research collaboration with Siemens Healthcare, outside the submitted work. F. Wacker reports grants from BMBF, DZL, Promedicus, Ltd., DFG, Rebirth-Cluster of Excellence, and Siemens Healthcare, outside the submitted work. D. Hartung reports grants from Rebirth-Cluster of Excellence, outside the submitted work.

AUTHOR CONTRIBUTIONS

Author contributions: K.H., H.H., F.W., W.G., and D.H. provided conception and design of research; K.H., M.G., F.L., N.R., N.H., M.J.P., P.M., S.T., and D.H. performed experiments; K.H., J.H.B., M.J.P., S.T., V.D.V.C., A.G., and D.H. analyzed data; K.H., F.G., J.H.B., M.G., M.-S.J., V.D.V.C., W.G., and D.H. interpreted results of experiments; K.H. prepared figures; K.H. drafted manuscript; K.H., F.G., J.H.B., M.G., M.-S.J., F.L., N.R., N.H., M.J.P., P.M., S.T., A.G., H.H., F.W., W.G., and D.H. edited and revised manuscript; K.H., F.G., J.H.B., M.G., M.-S.J., F.L., N.R., N.H., M.J.P., P.M., S.T., V.D.V.C., A.G., H.H., F.W., W.G., and D.H. approved final version of manuscript.

REFERENCES

1. **Barba J, Rioja J, Robles JE, Rincon A, Rosell D, Zudaire JJ, Berian JM, Pascual I, Benito A, Errasti P.** Immediate renal Doppler ultrasonography findings (<24 h) and its association with graft survival. *World J Urol* 29: 547–553, 2011.
2. **Blondin D, Lanzman RS, Mathys C, Grotemeyer D, Voiculescu A, Sandmann W, Rump LC, Modder U, Wittsack HJ.** [Functional MRI of transplanted kidneys using diffusion-weighted imaging]. *Rofo* 181: 1162–1167, 2009.
3. **Bonventre JV, Yang L.** Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest* 121: 4210–4221, 2011.
4. **Cano H, Castaneda DA, Patino N, Perez HC, Sanchez M, Lozano E, Perez MC.** Resistance index measured by Doppler ultrasound as a predictor of graft function after kidney transplantation. *Transplant Proc* 46: 2972–2974, 2014.
5. **Cavaille-Coll M, Bala S, Velidedeoglu E, Hernandez A, Archdeacon P, Gonzalez G, Neuland C, Meyer J, Albrecht R.** Summary of FDA workshop on ischemia reperfusion injury in kidney transplantation. *Am J Transplant* 13: 1134–1148, 2013.
6. **Chudek J, Kolonko A, Krol R, Ziaja J, Cierpka L, Wiecek A.** The intrarenal vascular resistance parameters measured by duplex Doppler ultrasound shortly after kidney transplantation in patients with immediate, slow, and delayed graft function. *Transplant Proc* 38: 42–45, 2006.
7. **Eisenberger U, Thoeny HC, Binser T, Gugger M, Frey FJ, Boesch C, Vermathen P.** Evaluation of renal allograft function early after transplantation with diffusion-weighted MR imaging. *Eur Radiol* 20: 1374–1383, 2010.
8. **Fenchel M, Martirosian P, Langanke J, Giersch J, Miller S, Stauder NI, Kramer U, Claussen CD, Schick F.** Perfusion MR imaging with FAIR true FISP spin labeling in patients with and without renal artery stenosis: initial experience. *Radiology* 238: 1013–1021, 2006.
9. **Franke M, Kramarczyk A, Taylan C, Maintz D, Hoppe B, Koerber F.** Ultrasound-guided percutaneous renal biopsy in 295 children and adolescents: role of ultrasound and analysis of complications. *PloS One* 9: e114737, 2014.
10. **Grosso G, Corona D, Mistretta A, Zerbo D, Sinagra N, Giaquinta A, Cimino S, Ekser B, Giuffrida G, Leonardi A, Gula R, Veroux P, Veroux M.** Delayed graft function and long-term outcome in kidney transplantation. *Transplant Proc* 44: 1879–1883, 2012.
11. **Han F, Xiao W, Xu Y, Wu J, Wang Q, Wang H, Zhang M, Chen J.** The significance of BOLD MRI in differentiation between renal transplant rejection and acute tubular necrosis. *Nephrol Dial Transplant* 23: 2666–2672, 2008.
12. **Heusch P, Wittsack HJ, Blondin D, Ljimani A, Nguyen-Quang M, Martirosian P, Zenginli H, Bilk P, Kropil P, Heusner TA, Antoch G, Lanzman RS.** Functional evaluation of transplanted kidneys using arterial spin labeling MRI. *J Magn Reson Imaging* 40: 84–89, 2014.
13. **Heusch P, Wittsack HJ, Heusner T, Buchbender C, Quang MN, Martirosian P, Bilk P, Kropil P, Blondin D, Antoch G, Lanzman RS.** Correlation of biexponential diffusion parameters with arterial spin-labeling perfusion MRI: results in transplanted kidneys. *Invest Radiol* 48: 140–144, 2013.
14. **Hueper K, Gutberlet M, Rong S, Hartung D, Mengel M, Lu X, Haller H, Wacker F, Meier M, Gueler F.** Acute kidney injury: arterial spin labeling to monitor renal perfusion impairment in mice-comparison with histopathologic results and renal function. *Radiology* 270: 117–124, 2014.
15. **Kolonko A, Chudek J, Zejda JE, Wiecek A.** Impact of early kidney resistance index on kidney graft and patient survival during a 5-year follow-up. *Nephrol Dial Transplant* 27: 1225–1231, 2012.
16. **Kolonko A, Szotowska M, Kuczera P, Chudek J, Wiecek A.** Extrarenal factors influencing resistance index in stable kidney transplant recipients. *Transplantation* 96: 406–412, 2013.
17. **Lanzman RS, Ljimani A, Pentang G, Zgoura P, Zenginli H, Kropil P, Heusch P, Schek J, Miese FR, Blondin D, Antoch G, Wittsack HJ.** Kidney transplant: functional assessment with diffusion-tensor MR imaging at 3T. *Radiology* 266: 218–225, 2013.
18. **Lanzman RS, Wittsack HJ, Martirosian P, Zgoura P, Bilk P, Kropil P, Schick F, Voiculescu A, Blondin D.** Quantification of renal allograft perfusion using arterial spin labeling MRI: initial results. *Eur Radiol* 20: 1485–1491, 2010.
19. **Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F.** Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 145: 247–254, 2006.
20. **Martirosian P, Boss A, Schraml C, Schwenzer NF, Graf H, Claussen CD, Schick F.** Magnetic resonance perfusion imaging without contrast media. *Eur J Nucl Med Mol Imaging* 37, *Suppl* 1: S52–S64, 2010.
21. **Martirosian P, Klose U, Mader I, Schick F.** FAIR true-FISP perfusion imaging of the kidneys. *Magn Reson Med* 51: 353–361, 2004.
22. **Nadasdy T.** Thrombotic microangiopathy in renal allografts: the diagnostic challenge. *Curr Opin Organ Transplant* 19: 283–292, 2014.
23. **Naesens M, Heylen L, Lerut E, Claes K, De Wever L, Claus F, Oyen R, Kuypers D, Evenepoel P, Bammens B, Sprangers B, Meijers B, Pirenne J, Monbaliu D, de Jonge H, Metalidis C, De Vusser K, Vanrenterghem Y.** Intrarenal resistive index after renal transplantation. *N Engl J Med* 369: 1797–1806, 2013.
24. **Perico N, Cattaneo D, Sayegh MH, Remuzzi G.** Delayed graft function in kidney transplantation. *Lancet* 364: 1814–1827, 2004.
25. **Radermacher J, Mengel M, Ellis S, Stuht S, Hiss M, Schwarz A, Eisenberger U, Burg M, Luft FC, Gwinner W, Haller H.** The renal arterial resistance index and renal allograft survival. *N Engl J Med* 349: 115–124, 2003.
26. **Ritt M, Janka R, Schneider MP, Martirosian P, Hornegger J, Bautz W, Uder M, Schmieder RE.** Measurement of kidney perfusion by magnetic resonance imaging: comparison of MRI with arterial spin labeling to para-aminohippuric acid plasma clearance in male subjects with metabolic syndrome. *Nephrol Dial Transplant* 25: 1126–1133, 2010.
27. **Schwarz A, Gwinner W, Hiss M, Radermacher J, Mengel M, Haller H.** Safety and adequacy of renal transplant protocol biopsies. *Am J Transplant* 5: 1992–1996, 2005.
28. **Sharif A, Borrows R.** Delayed graft function after kidney transplantation: the clinical perspective. *Am J Kidney Dis* 62: 150–158, 2013.
29. **Siedlecki A, Irish W, Brennan DC.** Delayed graft function in the kidney transplant. *Am J Transplant* 11: 2279–2296, 2011.
30. **Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, Cosio F, David DS, Drachenberg C, Einecke G, Fogo AB, Gibson IW, Glotz D, Iskandar SS, Kraus E, Lerut E, Mannon RB, Mihatsch M, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Roberts I, Seron D, Smith RN, Valente M.** Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 8: 753–760, 2008.
31. **Solez K, Racusen LC.** The Banff classification revisited. *Kidney Int* 83: 201–206, 2013.
32. **Thoeny HC, Zumstein D, Simon-Zoula S, Eisenberger U, De Keyzer F, Hofmann L, Vock P, Boesch C, Frey FJ, Vermathen P.** Functional evaluation of transplanted kidneys with diffusion-weighted and BOLD MR imaging: initial experience. *Radiology* 241: 812–821, 2006.
33. **Wang JJ, Hendrich KS, Jackson EK, Ildstad ST, Williams DS, Ho C.** Perfusion quantitation in transplanted rat kidney by MRI with arterial spin labeling. *Kidney Int* 53: 1783–1791, 1998.
34. **Winter JD, St Lawrence KS, Cheng HL.** Quantification of renal perfusion: comparison of arterial spin labeling and dynamic contrast-enhanced MRI. *J Magn Reson Imaging* 34: 608–615, 2011.
35. **Yarlagadda SG, Coca SG, Formica RN Jr, Poggio ED, Parikh CR.** Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol Dial Transplant* 24: 1039–1047, 2009.