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Donor Effect on Cortical Perfusion Intensity in Renal Allograft Recipients: A Paired Kidney Analysis

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Key Words
Dynamic tissue perfusion measurement · Paired kidney analysis · Renal hemodynamics · Renal transplantation · Renal resistance index · Ultrasound

Abstract
Background: The contributions of donor- and recipient-related factors to renal allograft hemodynamics are difficult to dissect due to methodological reasons. We analyzed 28 pairs of kidneys (each pair from the same donor) transplanted to 56 different recipients in order to define the contributions of the donor and the recipient to allograft hemodynamics. ***Methods:*** Two different techniques based on color-coded duplex ultrasound were used: renal resistance index (RI; measured in 3 different segmental arteries) and cortical perfusion intensity (PI; calculated as the average PI of selected cortical parenchymal regions during one heart cycle in standardized registered and processed ultrasound videos). All measurements were performed during the same study visit. ***Results:*** Donor age was 56 years (median, range 17–78) and recipient age at examination 54 years (range 30–77). Median time after transplant (at the date of examination) was 2.4 years (range 0.7–5.5). RI correlated with pulse pressure ($r = 0.64$; $p < 0.001$) and recipient age ($r = 0.42$; $p < 0.03$), but not with donor age or transplant function expressed as estimated glomerular filtration rate (eGFR) or PI. In within- and between-pairs ANOVA, donor-derived factors determined eGFR ($p < 0.02$) and cortical PI ($p < 0.03$), but not RI. ***Conclusions:*** Intrinsic donor-derived factors are associated with GFR and cortical parenchymal perfusion intensity, but not the RI of segmental arteries in renal allografts.

Introduction

Donor-related factors have a direct impact on renal function in kidney transplants [1–3], an effect closely related to the amount of glomerulosclerosis observed in 210 protocol biopsies taken at the time of engraftment [4]. Chronic structural damage translates to impaired renal hemodynamics. Color-coded duplex ultrasound is a non-invasive tool for exploring alterations in renal perfusion. In a seminal study on 601 renal allograft recipients, an increased renal resistance index (RI), measured by color-coded duplex ultrasound, was associated with poor kidney allograft and patient survival [5]. Prior studies provided correlations between RI measured in the segmental arteries of the transplant and renal transplant outcome or recipient-related determinants of vascular compliance, such as recipient age, pulse pressure, and other measures

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of arterial stiffness and atherosclerosis [6–9]. These associations differed in part between the various studies and did not unambiguously dissect donor and recipient influences upon RI. Of interest, the RI index does not allow quantification of the cortical perfusion of the kidney. Most of the glomeruli are however located in the cortex. Given the relevance of glomerulosclerosis for transplant function [4], the specific assessment of cortical perfusion is of potential relevance. Recently, a novel ultrasound-based technology for the assessment of renal cortical perfusion was introduced – the PixelFlux method [10]. Investigations analyzing cortical perfusion intensity (PI) by PixelFlux and color-coded duplex ultrasound measuring RI in the same kidney have not been performed so far.

Therefore, the aim of the present study was to dissect the donor-related influence from that of the recipient on renal allograft function measured by estimated glomerular filtration rate (eGFR) and on renal hemodynamics defined by RI measures from the segmental arteries and PI measurements of predefined cortical parenchymal regions of the allograft. For this purpose, 28 pairs of recipients (each transplanted from the same donor and followed in one transplant center) were investigated.

Patients and Methods

This single-center study was performed at the University Hospital of Bern, Switzerland, and was approved by the local ethics committee and registered with the Cochrane Renal Group database (CRG 110600098). We identified 32 consecutive pairs of kidneys (each pair from the same deceased donor) transplanted to 2 different recipients in our transplant center between May 2002 and March 2007. Twenty-eight pairs of kidneys transplanted from the same donor and with stable graft function for at least 3 months prior to the examination were eligible for the study. Exclusion criteria were patient death (1 pair), graft loss (2 pairs) and loss of follow-up at the center (1 pair). All measurements were performed on the same occasion under standardized conditions. The patients investigated had no atrial fibrillation or other arrhythmias, renal transplant artery stenosis, hydronephrosis grade II or more, significant compression of the graft due to a lymphocele or a hematoma, or signs of infection at the time of examination.

All assessments were made during a single study visit per patient. Sonographic and hemodynamic measurements were performed by one experienced physician (L.M.) following a standardized protocol. The observer was blinded with respect to the study pairs. Sequoia (Siemens) with a 2- to 6-MHz convex-array tracer was used to perform the ultrasound measurements. Following overnight fasting, patients were examined in the supine position after 5 min of recumbence. Initial sonographic assessment by B-mode analysis confirmed an appropriate renal volume, parenchymal width, shape and echogenicity of the allograft.

Doppler-based assessment of RI was performed on 3 representative and distinct segmental arteries located in the superior, middle and inferior parts of the transplanted kidney, as described previously [9]. The Doppler sample volume was adjusted according to the vessel size and the Doppler gain was set at its optimal condition to obtain a clear outline of flow waves with minimal background noise without extinguishing flow-related signals. Interrogation angles between the Doppler beam axis and the vessels examined were less than 60°. The pulse repetition frequency was set to avoid aliasing, and the wall filter was optimized as low as possible to detect slow diastolic flow. RI was calculated as the mean of 3 distinct segmental arteries as follows: RI = 1 – (end diastolic velocity/peak systolic velocity).

Dynamic color Doppler tissue perfusion measurement was performed as described elsewhere [10]. In brief, longitudinal and transversal sections of the transplant were recorded. Care was taken to investigate interlobar arteries that run straight towards the transducer to avoid angle correction of the Doppler signal. A constant color Doppler frequency (4 MHz) was applied and the following presets were fixed: spatial resolution (S1), edge (–1), color scale without variance display (V3), persistence (2), preset (low flow) and color gain (50%). To avoid aliasing, maximal color velocity (pulse repetition frequency) was adapted in a range from 4.3 to 8.6 cm/s.

Perfusion intensity was measured with commercially available software (PixelFlux; Chameleon Software, Freiburg, Germany). This software automatically calibrates distances and color hues as flow velocities and calculates color pixel area, flow velocity and perfusion intensity inside a region of interest from a video sequence in DICOM format. Videos contained 25–50 images obtained during at least one heart cycle. The region of interest was chosen in the renal parenchyma between the outer border of medullary pyramids and the kidney surface. A parallelogram was placed to enclose a complete vascular segment fed by one interlobar artery (fig. 1). The cortical tissue PI was calculated by a mean velocity multiplied by the area of all pixels integrated over one heart cycle.

Arterial stiffness was assessed by photoplethysmography with a Pulse Trace PCA (Micro Medical Limited, Rochester, UK) using a stiffness index (SI_{DVP}) based on digital volume pulse, as previously described in detail [11]. Brachial blood pressure was measured with a mercury sphygmomanometer, and phases I and V of Korotkoff sounds were considered to be systolic and diastolic blood pressure, respectively.

Routine laboratory parameters were assessed on the day of measurement.

Statistics

Statistical analyses were performed using SYSAT Version 12 (SPSS Inc., Chicago, Ill., USA) and R (The R Foundation for Statistical Computing, Vienna, Austria). Means ± SD were determined whenever appropriate. Correlation analysis was used to detect factors potentially influencing transplant function, RI or PI levels in all recipients independently of the donor. Correlation levels were calculated using the Pearson test corrected by Bonferroni.

To evaluate possible donor effects in the studied population, squared differences of parameters between and within pairs of recipients from the same donor were compared with an ANOVA procedure, as previously described in twin research [12]. To visualize the findings of the ANOVA procedure, 500 bootstrap data sets were generated by randomly creating 28 pairs from 56 patients of the original data set. The probability density function of

Table 1. Demographic data of all renal transplant patients

Donors (n = 28)	
Age, years	56 (17–78)
Males	13 (46)
Hypertensive	8 (29)
Recipients (n = 56)	
Age at examination, years	54 (30–77)
Time after transplant (at examination), years	2.4 (0.7–5.5)
Males	28 (50)
Smokers	13 (2)
Diabetics	16 (29)
Hypertensive	54 (96)
First transplant	48 (86)
HLA-A and B (≥2 mismatches)	47 (83)
HLA-DR (≥1 mismatch)	51 (91)
Cold ischemia time, h	9 (3–20)
Panel reactive antibodies >10%	3 (5)
Rejection	8 (14)
Primary cause of renal failure	
Glomerulonephritis	17 (29)
Diabetes mellitus	8 (14)
Polycystic kidney disease	15 (26)
Interstitial nephritis	5 (9)
Others/unknown	13 (22)
Immunosuppression at time of examination	
Corticosteroids	42 (75)
Calcineurin inhibitor	43 (76)
MMF/MPA	36 (64)
Sirolimus/everolimus	9 (16)
Antihypertensive and lipid-lowering medication	
Calcium antagonist	22 (39)
ACE/ARB	43 (77)
Diuretics	38 (68)
β-Blocker	43 (77)
Statins	41 (73)

Data presented as medians (range) or n (%). HLA = Human leukocyte antigen; MMF = mycophenolate mofetil; MPA = mycophenolic acid; ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker.

the mean square differences of parameters between pairs of the original dataset can then be visually compared with the 500 probability density functions resulting from random pairing of the patients from the original data set. For all results, statistical significance was assumed at $p < 0.05$.

Results

Fifty-six recipients transplanted from 28 donors were included in the study. Demographic data of all study patients are presented in table 1. The median time after transplant (at examination) was 2.4 years. Overall mean

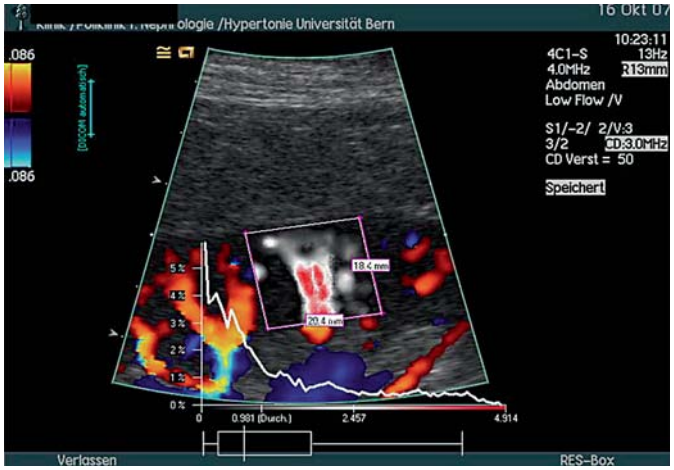

Fig. 1. Definition of the region of interest for the measurement of cortical perfusion intensity in a renal transplant by color-coded duplex ultrasound: A parallelogram is placed to enclose a complete vascular segment fed by one interlobar artery limited by the outer border of medullary pyramids and the kidney surface.

ages in donors and recipients were comparable. Recipients were mainly first transplants at a relatively low immunological risk with a short ischemic time and a low number of rejections during the follow-up period. The immunosuppressive regimen consisted of a calcineurin-inhibitor-based regimen in more than two thirds of the patients. Nearly all patients were hypertensive with an average number of 3.2 antihypertensive drugs. Hypertension was controlled with a mean systolic blood pressure of 133 ± 18 mm Hg and a diastolic blood pressure of 82 ± 11 mm Hg. After a mean post-transplant time of 2.4 years, renal function (measured as estimated GFR) was 61.4 ± 23.7 ml/min. Laboratory and hemodynamic parameters, as well as type of immunosuppressive and antihypertensive therapy, are displayed in table 2.

Independently from donor-recipient pairs, RI of segmental arteries, but not cortical PI, was correlated to pulse pressure ($r = 0.64$, $p < 0.001$) and recipient age ($r = 0.42$, $p < 0.03$). The use of diuretics ($r = 0.58$, $p < 0.001$) or calcium antagonists ($r = 0.49$, $p < 0.01$) influenced RI, but not PI, measurements. Renal function parameters, serum cholesterol, albumin and hematocrit, as well as the immunosuppressive drug therapy showed no relevant relation to either RI or PI. RI of segmental arteries was not related to cortical PI ($r = -0.17$, n.s.). Cortical PI in patients with normal renal function (defined as a serum creatinine level <100 μmol/l; n = 14) was significantly higher than in transplant patients with elevated serum

creatinine (n = 42) (1.4 ± 0.5 vs. 1.1 ± 0.45, respectively; $p < 0.05$).

To evaluate possible donor effects in the studied population, an ANOVA within and between recipient pairs from the same donor was performed (table 3). This analysis showed that RI values are not donor-dependent (r = 0.1, n.s.). On the other hand, cortical PI (r = 0.42, $p < 0.01$) as well as renal function measured as estimated glomerular filtration rate (r = 0.42, $p < 0.01$) or serum urea (r = 0.39, $p < 0.02$) are influenced by donor-derived factors. Other cardiovascular risk markers, as well as recipient age, were not donor related (table 3).

The most important findings of this paired kidney analysis are visualized in figure 2. The probability density function of the mean square difference of parameters within pairs of the original dataset was overlaid over the probability density functions of 500 data sets each generated from 28 randomly assigned pairs. Our findings with respect to eGFR and PI clearly demonstrated a smaller than expected mean square difference within recipients pairs. This was not the case for RI and systolic blood pressure values.

Table 2. Laboratory parameters at examination

Number of transplant recipients	56
BMI	27 ± 4.6
Serum creatinine, μmol/l	134 ± 61
eGFR, ml/min	61.4 ± 23.7
Serum urea, mmol/l	12.5 ± 5.9
Serum cholesterol, mmol/l	4.9 ± 1.4
Hematocrit, %	51 ± 16
HbA_{1C}, %	6.3 ± 1.2
Transplant length, cm	11.6 ± 1.2
Pulse pressure, mm Hg	37 ± 5
Systolic blood pressure, mm Hg	133 ± 18
Diastolic blood pressure, mm Hg	82 ± 11
Arterial stiffness index, m/s	10.0 ± 3.6
RI (segmental renal arteries)	0.71 ± 0.06
PI (cortical), cm/s	1.2 ± 0.5

Table 3. ANOVA between and within pairs of recipients from the same donor

	Mean square within pairs	Mean square between pairs	r (donor-derived)	p
eGFR	328	800	0.42	0.01
Serum urea	21	50	0.39	0.02
PI	0.15	0.32	0.35	0.03
Transplant length	0.9	1.6	0.29	0.06
Body weight	253	153	0.23	0.1
CHD	0.193	0.121	0.23	0.1
Recipient age	153	96	0.23	0.1
Smoking	0.2	0.16	0.13	0.3
Diabetes	0.23	0.19	0.1	0.3
RI	0.004	0.004	0.1	0.4
Serum cholesterol	1.97	1.98	–0.002	0.5
BP diastolic	112	113	–0.01	0.5
BP systolic	345	322	–0.03	0.6

CHD = Coronary heart disease.

Discussion

The present study investigated for the first time donor effects on renal allograft hemodynamics in a paired kidney analysis. For this purpose, two non-invasive hemodynamic measurements of vascular blood flow by color-coded Doppler ultrasound were applied. The analysis revealed that intragraft RI of the segmental arteries depended on age and pulse pressure of the recipient, whereas cortical perfusion appeared to be associated predominantly with donor-derived factors.

In the present study, renal allograft function was clearly dependent on the donor. Our observation is in line with that of Cosio et al. [1], who studied renal allograft function in 189 recipient pairs from the same donor. They calculated that 64% of the variability in serum creatinine at 6 months after transplantation was due to donor-related factors and 36% was due to recipient-related factors. This finding was confirmed by Bertoni et al. [2] in 103 pairs of cadaveric kidneys grafted in 206 recipients. Donor age appears to be important not only for short- but also for long-term allograft function [1–3]. A multivariate analysis in 7,209 cadaveric kidney transplant recipients revealed that donor age and donor-related factors, including cerebrovascular cause of death or a history of hypertension, are relevant risk factors for graft survival in the recipient [1].

Renal transplant and patient survival can be predicted by measuring the intrarenal RI non-invasively by color-coded duplex ultrasound [5]. It is still debated whether, and if so how importantly, donor-related factors are implicated in variations in the renal RI measured in the recipient. Saracino et al. [14] retrospectively analyzed RI measurements in 76 kidney transplant recipients during the first month after transplantation with regard to allograft function. A multivariate analysis of this data showed that donor and recipient age both had an independent predictive value for RI. In another prospective

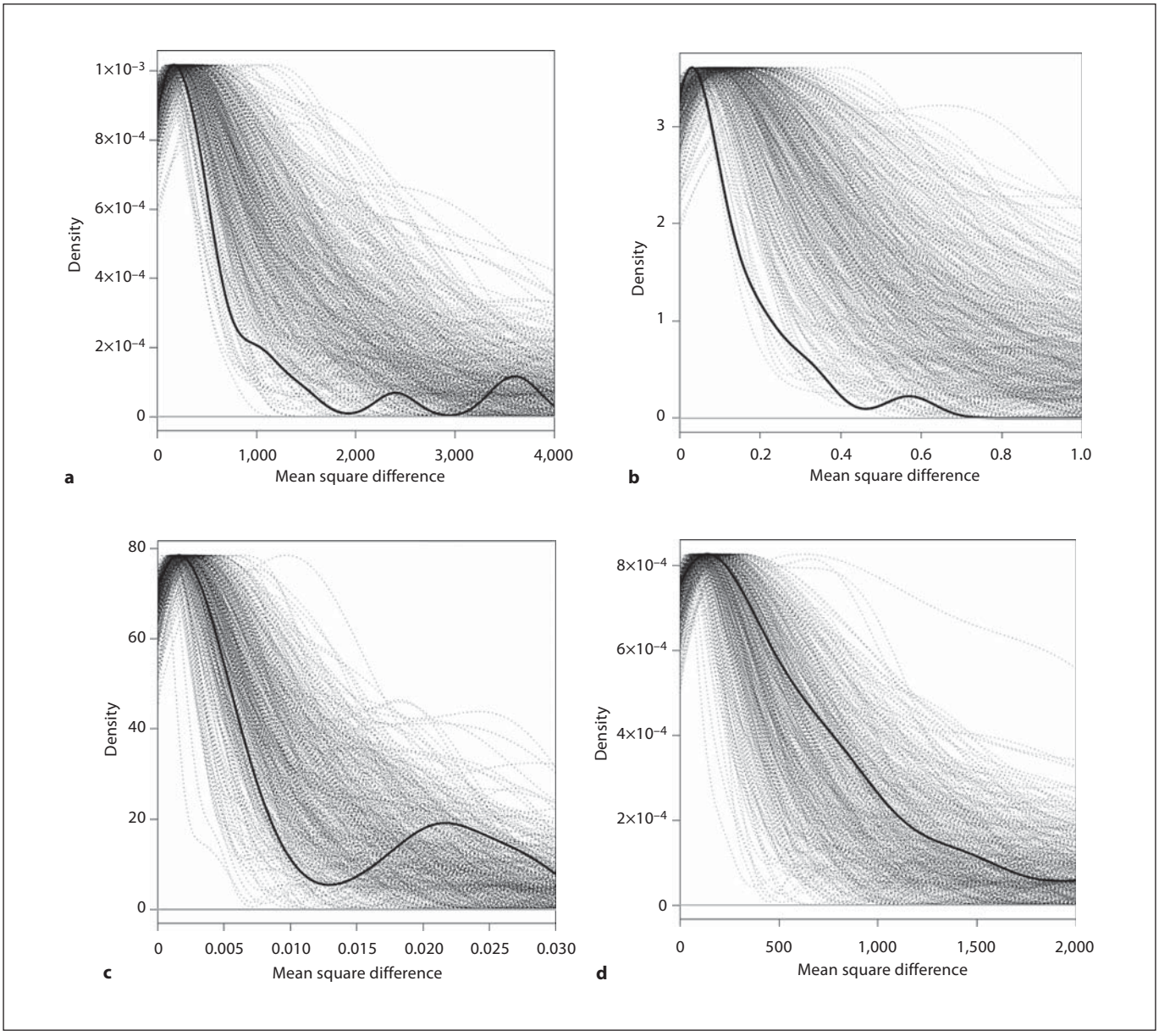

Fig. 2. Probability density functions of squared differences for eGFR (**a**), cortical PI (**b**), renal RI (**c**) and systolic BP (**d**) of the paired kidney analysis compared to a randomly created data set. Bold lines = probability density functions of squared differences within the original 28 pairs of recipients from the same donor; broken lines = 500 data sets generated by randomly creating 28 pairs from the 56 patients of the original dataset. Visual inspection reveals that mean square differences within pairs of recipients from the same donor are smaller than expected from random pairing for eGFR and PI, but not for RI or systolic BP.

cohort study of 76 renal transplant patients, RI measurements were performed on average 77.5 months after transplantation and identified recipient age, pulse wave velocity and pulse pressure, but not donor age, as independent predictors for RI [7]. We have previously evaluated a cohort of 200 renal transplant recipients on average 7 years after transplantation for relevant determinants for RI [9]. RI values were primarily dependent on recipient-dependent factors, such as age, pulse pressure, diabetes and serum asymmetrical dimethylarginine, an endogenous inhibitor of NO synthase. However, such cross-sectional cohort studies are not the most satisfactory

approach to unambiguously determine the donor impact on renal allograft recipients. According to current transplant procedures, donor and recipient age are often related.

More promising is the current study design allowing the analysis of recipient pairs from the same donor in order to differentiate between donor and recipient effects on renal hemodynamic measurements. In this model, a relevant donor-derived effect on RI values in recipient pairs was absent. This observation is not surprising in the light of the findings of Aschwanden et al. [15] who compared RI values in 80 donor-recipient pairs before and after living donor kidney transplantation. They observed a rapid recipient adaption of RI values after transplantation. Taken together, RI values of segmental arteries in a renal transplant recipient depend mainly on recipient-related factors affecting vascular compliance, such as pulse pressure or recipient age, but not on donor-derived factors.

Here, in addition to RI, we quantified the dynamic intrarenal PI in the cortical parenchyma during one heart cycle by color-coded duplex ultrasound using a new non-invasive method. This method was previously applied in 38 young renal transplant recipients with a mean age of 15 years. The study provided cortical PI values of 1.06 cm/s on average during the first year of transplantation and demonstrated a decrease during the subsequent transplant follow-up [10]. Our study is the first to evaluate cortical PI values in an adult population of renal transplant recipients. Cortical PI values in our investigation were within the range of a previous study in children [10].

Interestingly, cortical PI values were related within recipient pairs from the same donor, suggesting an influence of donor-derived factors on parenchymal renal blood flow. Cortical PI values were neither correlated with RI of segmental arteries, nor with pulse pressure, serum creatinine or recipient age – indicating that the dynamic measurement of cortical PI by the PixelFlux method is a distinct intrarenal hemodynamic parameter.

It has been shown that RI is strongly related to the systemic vascular bed, potentially masking local perfusion changes within the kidney [6–8]. It is therefore not unexpected that donor-related factors are, if at all, poorly reflected by RI. On the other hand, the finding that PI is related to donor-derived factors suggests that it is less dependent on systemic hemodynamics. Hence, PI has the potential to become a better non-invasive measure to detect and follow local parenchymal perfusion changes within the kidney as compared with the conventional measurement of RI in segmental arteries.

Additional studies are necessary to prove the clinical utility of cortical PI for exploration of allograft function when compared with renal scintigraphy or MRI – both established methods for perfusion measurement in the kidney [16, 17]. A methodological drawback of PI measurements is the time-consuming post-processing of the ultrasound video sequences, which currently complicates the clinical application in everyday practice. This issue can be resolved with integrated ultrasound application software in the future.

The present study design of a paired kidney analysis in a single center has some limitations. First, the number of recipient pairs eligible for analysis was relatively limited; second, the examinations in different patients were performed at variable time points after transplantation; third, drug therapy was not absolutely congruent in all the pairs even though all patients were followed in the same center with a standardized regime of care.

Despite these limitations, we identified a donor-related influence on cortical perfusion intensity, quantified by color-coded duplex with the PixelFlux method. Our results will hopefully stimulate more clinical research to use this method as a tool for non-invasive evaluation of impaired renal allograft blood flow.

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