

Article

Influence of Immunosuppressive Regimen on Diffusivity and Oxygenation of Kidney Transplants—Analysis of Functional MRI Data from the Randomized ZEUS Trial

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Abstract: The ZEUS study was a multi-center randomized controlled trial investigating the effect of early conversion from a ciclosporin-based to an everolimus-based regimen on graft function twelve months post-transplantation. In this investigator-initiated sub-study, functional magnetic resonance imaging (fMRI) of kidney grafts was prospectively performed to non-invasively assess differences in graft oxygenation, diffusion and perfusion between groups and time-points using diffusion-weighted imaging (DWI) and blood oxygen level-dependent (BOLD)-MRI. Sixteen patients underwent DWI and BOLD-MRI at months 4.5 and 12 post-transplantation on a 3 Tesla and 1.5 Tesla ($n = 3$) MR scanner. After exclusion due to image quality, outlier values or missing data, DWI was analyzed for ten subjects; BOLD for eight subjects. The diffusion coefficient ADC_D decreased in the CsA-treated group over time, whereas it increased in the EVE group ($p = 0.046$, medulla). The change in ADC_D from months 4.5 to 12 significantly differed between groups in the cortex ($p = 0.033$) and medulla ($p = 0.019$). In BOLD, cortico-medullary transverse relaxation rate $R2^*$ increased (decreased tissue oxygen) in the CsA-treated and decreased in EVE-treated groups over time. Similarly, $R2^*$ values at month 12 were higher in the CsA-treated group compared to the EVE-treated group. There was no significant difference for the perfusion fraction F_p . In conclusion, this prospective sub-study of the ZEUS trial suggests an impact of immunosuppressive regimen on fMRI parameters of the kidney graft.

Keywords: kidney transplantation; functional MRI; diffusion-weighted imaging (DWI); blood oxygen level-dependent (BOLD); intravoxel incoherent motion imaging (IVIM); ZEUS study; cyclosporin; everolimus; calcineurin inhibitors; mTOR inhibitors



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1. Introduction

Kidney transplantation is the treatment of choice for patients with end-stage kidney disease, prolonging survival [1]. Thanks to highly efficient immunosuppressive therapies, a dramatic reduction in the risk of allograft rejection has been achieved since its beginnings. Until now, the mainstay of post-transplant immunosuppressive regimens are calcineurin inhibitors acting on T cell and T cell-dependent B cell activation. However, major adverse effects, including nephrotoxicity, arterial hypertension and de novo diabetes mellitus, limit their usefulness in kidney transplant recipients [2]. Hence, existing efforts target therapeutic strategies maximally limiting the exposition to calcineurin inhibitors by dose reduction, by shortening of administration period or by replacement with other drugs [3–5].

The ZEUS study was a multi-center, open-label randomized controlled trial designed to investigate the effect of an early conversion from a calcineurin inhibitor-based immunosuppression with ciclosporin (CsA) to a mammalian-target-of-rapamycin inhibitor-based regimen with everolimus (EVE) on graft function twelve months post-transplantation [6]. An improvement in the estimated glomerular filtration rate (eGFR) at twelve months was demonstrated in the EVE-treated group compared to the CsA-treated group, which persisted five years post-transplant, despite a non-significantly higher number of rejection episodes [6,7].

Functional magnetic resonance imaging (fMRI) techniques represent an attractive diagnostic tool for renal investigations allowing the non-invasive simultaneous assessment of several aspects of kidney function, such as tissue structure, perfusion and oxygenation, in addition to morphological imaging, without the need for contrast media [8,9].

Diffusion-weighted imaging (DWI) evaluates organ diffusivity and microperfusion, while blood oxygen level-dependent (BOLD)-MRI assesses tissue oxygenation. Whereas DWI has been investigated as a non-invasive marker of kidney function, BOLD has proven particularly useful to study the acute and chronic effects of various interventions [9–12].

The purpose of this investigator-initiated local sub-study of the ZEUS trial was the evaluation of additional aspects of kidney graft function according to the immunosuppressive regimen using fMRI methods. With the exception of one study performing BOLD after acute intake of ciclosporin, this question has not been addressed so far to the best of our knowledge [13]. Due to the known acute and chronic effects of calcineurin inhibitor nephrotoxicity, including vasoconstriction of afferent arterioles and development of interstitial fibrosis and tubular atrophy (IFTA), we hypothesized that diffusivity and micro-perfusion as measured by DWI and tissue oxygenation measured by BOLD-MRI of kidney grafts differ between patients treated with ciclosporin or everolimus [14].

2. Materials and Methods

The protocol of the present investigator-initiated, prospective, single-center sub-study of the ZEUS trial was approved by the local ethics committee (Canton of Bern, Switzerland, approval number 2004/213) and conducted in accordance with the Declarations of Helsinki and Istanbul [15,16].

2.1. Study Population

All kidney transplant recipients included in the ZEUS trial from our study center were eligible for the current study [6]. Specific exclusion criteria were lack of consent to participate in the sub-study, body weight > 200 kg, classical contraindications to MRI and implanted metallic material without prior 3T-MRI after implantation. Among the 300 study participants of the ZEUS study, 37 patients had been enrolled from our center and represented the screening population for this sub-study.

2.2. Study Design

The ZEUS study was a 12-month multi-center randomized controlled parallel-group trial, the protocol of which has been published previously [6]. Patients were screened among participants of the ZEUS trial before randomization at the outpatient University Clinic for Nephrology and Hypertension in Bern (Figure 1). Written informed consent was obtained from each participant prior to inclusion. The two study visits took place at baseline (4.5 months after transplantation, before randomization within the ZEUS trial) and at month 12 after transplantation. A light meal was allowed on the study day. Laboratory tests, including serum creatinine, serum urea, CsA trough levels and urinary protein as well as ambulatory blood pressure measurement (ABPM), duplex ultrasound scan and functional MRI of the kidney graft were performed at both visits. Baseline clinical characteristics based on medical chart review and EVE trough level were determined at baseline- and 12 month-visits respectively. In addition, protocol transplant biopsies were performed at baseline and 12 months as part of this study amendment.

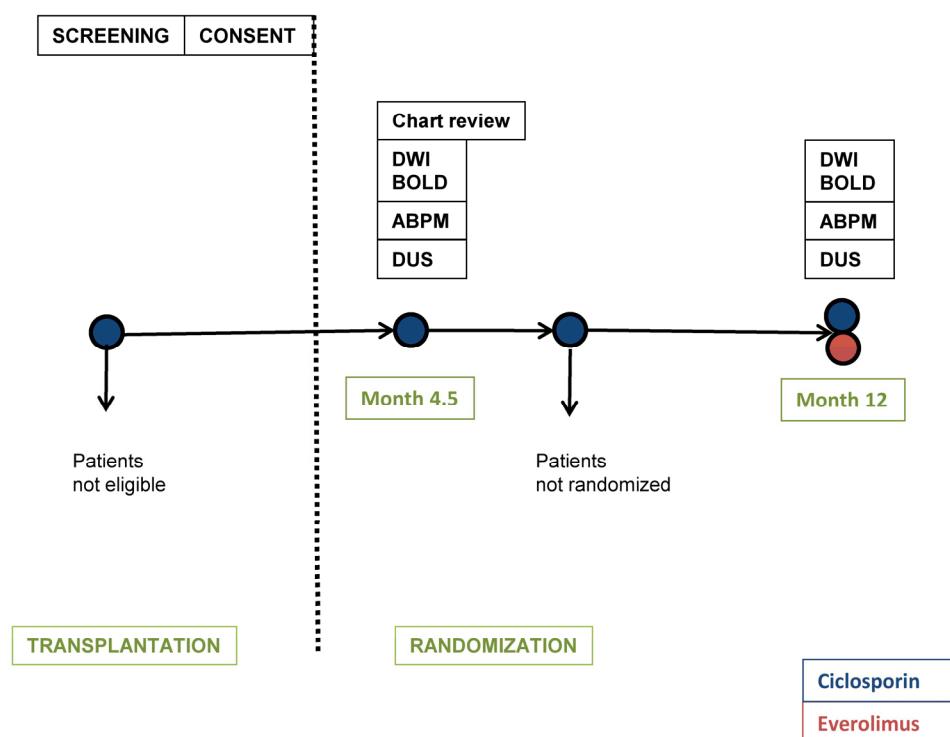


Figure 1. Study design. Diffusion-weighted imaging (DWI); blood oxygen level dependent-magnetic resonance imaging (BOLD-MRI); ambulatory blood pressure measurement (ABPM); duplex ultrasound scan (DUS).

2.3. MRI Protocol

MRI data were acquired on a 3.0 T whole body MR Scanner (Tim Trio[®]; Siemens Healthcare, Erlangen, Germany) for all subjects except three who underwent MRI on a 1.5 T MR scanner (Sonata[®]; Siemens Healthcare, Erlangen, Germany). During each of the two MRI sessions, anatomical MRI, DWI and BOLD-MRI were performed.

Intravoxel incoherent motion (IVIM)-DWI yielded the perfusion-cleared apparent diffusion coefficient ADC_D and the perfusion fraction F_p . Coronal multisection echoplanar DWI was performed with the following parameters: 11 slices (thickness: 5 mm, intersection gap: 1 mm), field of view (FOV) = 400×400 mm², matrix = 128×128 , six averages, bandwidth = 2300 Hz/pixel and partial Fourier 6/8. Ten diffusion gradient b-values were applied (in s/mm²): b = 0, 10, 20, 50, 100, 180, 300, 420, 550 and 700. The gradients were applied in three orthogonal directions and subsequently averaged, minimizing effects of diffusion anisotropy. Parallel imaging (iPAT, mSENSE) with a reduction factor of 2 was applied. A TE of 64 ms was used. Acquisition time for DWI was 8:06 min.

BOLD-MRI takes advantage of deoxygenated hemoglobin as an endogenous contrast agent, which influences the relaxation time T_2^* , yielding the transverse relaxation rate R_2^* (equal to $1/T_2^*$), which correlates to tissue oxygen content provided that confounding factors such as blood volume or hydration state are excluded [8,9]. For BOLD-MRI, a multiple gradient recalled echo sequence (mGre) was used. Four to six coronal slices were acquired with a slice thickness of 5 mm and an intersection gap of 1 mm, a FOV of 400×400 mm² and a matrix size of 256×256 , on average. Other parameters were: TR of 65 ms, TE of 6–52 ms, inter-echo spacing time of 4.2 ms, flip angle of 30° and bandwidth of 330 Hz/pixel. Twelve T_2^* -weighted images, corresponding to 12 different echoes, were acquired for each slice within a single breath-hold of 17 s.

A maximum of three regions of interest (ROIs) traced in medulla and cortex were analyzed in every slice (BOLD: 4–6, DWI: 4 slices) for each medulla and cortex. ROIs were manually defined by the same blinded investigator on images handed over in a random fashion. ROIs were traced in the medulla and cortex. Data were analyzed using in-house

custom-scripts written in IDL[®] and MATLAB[®]. The obtained values were read into MS Excel[®] for further statistical processing. GraphPad Prism 9[®] and MS Powerpoint[®] were used for figure preparation.

2.4. Duplex Ultrasound Scan

Duplex ultrasound scan (DUS) was performed at both study visits by the same experienced nephrologist on a Siemens Acuson Sequoia 512[®] machine. Recorded parameters were resistive indices (RI) measured at intralobar arteries (as a mean of superior, median and lower).

2.5. Ambulatory Blood Pressure Measurement

Ambulatory blood pressure measurement was performed at both study visits using a Profimat II[®] device (Disetronic Medical Systems, Burgdorf, Switzerland). Recorded variables were the overall mean arterial systolic, diastolic and mean pressures and dipping effect (in mmHg).

2.6. Laboratory Analyses

Laboratory analyses were performed according to the ZEUS study protocol as specified in Section 2.2 in the central laboratory of the Bern University Hospital. Glomerular filtration rate was estimated (eGFR) according to chronic kidney disease epidemiology 2021 formula.

2.7. Histopathological Analysis of Kidney Grafts

Histopathological analysis of renal tissue obtained from protocol graft biopsies was performed according to clinical routine in the department of pathology of the University of Bern. Pathologists were not blinded to patient treatment. Biopsy reports were retrospectively assessed for the presence of IFTA, arteriolar hyalinosis and rejection in a semi-quantitative manner. There was no study-specific histological re-analysis.

2.8. Outcome Measures

Primary outcomes were the differences between the CsA-treated and EVE-treated patient groups in ADC_D , F_p , $R2^*$ and medulla/cortex ratios (MCR) $MCR ADC_D$, $MCR F_p$ and $MCR R2^*$ at month 12; as well as in the changes in ADC_D , F_p , $R2^*$ and the MCR from month 4.5 to month 12. Secondary outcomes were the changes in ADC_D , F_p , $R2^*$ and cortico-medullary ratios from month 4.5 to month 12 in each medication group; the differences in mean RI and overall mean systolic, diastolic and mean blood pressure and dipping values between time-points for medication groups and the difference in changes from baseline to month 12 between groups; and the correlations of fMRI parameters and their changes over time across and within medication groups for eGFR, RI, overall mean systolic, diastolic and mean blood pressure and dipping values.

2.9. Statistical Analysis

Continuous variables are expressed as means with standard deviation (SD) or medians and range (between minimal and maximal values). Wilcoxon-signed-rank-test was used for cortico-medullary differences and longitudinal changes in fMRI, RI and ABPM parameters within groups. Mann–Whitney U-test was performed for comparisons across groups of absolute fMRI, RI and ABPM values and of longitudinal changes in these parameters. Correlations between fMRI absolute values and changes over time and differences across groups in eGFR, medication levels, RI and ABPM were determined by Kendall's Tau correlation coefficient analysis. Data were analyzed using IBM SPSS Statistics 24[®] and MS Office 2007[®].

3. Results

3.1. Study Population

3.1.1. Patient Enrolment

From September 2005 until December 2006, 70 patients received a kidney transplant in our center and were screened at the outpatient University Clinic for Nephrology and Hypertension in Bern for participation in the ZEUS trial and the MRI substudy. A total of 37 subjects were enrolled in the ZEUS trial, 16 of which were included in the current study and underwent MR measurements. One patient was subsequently excluded because of lack of randomization, one patient resigned from participation (Figure 2).

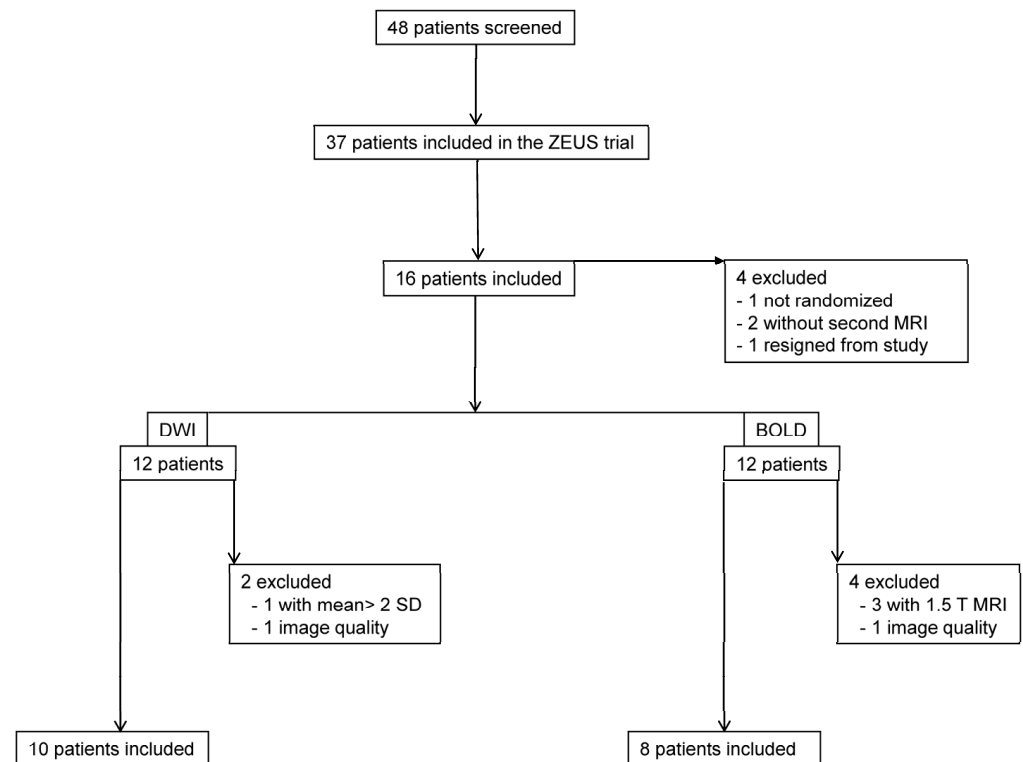


Figure 2. Flow chart. Patient screening and data exclusion.

3.1.2. Patient Characteristics

Clinical characteristics of the subjects are shown in Tables 1 and S1. All of the patients were of Caucasian origin and were mostly first transplant recipients with relatively preserved transplant function. Four and eight subjects had been randomized to the CsA and EVE groups, respectively. There were more female subjects and second transplant recipients in the CsA group, whereas the living donor type was more frequent in the EVE group. Baseline transplant function was lower in the CsA group compared to the EVE group, and baseline mean blood pressure was lower. The proportion of extended criteria donors was higher and the cold ischemia time longer in the CsA group compared to the EVE group. Other variables were homogeneously distributed among medication groups. There were no transplantations from donors deceased from cardiac death in this study.

3.2. Data Quality

The MRI protocol, including morphological sequences, DWI and BOLD, could be performed in all of the patients at baseline and in 12 patients on study day 2. Subjects without a second measurement were not included in the analysis (Figure 2). Data of two patients had to be excluded due to poor image quality, resulting in analyzable data for 11 patients from each MR modality. For DWI, one patient had to be excluded because of outlier values (deviation $> \text{mean} \pm 2 \text{SD}$); for BOLD, three patients were excluded due to

field strength (1.5 T). This resulted in different patient populations undergoing DWI and BOLD for final analysis. A mean scanning time of one hour was met per session. DWI- and BOLD-derived mean values and SD ranges were roughly in line with previously reported values (Table 2) [17–20]. Low SD values confirmed measurement stability.

Table 1. Clinical characteristics of the subjects (*n* = 12).

	CsA Group (<i>n</i> = 4)	EVE Group (<i>n</i> = 8)
Age at transplantation (years)	44 ± 10 (46; 30–53)	45 ± 12 (47; 29–62)
Female gender (%)	75	38
Living donor (%)	0	25
Donor age (years)	58 ± 8 (61; 47–65)	48 ± 15 (53; 23–65)
Extended criteria donor (%)	50	25
Transplant episode (%)		
- first	50	88
Cold ischemia time (min.)	567 ± 232	401 ± 227
Delayed graft function (%)	0	13
eGFR (mL/min/1.73 m ²)	59 ± 11 (62; 45–69)	66 ± 27 (57; 35–109)
CsA trough level (ng/mL)		
- at baseline	174 ± 81 (174; 116–231)	165 ± 57 (144; 87–241)
- at 12 months	131 ± 6 (131; 126–135)	na
24 h-blood pressure (mmHg)		
- systolic	116 ± 10	121 ± 14
- diastolic	73 ± 6	80 ± 13
- mean	95 ± 7	101 ± 13
Hemoglobin (g/L)		
- at baseline	118 ± 17	126 ± 15
- at 12 months	116 ± 18	123 ± 16
Biopsy findings at baseline		
- BPAR (%)	0	0
- arteriolar hyalinosis (0–3)	0.4 ± 0.8	0.5 ± 1.0
- IFTA (0–3)	0.5 ± 0.6	0.5 ± 0.6
Time to MR scan (months)		
- baseline MR	4.3 ± 0.5	3.6 ± 0.5
- second MR	11.3 ± 0.5	11.9 ± 0.6

Values are expressed as mean ± standard deviation (median; range) or as percentages of patients, as appropriate. Number (*n*); ciclosporin (CsA); everolimus (EVE); estimated glomerular filtration rate (eGFR) estimated according to the chronic kidney disease epidemiology collaboration formula. Delayed graft function was defined as the necessity of dialysis therapy during the first week post-transplantation.

Table 2. Overall mean values and standard deviations of DWI- and BOLD-MRI-derived parameters.

	Medulla	Cortex
ADC _D	204.4 ± 15.0	203.0 ± 16.1
F _P	13.0 ± 4.0	14.6 ± 3.1
R2*	26.1 ± 3.0	17.5 ± 2.7

Diffusion coefficient (ADC_D); perfusion fraction (F_P); transverse relaxation rate (R2*).

3.3. Diffusion-Weighted Imaging

3.3.1. Diffusion Coefficient ADC_D

Mean overall medullary and cortical values for the apparent diffusion coefficient ADC_D as a marker of pure diffusion are shown in Tables 2 and 3. No cortico-medullary difference in the ADC_D was noted (*p* = 0.65 at month 4.5, *p* = 0.51 at month 12). In the CsA-treated group, medullary and cortical ADC_D values decreased in all but one subject from month 4.5 to month 12 (*p* = 0.14 and *p* = 0.14), whereas in the EVE-treated group, medullary and cortical ADC_D values increased from month 4.5 to month 12, reaching statistical significance for the medulla (*p* = 0.046 and *p* = 0.12, respectively). At baseline, there was a tendency for higher ADC_D values in the group randomized to CsA as compared to the group randomized to EVE. In contrast, at month 12, ADC_D values were higher by

trend in the EVE-treated group vs. the CsA-treated group (Table 3, Figure 3). This was the result of a significant difference of the mean change in ADC_D from month 4.5 to month 12 over time, which was negative in the CsA-treated group and positive in the EVE-treated group (Table 3, Figure 4). There were no differences in MCR ADC_D between time-points ($p = 0.47$ for CsA-group; $p = 0.17$ for EVE-group) or medication groups.

Table 3. DWI-derived pure diffusion coefficient according to medication group and time-point.

	CsA (n = 4)	EVE (n = 6)	p-Value ¹
Month 4.5			
ADC _D medulla	210.4 ± 25.0	201.0 ± 7.5	0.26
ADC _D cortex	212.1 ± 22.2	201.1 ± 10.0	0.26
MCR ADC _D	0.99 ± 0.02	1.01 ± 0.03	0.87
Month 12			
ADC _D medulla	195 ± 14.5	210.0 ± 12.2	0.17
ADC _D cortex	193.5 ± 20.0	206.3 ± 13.7	0.26
MCR ADC _D	1.01 ± 0.05	1.02 ± 0.04	0.78
Change month 4.5–12			
ΔADC _D medulla	−15.4 ± 18	9.0 ± 7.6	0.019
ΔADC _D cortex	−18.6 ± 23.8	6.2 ± 7.3	0.038
ΔMCR ADC _D	0.02 ± 0.04	0.01 ± 0.003	0.54

¹ Mann–Whitney U-test. Values are expressed as mean ± standard deviation. ADC_D in 10^{−5} mm²/s; MCR (medulla/cortex ratio).

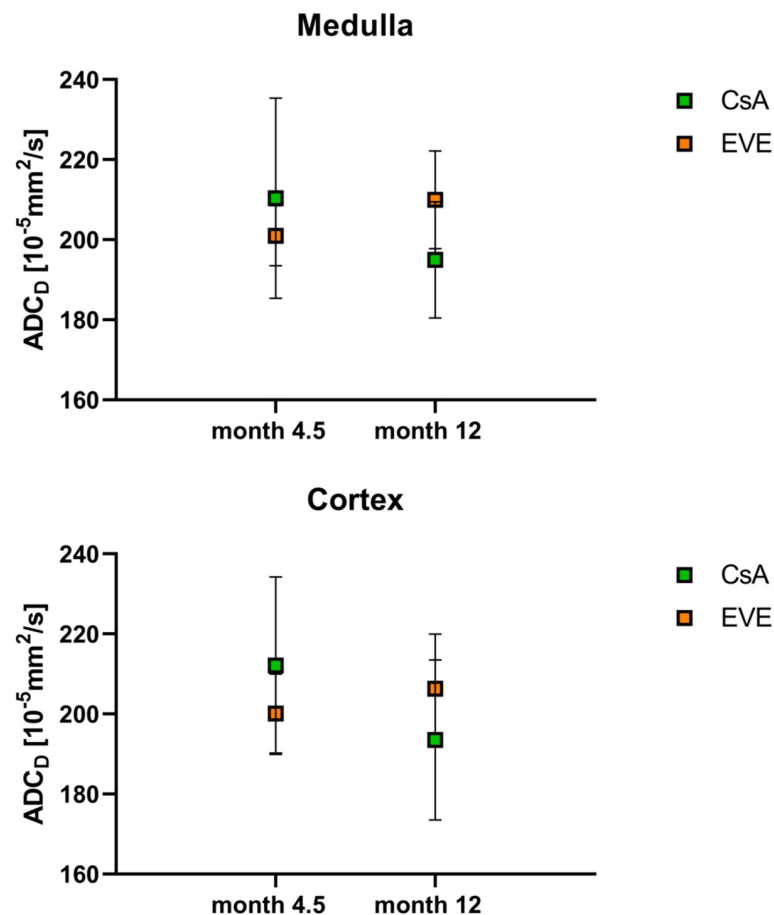


Figure 3. DWI-derived diffusion coefficient. Diffusion coefficient ADC_D according to medication group and time-point in medulla and cortex.

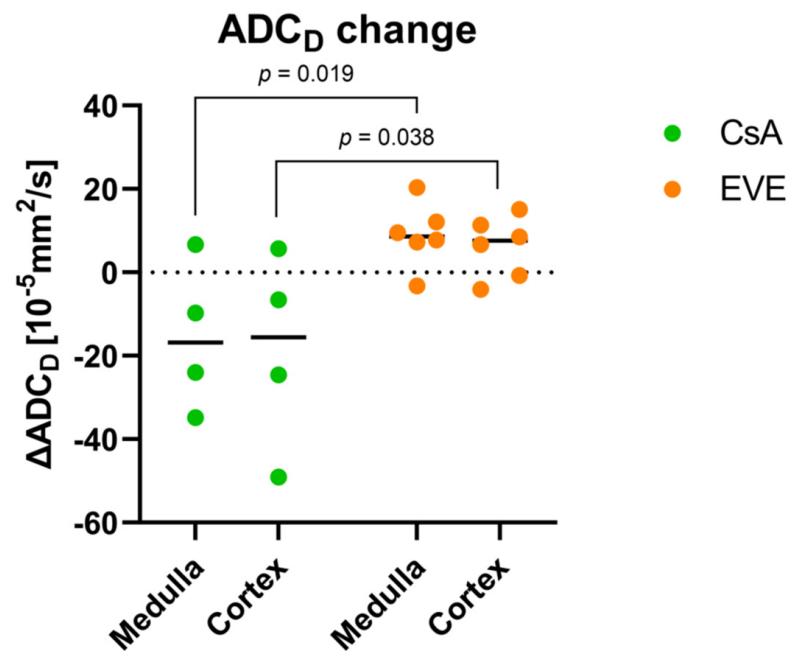


Figure 4. Change in DWI-derived diffusion coefficient. Change in diffusion coefficient ADC_D from month 4.5 to month 12. p -value calculated according to Mann–Whitney U-test.

3.3.2. Perfusion Fraction F_P

Mean medullary and cortical values for the fraction of perfusion F_P are shown in Tables 2 and 4. As with ADC_D , no significant cortico-medullary differences were found for F_P at either time-point ($p = 0.58$ at month 4.5, $p = 0.059$ at month 12). There was no difference in F_P or $MCRF_P$ between medication groups at baseline nor at twelve months (Figure 5) Furthermore, no difference in the change over time of F_P or $MCRF_P$ according to medication group was noted. Overall, no change was seen from baseline to month 12 across or within medication groups in F_P in the medulla ($p = 0.17$ for both groups; $p = 0.72$ for the CsA-group; $p = 0.12$ for the EVE-group), in the cortex ($p = 0.29$ for both groups; $p = 0.72$ for the CsA-group; $p = 0.35$ for the EVE-group) or in the $MCRF_P$ ($p = 0.45$ for both groups; $p = 0.47$ for the CsA-group; $p = 0.25$ for the EVE-group).

Table 4. DWI-derived perfusion fraction according to medication group and time-point.

	CsA (n = 4)	EVE (n = 6)	p-Value ¹
Month 4.5			
F_P medulla	12.8 ± 1.5	15.4 ± 5.9	0.26
F_P cortex	15.3 ± 3.8	15.4 ± 4.4	1.00
MCR F_P	0.9 ± 0.3	1.0 ± 0.2	0.87
Month 12			
F_P medulla	13.4 ± 3.5	10.6 ± 2.1	0.17
F_P cortex	14.4 ± 1.5	13.4 ± 1.9	0.48
MCR F_P	0.9 ± 0.3	0.8 ± 0.1	0.28
Change month 4.5–12			
ΔF_P medulla	0.6 ± 2.5	−4.8 ± 7.1	0.17
ΔF_P cortex	−0.9 ± 4.0	−2.0 ± 4.4	0.76
$\Delta MCR F_P$	0.05 ± 0.36	−0.2 ± 0.3	0.34

¹ Mann–Whitney U-test. Values are expressed as mean ± standard deviation. CsA (ciclosporin); EVE (everolimus); perfusion fraction (F_P) in %.

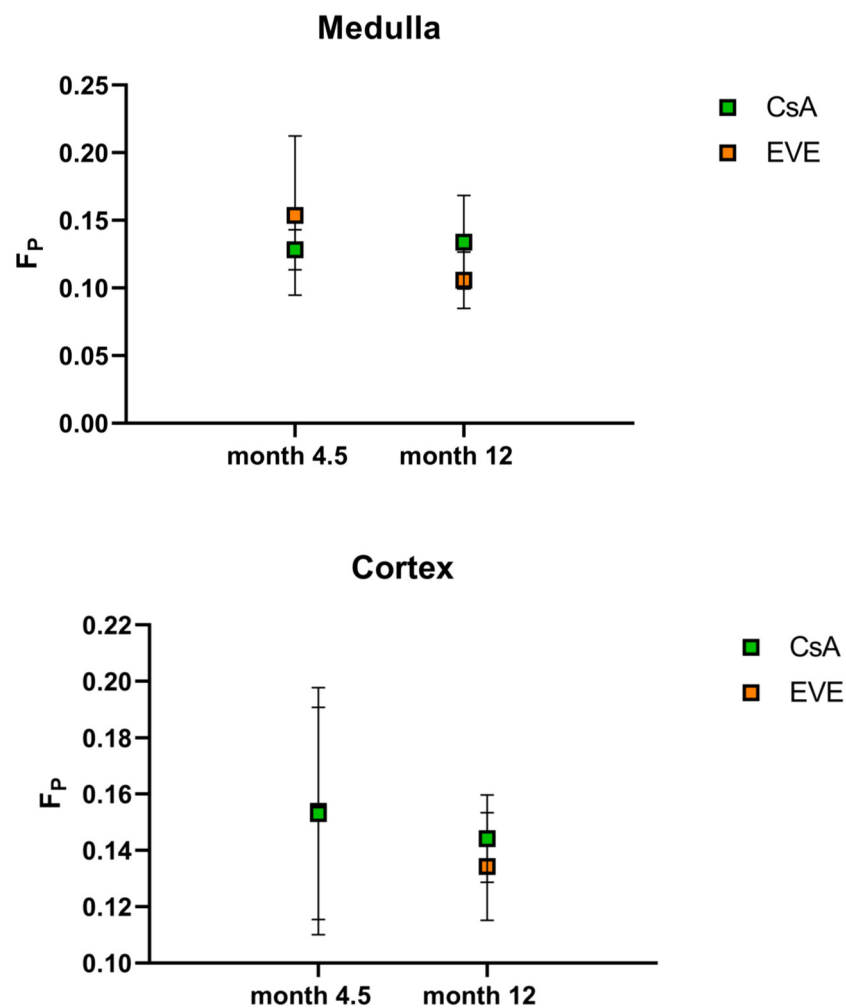


Figure 5. DWI-derived perfusion fraction. Perfusion fraction F_p according to medication group and time-point in medulla and cortex.

3.4. Blood Oxygen Level Dependent-Imaging

Mean medullary and cortical values for the transverse relaxation rate $R2^*$ are shown in Tables 2 and 5. As expected, significant cortico-medullary differences were found at both time-points ($p = 0.012$) with lower cortical $R2^*$ values, compatible with the known relative medullary hypoxia. Inverse trends were noted according to medication assignment, including increasing medullary $R2^*$ values (i.e., reduced tissue oxygenation) in all of the CsA-treated patients and decreasing medullary and cortical $R2^*$ values in five and four of six EVE-treated patients, respectively (Figure 6). However, the number and distribution of cases across groups at month 12 precluded formal statistical analysis. No changes in medullo-cortical distribution were seen between medication groups or time-points.

3.5. Resistive Indices

Mean resistive indices measured by duplex ultrasound scanning are shown in Table 6. At baseline, measured RI were by trend higher in the group randomized to CsA than in the group randomized to EVE. At month 12, however, the difference reached statistical significance due to a tendency to a greater increase in RI over time in the CsA-treated group. No change was seen separately in the groups of CsA- and EVE-treated patients over time ($p = 0.35$ and $p = 0.80$ respectively).

Table 5. BOLD-derived transverse relaxation rate according to medication group and time-point.

	CsA (n = 2)	EVE (n = 6)
Month 4.5		
R2* medulla	24.7 ± 2.7	27.4 ± 3.7
R2* cortex	17.0 ± 3.2	18.6 ± 3.1
MCR R2*	1.5 ± 0.1	1.5 ± 0.1
Month 12		
R2* medulla	27.1 ± 4.1	25.0 ± 1.9
R2* cortex	17.2 ± 0.3	16.7 ± 2.7
MCR R2*	1.6 ± 0.3	1.5 ± 0.3
Change month 4.5–12		
ΔR2* medulla	2.4 ± 1.4	−2.4 ± 3.5
ΔR2* cortex	0.2 ± 3.5	−1.9 ± 3.3
ΔMCR R2*	0.1 ± 0.4	0.1 ± 0.3

Values are expressed as mean ± standard deviation. R2* in s^{−1}.

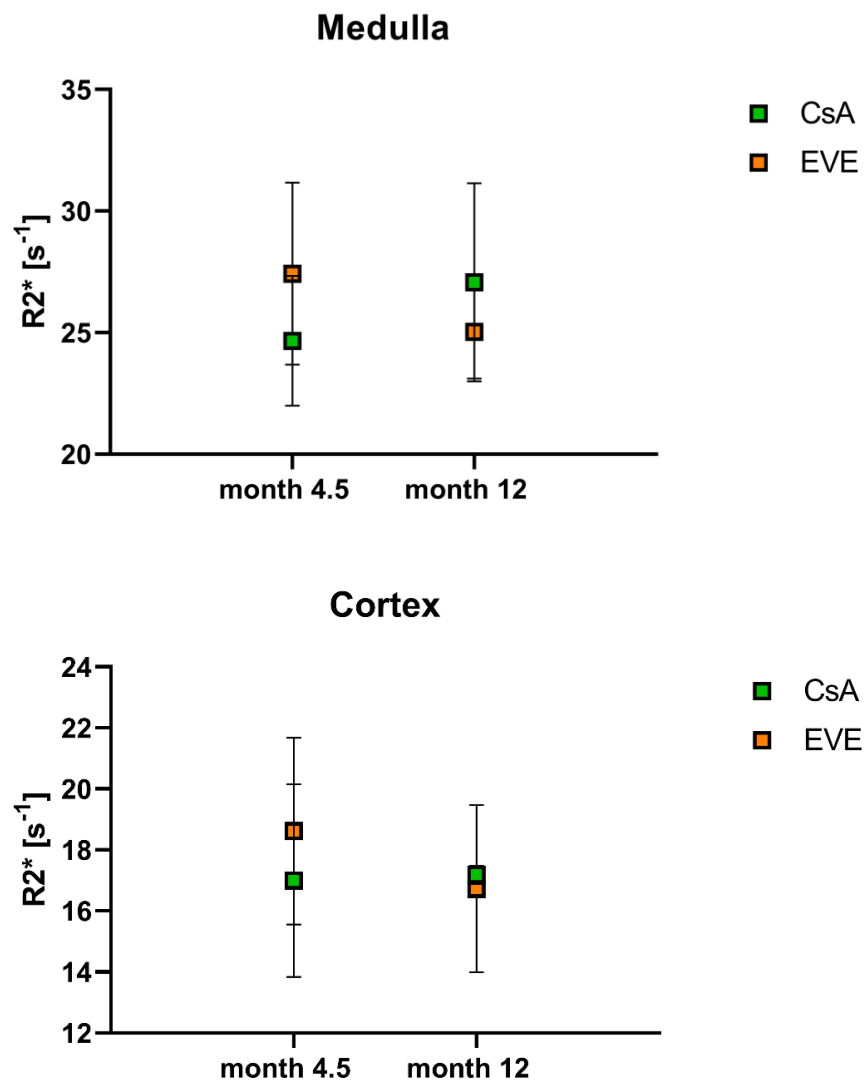


Figure 6. BOLD-derived transverse relaxation rate. Transverse relaxation rate R2* according to medication group and time-point in medulla and cortex.

Table 6. Mean resistive indices measured by duplex ultrasound according to medication group and time-point.

	CsA (n = 6)	EVE (n = 8)	p-Value ¹
RI month 4.5	70.3 ± 4.5	65.0 ± 8.2	0.18
RI month 12	71.7 ± 4.5	65.8 ± 7.3	0.04
ΔRI (month 4.5–12)	1.4 ± 3.7	0.8 ± 4.5	0.76

¹ Mann–Whitney U-test. Values are expressed as mean ± standard deviation. Resistive index (RI).

3.6. Ambulatory Blood Pressure Measurement

Mean ABPM-derived blood pressure values are shown in Table 7. There was a trend for increase in blood pressure from months 4.5 to 12 in the CsA-treated group, whereas blood pressure values in the EVE-treated groups showed no evident change, even though formal statistical analysis was not possible due to the number and distribution of cases at month 12. Conversely, the nocturnal blood pressure dipping tended to increase in the CsA-treated group compared to the EVE-treated group.

Table 7. Mean ABPM-derived blood pressure profile according to medication group and time-point.

	CsA (n = 7) ¹	EVE (n = 8)
Month 4.5		
BP sys	121.3 ± 10.3	120.9 ± 13.5
BP dia	77.1 ± 7.6	79.9 ± 12.8
BP mean	99.6 ± 8.6	100.9 ± 13.2
Dipping (sys/dia/mean)	1.0 ± 6.9/5.0 ± 9.1/2.7 ± 7.3	7.3 ± 12.1/9.5 ± 12.9/8.9 ± 12.9
Month 12		
BP sys	121.0 ± 1.0	122.1 ± 8.1
BP dia	77.3 ± 2.1	79.8 ± 4.8
BP mean	99.7 ± 1.5	101.1 ± 6.2
Dipping (sys/dia/mean)	4.3 ± 10.2/6.3 ± 11.4/5.0 ± 10.6	3.5 ± 11.9/9.3 ± 11/6.0 ± 11.1
Change month 4.5–12		
ΔBP sys	9.7 ± 7.4	1.25 ± 13.5
ΔBP dia	6.3 ± 6.8	−0.1 ± 13.2
ΔBP mean	8.0 ± 6.9	0.3 ± 13.2
ΔDipping (sys/dia/mean)	3.7 ± 16.7/0.3 ± 22.6/2.0 ± 18.5	−3.8 ± 10.0/−0.3 ± 13.2/−2.9 ± 10.9

¹ n = 3 at month 12. Values are expressed as mean ± standard deviation. Blood pressure (BP) in mmHg.

3.7. Laboratory Parameters of Graft Function

Mean serum creatinine values and estimated glomerular filtration rates are shown in Table 8. At baseline, there was a tendency for lower eGFR in the group randomized to CsA. Whereas graft function was stable in the EVE-treated patients over time, it worsened in general in the CsA-treated group. However, values at twelve months and changes from month 4.5 were not significantly different between medication groups.

3.8. Protocol Biopsies

The number of available biopsies was six and five at baseline and five and five at month 12 in the groups randomized to CsA and EVE, respectively. No difference in the degree of IFTA (reaching from none to mild) or arteriolar hyalinosis (reaching from none to severe) was revealed between nor within medication groups (Table S2).

3.9. Correlation of fMRI Parameters

To investigate medication-induced changes in fMRI parameters and correlations of fMRI parameters with selected clinical, biological and histological parameters, exploratory correlation testing was performed.

Table 8. Mean serum creatinine values and estimated glomerular filtration rate according to medication group and time-point.

	CsA (n = 7)	EVE (n = 8)	p-Value ¹
Month 4.5			
Serum creatinine	112.0 ± 21.0	121.0 ± 40.0	0.46
eGFR	60.9 ± 8.1	65.6 ± 26.8	0.61
Month 12			
Serum creatinine	118.9 ± 25.6	117 ± 35.0	1.00
eGFR	57.1 ± 11.0	65.5 ± 19.7	0.40
Change month 4.5–12			
ΔSerum creatinine	6.9 ± 17.2	−4.4 ± 22.3	0.46
ΔeGFR	−3.7 ± 10.3	−0.1 ± 16.5	0.87

¹ Mann–Whitney U-test. Values are expressed as mean ± standard deviation. eGFR according to chronic kidney disease epidemiology formula. Serum creatinine in μmol/l.

Changes in ADC_D over time were not correlated with changes in eGFR or histological parameters. Cortical ADC_D at twelve months correlated negatively with the resistive indices measured by DUS ($\tau = -0.511, p = 0.040$).

The perfusion fraction F_P correlated positively with eGFR at twelve months ($\tau = 0.629, p = 0.012$). A negative correlation was found between F_P and the diastolic and mean blood pressure by ABPM ($\tau = -0.584, p = 0.020$ and $\tau = -0.523, p = 0.038$ respectively).

No correlation of R2* with blood pressure, RI or graft function was noted.

4. Discussion

In this investigator-initiated study, we tested the hypothesis that diffusivity, perfusion and tissue oxygenation as measured by fMRI techniques differ between kidney transplant recipients included in the ZEUS trial randomized to continuing ciclosporin or to being switched to everolimus. The results show an improvement in graft diffusivity after switch to everolimus as compared to patients maintained on ciclosporin.

The main findings are the following: (1) The mean changes in medullary and cortical ADC_D significantly differed between the medication groups showing increase in EVE-treated patients while decreasing in CsA-treated patients. (2) Medullary ADC_D significantly increased after switching to everolimus. (3) Medullary and cortical R2* values showed inverse trends in medication groups with increasing values (i.e., reduced tissue oxygenation) in the CsA-treated group and decreasing values in the EVE-treated group.

First, ADC_D as a marker of perfusion-free diffusion, decreased in patients maintained on CsA, whereas it increased in patients converted to everolimus. The ADC_D has previously been shown to be reduced in various states of acute and chronic kidney diseases and to correlate to biological kidney function markers and interstitial fibrosis [9,18,21–24]. This course parallels the improvement of eGFR in EVE-treated patients reported in the ZEUS trial, which was by trend also observed in our sub-study population. Further, the decrease in diffusivity in CsA-treated patients might point to changes in tissue structure, possibly due to chronic nephrotoxicity patterns of calcineurin inhibitors with development of IFTA. Review of the performed protocol biopsies did however not suggest increased proportion or severity of IFTA in the CsA-treated group, even though the number of biopsies available was low and a sampling error cannot be excluded. In addition, the reversibility after 4.5 months of CsA treatment in the intervention group might suggest early injury or alternative, potentially functional mechanisms, including CsA-induced hemodynamic effects. Microperfusion as measured by F_P was not affected in this study, possibly due to lack of statistical power; moreover, in addition to capillary perfusion, this parameter is believed to reflect tubular processes. Lastly, an influence of the higher proportion of recipients of living donor kidneys in the EVE-treated group together with a higher

number of transplantations from extended criteria donors in the CsA-treated group cannot be excluded.

Second, $R2^*$, as a marker of tissue oxygenation—if confounding factors are excluded—increased (lower tissue oxygenation) in CsA-maintained patients, whereas it decreased (higher tissue oxygenation) in EVE-treated patients. This may suggest ameliorated graft tissue oxygenation under everolimus as compared to ciclosporin, possibly due to the expected vasoconstrictor effect of calcineurin inhibitors [14]. However, hematocrit represents a major confounding factor for BOLD-MRI with anemia leading to falsely low $R2^*$ values, and is expected as part of the everolimus side effect profile [25]. Despite lower hemoglobin values in the core study in EVE-treated patients, hemoglobin values were not significantly different between the medication groups at both time-points in the current study [6].

The DWI-derived fraction of perfusion F_P showed no significant changes over time nor between medication groups. Studies in kidney grafts have suggested lower F_P values in cases of decreased graft function and acute rejection and a correlation with chronic tubulo-interstitial damage [18,23,26]. In our study, the number of cases might have prevented a significant finding. F_P correlated positively with eGFR at month 12, which corresponds to previous reports [18].

In this study, no cortico-medullary differences were found for ADC_D and F_P . This is in accordance with previous studies from our center showing a loss of cortico-medullary differentiation over time in transplanted kidneys from living donors [18,21,22].

Other relevant findings include significantly higher resistive indices as measured by DUS in the CsA-treated group as compared to the EVE-treated group at month 12, possibly in the context of calcineurin vasculopathy. Baseline RI values 4.5 months after transplantation corresponded to reference values in kidney grafts [27]. However, this difference, albeit non-significant, was present at baseline as well. In addition, higher resistive indices were associated with lower cortical ADC_D values at month 12. Similarly and expectedly, mean blood pressure values as measured by ABPM increased by trend in the CsA-treated group over time but remained stable in the EVE-treated group.

There are important limitations to our study: First, the number of subjects available for final analysis was limited, precluding formal statistical analysis of the BOLD data and multivariate analysis of clinical correlations. Second, for technical reasons, three patients underwent MRI at 1.5 T (two of them at baseline, one of them at both time-points), as opposed to 3 T for the other patients. For DWI, the results for ADC_D are nominally independent from field strength, whereas for F_P the sequence can be adapted so that values are comparable even at different field strengths; in contrast, BOLD-derived parameters are not comparable across different field strengths. Consequently, these data were excluded for BOLD-MRI. Lastly, post hoc correction for multiple comparisons was not carried out in this exploratory analysis of clinical correlations.

In conclusion, this prospective small cohort from the ZEUS trial suggests early MRI modifications following CsA to EVE switch. Whether these modifications are linked to the absence of vasoconstrictive effects of calcineurin inhibitors or reflect an early signal of allograft protection needs to be clarified by further studies involving a larger number of patients with systematical and standardized histological analysis.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jcm11123284/s1>. Table S1: Clinical characteristics of the total study population ($n = 16$). Table S2: Histological characteristics at baseline and follow-up.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy reasons.

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References

1. Wolfe, R.A.; Ashby, V.B.; Milford, E.L.; Ojo, A.O.; Ettenger, R.E.; Agodoa, L.Y.; Held, P.J.; Port, F.K. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N. Engl. J. Med.* **1999**, *341*, 1725–1730. [[CrossRef](#)] [[PubMed](#)]
2. Halloran, P.F. Immunosuppressive drugs for kidney transplantation. *N. Engl. J. Med.* **2004**, *351*, 2715–2729. [[CrossRef](#)] [[PubMed](#)]
3. Ekberg, H.; Tedesco-Silva, H.; Demirbas, A.; Vitko, S.; Nashan, B.; Gurkan, A.; Margreiter, R.; Hugo, C.; Grinyo, J.M.; Frei, U.; et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N. Engl. J. Med.* **2007**, *357*, 2562–2575. [[CrossRef](#)] [[PubMed](#)]
4. Webster, A.C.; Lee, V.W.; Chapman, J.R.; Craig, J.C. Target of rapamycin inhibitors (TOR-I; sirolimus and everolimus) for primary immunosuppression in kidney transplant recipients. *Cochrane Database Syst. Rev.* **2006**, CD004290. [[CrossRef](#)] [[PubMed](#)]
5. Masson, P.; Henderson, L.; Chapman, J.R.; Craig, J.C.; Webster, A.C. Belatacept for kidney transplant recipients. *Cochrane Database Syst. Rev.* **2014**, CD010699. [[CrossRef](#)] [[PubMed](#)]
6. Budde, K.; Becker, T.; Arns, W.; Sommerer, C.; Reinke, P.; Eisenberger, U.; Kramer, S.; Fischer, W.; Gschaidmeier, H.; Pietruck, F.; et al. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: An open-label, randomised, controlled trial. *Lancet* **2011**, *377*, 837–847. [[CrossRef](#)]
7. Budde, K.; Lehner, F.; Sommerer, C.; Reinke, P.; Arns, W.; Eisenberger, U.; Wuthrich, R.P.; Muhlfield, A.; Heller, K.; Porstner, M.; et al. Five-year outcomes in kidney transplant patients converted from cyclosporine to everolimus: The randomized ZEUUS study. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* **2015**, *15*, 119–128. [[CrossRef](#)]
8. Prasad, P.V. Functional MRI of the kidney: Tools for translational studies of pathophysiology of renal disease. *Am. J. Physiol. Ren. Physiol.* **2006**, *290*, F958–F974. [[CrossRef](#)]
9. Mani, L.Y.; Vogt, B.; Vermathen, P. Functional Magnetic Resonance Imaging of the Kidney. In *Current Progress in Nephrology*; Ponticelli, C., Visweswaran, R.K., Eds.; TreeLife Media (A Div of Kothari Medical): Maharashtra, India, 2022; pp. 23–48.
10. Caroli, A.; Schneider, M.; Friedli, I.; Ljmani, A.; De Seigneux, S.; Boor, P.; Gullapudi, L.; Kazmi, I.; Mendichovszky, I.A.; Notohamiprodjo, M.; et al. Diffusion-weighted magnetic resonance imaging to assess diffuse renal pathology: A systematic review and statement paper. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. Eur. Ren. Assoc.* **2018**, *33* (Suppl. 2), ii29–ii40. [[CrossRef](#)]
11. Pruijm, M.; Mendichovszky, I.A.; Liss, P.; Van der Niepen, P.; Textor, S.C.; Lerman, L.O.; Krediet, C.T.P.; Caroli, A.; Burnier, M.; Prasad, P.V. Renal blood oxygenation level-dependent magnetic resonance imaging to measure renal tissue oxygenation: A statement paper and systematic review. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. Eur. Ren. Assoc.* **2018**, *33* (Suppl. 2), ii22–ii28. [[CrossRef](#)]
12. Mani, L.-Y.; Seif, M.; Nikles, F.; Tshering Vogel, D.W.; Diserens, G.; Martirosian, P.; Burnier, M.; Vogt, B.; Vermathen, P. Hip Position Acutely Affects Oxygenation and Perfusion of Kidney Grafts as Measured by Functional Magnetic Resonance Imaging Methods—The Bent Knee Study. *Front. Med.* **2021**, *8*, 697055. [[CrossRef](#)] [[PubMed](#)]
13. Hofmann, L.; Simon-Zoula, S.; Nowak, A.; Giger, A.; Vock, P.; Boesch, C.; Frey, F.J.; Vogt, B. BOLD-MRI for the assessment of renal oxygenation in humans: Acute effect of nephrotoxic xenobiotics. *Kidney Int.* **2006**, *70*, 144–150. [[CrossRef](#)] [[PubMed](#)]
14. Naesens, M.; Kuypers, D.R.; Sarwal, M. Calcineurin inhibitor nephrotoxicity. *Clin. J. Am. Soc. Nephrol.* **2009**, *4*, 481–508. [[CrossRef](#)] [[PubMed](#)]
15. World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA J. Am. Med. Assoc.* **2013**, *310*, 2191–2194. [[CrossRef](#)] [[PubMed](#)]
16. Steering Committee of the Istanbul Summit. Organ trafficking and transplant tourism and commercialism: The Declaration of Istanbul. *Lancet* **2008**, *372*, 5–6. [[CrossRef](#)]
17. Seif, M.; Mani, L.Y.; Lu, H.; Boesch, C.; Reyes, M.; Vogt, B.; Vermathen, P. Diffusion tensor imaging of the human kidney: Does image registration permit scanning without respiratory triggering? *J. Magn. Reson. Imaging* **2016**, *44*, 327–334. [[CrossRef](#)]

18. Eisenberger, U.; Thoeny, H.C.; Binser, T.; Gugger, M.; Frey, F.J.; Boesch, C.; Vermathen, P. Evaluation of renal allograft function early after transplantation with diffusion-weighted MR imaging. *Eur. Radiol.* **2010**, *20*, 1374–1383. [[CrossRef](#)]
19. Vermathen, P.; Binser, T.; Boesch, C.; Eisenberger, U.; Thoeny, H.C. Three-year follow-up of human transplanted kidneys by diffusion-weighted MRI and blood oxygenation level-dependent imaging. *J. Magn. Reson. Imaging* **2012**, *35*, 1133–1138. [[CrossRef](#)]
20. Seif, M.; Eisenberger, U.; Binser, T.; Thoeny, H.C.; Krauer, F.; Rusch, A.; Boesch, C.; Vogt, B.; Vermathen, P. Renal Blood Oxygenation Level-dependent Imaging in Longitudinal Follow-up of Donated and Remaining Kidneys. *Radiology* **2016**, *279*, 795–804. [[CrossRef](#)]
21. Thoeny, H.C.; Zumstein, D.; Simon-Zoula, S.; Eisenberger, U.; De Keyzer, F.; Hofmann, L.; Vock, P.; Boesch, C.; Frey, F.J.; Vermathen, P. Functional evaluation of transplanted kidneys with diffusion-weighted and BOLD MR imaging: Initial experience. *Radiology* **2006**, *241*, 812–821. [[CrossRef](#)]
22. Eisenberger, U.; Binser, T.; Thoeny, H.C.; Boesch, C.; Frey, F.J.; Vermathen, P. Living renal allograft transplantation: Diffusion-weighted MR imaging in longitudinal follow-up of the donated and the remaining kidney. *Radiology* **2014**, *270*, 800–808. [[CrossRef](#)] [[PubMed](#)]
23. Sulkowska, K.; Palczewski, P.; Wojcik, D.; Ciszek, M.; Sanko-Resmer, J.; Wojtowicz, J.; Leszkiewicz, M.; Golebiowski, M. Intravoxel incoherent motion imaging in monitoring the function of kidney allograft. *Acta Radiol.* **2019**, *60*, 925–932. [[CrossRef](#)] [[PubMed](#)]
24. Hueper, K.; Khalifa, A.A.; Bräsen, J.H.; Vo Chieu, V.D.; Gutberlet, M.; Wintterle, S.; Lehner, F.; Richter, N.; Peperhove, M.; Tewes, S.; et al. Diffusion-Weighted imaging and diffusion tensor imaging detect delayed graft function and correlate with allograft fibrosis in patients early after kidney transplantation. *J. Magn. Reson. Imaging* **2016**, *44*, 112–121. [[CrossRef](#)] [[PubMed](#)]
25. Niendorf, T.; Pohlmann, A.; Arakelyan, K.; Flemming, B.; Cantow, K.; Hentschel, J.; Grosenick, D.; Ladwig, M.; Reimann, H.; Klix, S.; et al. How bold is blood oxygenation level-dependent (BOLD) magnetic resonance imaging of the kidney? Opportunities, challenges and future directions. *Acta Physiol.* **2015**, *213*, 19–38. [[CrossRef](#)] [[PubMed](#)]
26. Poynton, C.B.; Lee, M.M.; Li, Y.; Laszik, Z.; Worters, P.W.; Mackenzie, J.D.; Courtier, J. Intravoxel incoherent motion analysis of renal allograft diffusion with clinical and histopathological correlation in pediatric kidney transplant patients: A preliminary cross-sectional observational study. *Pediatric Transplant.* **2017**, *21*, e12996. [[CrossRef](#)]
27. Jakobsen, J.A.; Brabrand, K.; Egge, T.S.; Hartmann, A. Doppler examination of the allografted kidney. *Acta Radiol.* **2003**, *44*, 3–12. [[CrossRef](#)]