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Paper accepted by *European Radiology*

The prevalence and significance of renal perfusion defects in early kidney transplants quantified using 3D contrast enhanced ultrasound (CEUS)

Stenberg B, Wilkinson M, Elliott S, Caplan N

Abstract

Objectives Vascular complications are one of the most common causes of early kidney transplant dysfunction. Contrast enhanced ultrasound increases sensitivity to tiny vascular changes.. The aim was to assess the prevalence and size of vascular abnormalities in renal transplantation patients following surgery using 3D CEUS to determine the significance of perfusion defects on renal function.

Methods Ninety nine renal transplant patients underwent 3D CEUS after surgery to quantify perfusion defects as percentage total renal volume (TRV). Serum creatinine and estimated glomerular filtration rate (eGFR) were recorded up to three months post-surgery.

Results In the 99 patients, 20 had perfusion defects (0.2 – 43% TRV). There was a meaningful difference in patients with perfusion defects in eGFR at 1 month (90% CI 2.7 to 19.2 ml/min/1.73m²) and 3 months (90% CI 1.9 to 19.6 ml/min/1.73m²) and in Creatinine at 3 months (90% CI -56 to -8 µmol/L) using a predetermined clinical threshold.

Perfusion defect size correlated well with both serum creatinine and eGFR at 3 months (R= 0.80 (p ≤ 0.000) and 0.58 (p= 0.038)). No correlation seen prior to 3 months.

Conclusions Perfusion defects in kidney transplants were more common than expected and were highly likely to reduce renal function at 1-3 months and the size of the defect affected the degree of functional change at 3 months.

Keywords: Contrast enhanced ultrasound
Three-dimensional ultrasound
Kidney transplant
Perfusion defect
Kidney function

Keypoints:

- Perfusion defects were more common than previously thought and could be quantified using 3D CEUS.
- The presence of even small perfusion defects may affect function of the kidney.
- The size of perfusion defects correlated with subsequent kidney function at 3 months.
- This may be useful in informing clinician expectations of kidney function following surgery.

Introduction

In the immediate post-surgical phase of recovery from kidney transplantation, vascular complications are infrequent but still one of the most likely causes of dysfunction and loss of the graft [1]. The most common complications include renal vein thrombosis, both major and minor arterial occlusion and acute tubular necrosis, often due to the periods of warm ischaemia prior to, and during, retrieval [2].

Traditionally, a combination of colour and spectral Doppler ultrasound and nuclear medicine, Technetium 99m-diethylene triamine penta-acetic acid (Tc-DTPA) renogram, has been used to assess the vasculature in the new graft [3]. More recently, contrast enhanced ultrasound (CEUS) has emerged as a viable technique that can offer a high temporal and spatial resolution map of the microvasculature of the kidney [4, 5]. This provides new and more detailed information about the blood flow throughout the kidney and in particular areas of non-perfusion and is well established in native kidneys [6]. Several studies have described an increase in the detection of perfusion defects in kidney transplants when using CEUS compared to traditional techniques in both pathological and surgical arterial occlusion, suggesting that that perfusion defects may be more common than previously thought [7, 8].

The value of 3D ultrasound is well documented with regards to the detection of pathology and improving the accuracy of volume measurements using stacked contours to build up a model of the area being measured, being particularly useful in complex shapes [9,10]. Studies have shown that inter

and intra-operator variability is reduced in 3D volumetric studies of the kidneys with error margins of approximately 11% compared with 23% in standard ellipsoid measurements [8,9] Three dimensional CEUS enables the assessment of any abnormalities in the blood flow to a kidney transplant and to measure volumes of perfused tissue with greater accuracy than standard ultrasound and radio-isotope renograms [10]. Contrast enhanced ultrasound has been used extensively in liver, breast and cardiac applications but there are limited reports on renal transplant use [11].

Although the increased accuracy of the assessment of renal perfusion is known to be increased using CEUS, the clinical relevance of these tiny perfusion defects is currently not known, although it has been suggested that they may be a cause for patients with “stuck” creatinine [12]. The aim of this study, therefore, was to assess the prevalence and size of perfusion defects in renal transplantation patients following surgery using 3D CEUS in an attempt to determine the significance of these perfusion defects on renal function.

Method

One hundred and five consecutive kidney transplant recipients were recruited prospectively regardless of donor type or post-surgical course. Ethical approval was obtained from the local ethics committee and the trial was registered with the International Standard Randomised Controlled Trial

Number (ISRCTN) registry. Written informed consent was obtained from each participant to be included in the study.

Following each patient's routine ultrasound examination at day 1 post-surgery, each participant underwent a focused CEUS of the kidney transplant. This was performed using a standard dose of 2.4 ml Sonovue (Bracco, Milan, Italy) injected intravenously as a bolus, ideally in a peripheral cannula. A central venous line was used on two occasions, however, due to poor peripheral access. The patients were scanned using an iu22 ultrasound system (Philips Healthcare, Bothell, USA) with vision 2011 software and an X6-1 matrix volume probe (broad bandwidth transducer operating between 1 and 6 MHz). A low mechanical index (0.05), power modulated technique with side by side, tissue suppressed, imaging was used for the contrast examination.

Each kidney was observed for 1 minute to allow for the medullary filling phase to complete (Figure 1). It was subsequently assessed for perfusion defects using standard 2D contrast-specific ultrasound. An electronically steered volume data set was then acquired for offline quantification of any detected defect. This dataset was assessed for quality and inclusion of entire organ at time of scan. The offline assessment was performed together by a sonographer and a radiologist (both >5 years' experience with CEUS and 3D manipulation) using QLab ultrasound analysis software (Philips Healthcare, Bothell, USA). This allowed the block of data to be assessed in all three planes simultaneously using a multi-planar reconstruction format (MPR) and any quantification to be performed. Any areas of non-perfusion were

quantified using manually segmented stacked contours volume measurement of the entire kidney and of the focal area of non-perfusion. The percentage of total renal volume (TRV) that was non-perfused was then calculated (Figure 1).

Each patient was followed up for three months with regular inpatient and outpatient blood tests. Serum creatinine was measured at 1 day, 1 week, 2 weeks, 1 month and 3 months post-surgery as well as estimated glomerular filtration rate (eGFR) at 1 month and 3 months post-surgery as an indicator of renal function. Local serum creatinine reference levels were 0.5 - 1.0 mg/dl (45-90 $\mu\text{mol/l}$) for women, 0.7 - 1.2 mg/dl (60-110 $\mu\text{mol/L}$) for men. The eGFR was calculated using the abbreviated MDRD equation [13].

Patients were separated into two independent groups based on the presence or absence of a perfusion defect. Mean differences between the groups on measures of renal function were then examined. The likely magnitude of the true (population) mean difference was estimated using 90% confidence intervals and the probability of the true effect exceeding a smallest clinically important change of 26 $\mu\text{mol/L}$ for creatinine and 5.0 ml/min/1.73m² for eGFR was calculated using the methods of Hopkins et al [14].

Analysis was performed using magnitude-based inference spreadsheets [15]. The smallest meaningful differences were taken from the renal association guidelines [14] as the difference between normal function and stage 1 acute kidney injury (AKI), and from discussions with the local transplant nephrology

team as the smallest clinical difference in eGFR which would require investigation. Findings were considered clinically clear if the chance of benefit exceeded 25% while the chance of harm was less than 0.5%. If chance of harm exceeded 0.5%, the finding was deemed clinically unclear and more data needed [14].

A linear regression model was used to analyse whether the size of perfusion defect correlated with subsequent renal function using IBM SPSS statistics software, version 21 (IBM, UK).

Results

Three dimensional CEUS datasets of the entire kidney were achieved in all patients.

Homogeneous global perfusion was seen in 81 patients. Focal areas of non-perfusion were seen in 22 patients and a complete absence of enhancement was seen in two patients (100% TRV). No cases of acute cortical necrosis, with its idiosyncratic, peripheral rim infarction were seen in this patient group. The focal perfusion defects detected ranged in size from 0.2 to 76% TRV (mean 10.86 ± 17.13 % TRV) (Figure 2). Retrospective analysis of the operation records demonstrated ligated polar arteries in seven of the patients with focal perfusion defects. No ligated arteries were seen in the normal perfusion group.

The majority of perfusion defects were confined to the peripheral cortex of the poles of the graft (18/22, 81%), two patients had small focal cortical perfusion defects in the equatorial region (2/22, 9%), one (43% TRV) encompassed the entire upper pole and a portion of the equatorial region and one (76% TRV) encompassed the whole of the upper pole and equatorial region.

Six patients were excluded from the longitudinal analysis as three underwent nephrectomies for graft ischaemia (the two patients with 100% perfusion loss and the patient with 76% perfusion loss) within the first two weeks after surgery, two patients were lost to follow up and one had primary non-function of the kidney. Creatinine was measured in all 99 remaining patients, of which 20 perfusion defects were seen. Estimated glomerular filtration rate was measured in 85 patients, of which 15 showed perfusion defects. Estimated glomerular filtration rate measurements were not available in some follow up sites hence the reduction in sample size.

Groups with non-surgically caused perfusion defects and those without perfusion defects were compared to assess any factors which might predispose transplanted kidneys to perfusion defects. No significant difference was seen in these groups (Table 1).

There was no significant difference in factors which would change renal function in the initial post-surgical period. Nine patients had ATN (8.6%) (3 with perfusion defect, 6 without), 7 (6.7%) had rejection (3 with perfusion

defect, 4 without) and two had both ATN and rejection (1 with perfusion defect, 1 without) within the first two weeks following surgery

There was a difference between patients with and without perfusion defects in eGFR at 1 month (90% CI 2.7 to 19.2 ml/min/1.73m²) and 3 months (90% CI 1.9 to 19.6 ml/min/1.73m²). There was an 88% and 86% chance respectively that the true differences exceeded a pre-determined clinical threshold magnitude of 5.0 ml/min/1.73m².

There was also a difference between patients with and without perfusion defects in creatinine at 3 months (90% CI -56 to -8 µmol/L) with a 66% chance that the true difference exceeded a predetermined clinical threshold of 26 µmol/L (Table 2). Differences between the groups in mean values for creatinine with reference to the smallest clinically meaningful difference at 1 day (90% CI 42 ± 90, 62% beneficial, 10.6% harmful), 1 week (90% CI 34 ± 110, 55% beneficial, 17.5% harmful), 2 weeks (90% CI 2.6 ± 66, 27.9% beneficial, 23.7% harmful) and 1 month (90% CI 10 ± 29, 18.6% beneficial, 2.0% harmful) were clinically unclear.

Little or no meaningful linear relationship was demonstrated between perfusion defect size and either creatinine at 1 day ($R^2=0.013$, $p = 0.651$), 1 week ($R^2=0.032$, $p =0.480$), 2 weeks ($R^2=0.036$, $p= 0.0452$) and 1 month ($R^2=0.139$, $p= 0.127$), or eGFR at 1 month ($R^2=0.101$, $p = 0.289$), but there was a high degree of correlation with eGFR at 3 months ($R^2= 0.34$, $p= 0.038$, 66% likely clinically beneficial) and a very high correlation with 3 month

creatinine ($R^2= 0.64$, $p < 0.000$, 99% likely clinically beneficial) (Table 3). Confidence intervals for the linear regression slope suggested that at 3 months, a 4.11 ± 0.76 $\mu\text{mol/L}$ increase in creatinine and a 0.89 ± 0.38 ml/min/1.73m^2 reduction in eGFR would be seen for each 1% increase in perfusion defect size.

Discussion

The aim of this study was to assess the prevalence and size of vascular abnormalities in renal transplantation patients following surgery using 3D CEUS in an attempt to determine the clinical relevance of the tiny perfusion defects which are seen when using this technique, on renal function. In this study 22 focal defects were seen in 105 patients (21% cases), and the majority were pathological rather than surgical (17 vs 7 cases), suggesting that kidney transplant perfusion defects are more prevalent than previously thought [17].

All perfusion defects were wedge shaped cortical infarctions or crescent shaped polar infarctions rather than the rare but significant finding of peripheral rim ischaemia seen in acute cortical necrosis.

These perfusion defects are likely to be clinically meaningful with increased creatinine and reduced eGFR seen at 3 months post-surgery in patients with a perfusion defect. Three-dimensional defect size quantification also correlated with the subsequent serum creatinine and eGFR at 3 months post-

surgery, which should lead to a better understanding of the likely function of the kidney in patients with these perfusion defects.

Large perfusion defects have previously been detected using ultrasound and Tc-DTPA [18]. However, smaller defects, almost all of which were peripheral and polar in position in this study, are difficult to assess as poor Doppler angle, low velocity flow in the peripheral cortex and edge dropout on ultrasound and the poor spatial resolution and static position in Tc-DTPA all play a factor in confidently making the diagnosis [3]. The resolution and dynamic nature of the CEUS made it relatively straightforward to detect even small areas of non-perfusion in this and similar studies [6].

The lack of a stable, unvarying initial post-surgical course makes it unsurprising that there was little correlation between perfusion defects and kidney function seen within the first few weeks following surgery, and that the interval for the correlation coefficient was broad. Large variability was observed in serum creatinine and eGFR measurement obtained at the initial follow up points, illustrating the variability often seen in function of early kidney transplantation [19]. This variability was reduced at the later follow up time points. This is likely to be due to a number of factors including prolonged retrieval, post-surgical complications, biopsies and biopsy complications and delayed graft function. Delayed function is common, lasting for between a few days to several months, and is caused by a variety of pre-, peri- and post-transplant factors including ischaemic time, fluid management, age of donor and pharmacological regimes [20].

The highly significant correlation seen between perfusion defect size and drop in renal function at three months is understandable as there is less functional tissue and there are fewer functioning nephrons. This has an impact on both the acute and long term function, as there are changes in the haemodynamics of the kidney which can cause and perpetuate chronic dysfunction [21]. The greater the size of the perfusion defect, the greater the loss of nephrons would be. In the present study, perfusion defect size and 3 month creatinine and eGFR were found to have B values of $4.1 \pm 0.76 \mu\text{mol/L}$ and $-0.89 \pm 0.38 \text{ ml/min/1.73m}^2$. In real terms, this means that we can expect a patient with a modest perfusion defect of 10% TRV to present in clinic at 3 months post-op with serum creatinine between 33.5 and 48.7 $\mu\text{mol/L}$ higher than if they had no defect at all. Patients with a 10% TRV perfusion defect would be expected to present to clinic at 3 months with an eGFR of between 5.1 and 12.7 ml/min/1.73m^2 lower.

Magnitude-based inference is a good way of determining the clinical usefulness of any test [13]. In this case, justifiable smallest clinically meaningful changes in serum creatinine (26 $\mu\text{mol/L}$) and eGFR (5.0 ml/min/1.73m^2) were used to determine the proportion of the confidence interval which lies above this value. This gives a likelihood that the result will be clinically beneficial, trivial or harmful. In this study, it was seen that not only will the presence of a perfusion defect result in a statistically significant rise in serum creatinine and eGFR at 3 months, but it is likely to produce a clinically meaningful change (67% and 86% likelihood, respectively).

These findings should modify expectation from the transplant team when follow up is being performed. Allowances can, therefore, be made for “stuck” creatinine just above normal values if a suitably sized perfusion defect was observed after surgery. This could prevent or reduce the number of biopsies being performed to explain these borderline results, reducing the associated risks of complications, quoted as between 0.06 and 13% [22] with this procedure and cost, both procedural and during overnight stays.

Limitations

The sample size in this study is quite small with 105 participants and subsequently only 22 perfusion defects were seen. While a 3D stacked contours technique was used to minimise operator variability this is still a recognised source of error (+/- 10%) [9] in measuring the size of the perfusion defects.

Conclusion

In conclusion, focal perfusion defects were more common than expected occurring in 21% of patients and ranged in size from 0.2 - 76%. CEUS allowed detection of tiny perfusion defects as small as 0.2% TRV. An increase in creatinine at 3 months and reductions in eGFR at 1 and 3 months post-

surgery were seen in patients with a perfusion defects in their kidney transplant and these changes are likely to be at a clinically meaningful level. The magnitude of the perfusion defect correlates with the degree of change in serum creatinine and eGFR seen at 3 month. This correlation is also highly likely to be clinically meaningful. This may be useful in informing clinician expectations of kidney function following surgery.

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Table 1

Table 1 demonstrating average values of population and surgical factors involved in kidney transplantation between patients with a non-surgically caused perfusion defect and those with no perfusion defect.

Factor	Non-surgical perfusion defect group averages (n=16)	No perfusion defect group averages (n=77)	Statistical divergence (t-test)
Recipient body mass index	28.90	28.15	t = -0.014, df 95, p = 0.495
Donor Age (years)	47.13	48.49	t = -0.762, df 96, p = 0.224
Donor type	9 DCD (56%)	41 DCD (53%)	
	3 DBD (19%)	12 DBD (16%)	
	4 LD (25%)	24 LD (31%)	
Warm storage time (minutes)	16.07	10.54	t = -1.270, df 97, p = 0.104
Cold storage time (minutes)	874.07	719.85	t = -0.030, df 96, p = 0.488
Number of donor arteries	1.5	1.37	t = -0.002, df 97, p = 0.499

Table 2

Table 2: Magnitude-based clinical inference based on the smallest meaningful change in kidney function results.

Compared Groups	Mean difference with 90% confidence limits $Z_{\alpha/2} * \sigma/\sqrt{n}$	Clinical inference ^a
Creatinine day 1 with and without perfusion defect	42 ± 90	Possibly beneficial (61.6%) Possibly trivial (27.8%) Unlikely harmful (10.6%)
Creatinine 1 week with and without perfusion defect	34 ± 110	Possibly beneficial (55%) Possibly trivial (27.5%) Unlikely harmful (17.5%)
Creatinine 2 week with and without perfusion defect	2.6 ± 66	Possibly beneficial (27.9%) Possibly trivial (48.4%) Unlikely harmful (23.7%)
Creatinine 1 month with and without perfusion defect	10 ± 29	Unlikely beneficial (18.6%) Unlikely Trivial (79.4%) Very unlikely harmful (2.0%)
Creatinine 3 month with and without perfusion defect	32 ± 24	Probably beneficial (66%) Possibly trivial (34%) Most unlikely harmful (0.0%)
eGFR 1 month with and without perfusion defect	11 ± 8.2	Likely beneficial (88.2%) Unlikely trivial (11.7 %) Most unlikely harmful (0.1%)
eGFR 3 month with and without perfusion defect	11 ± 9	Likely beneficial (85.9%) Unlikely trivial (13.9%) Very unlikely harmful (0.2%)
^a with reference to smallest worthwhile change of 26 micromol/L creatinine and 5.0 mls/min/1.73m ² eGFR		

Table 3

Table 3: Magnitude-based inference of clinical usefulness in predicting changes to renal function at 1 and 3 month following surgery based on perfusion defect size with reference to a high correlation of 0.5.

Compared Groups	Mean correlation coefficient with 90% confidence limits	Clinical inference ^a
Creatinine 1 month – perfusion defect size	0.37 ± 0.32	Unlikely beneficial (24.7%) Likely Trivial (75.3 %)
Creatinine 3 month – perfusion defect size	0.8 ± 0.14	Very likely beneficial (99.1%) Very unlikely trivial (0.9%)
eGFR 1 month – perfusion defect size	0.32 ± 0.39	Unlikely beneficial (21.4%) Likely trivial (78.5 %) Very unlikely Harmful (0.1 %)
eGFR 3 month – perfusion defect size	0.58 ± 0.3	Probably beneficial (65.8%) Possibly trivial (34.2%)
^a with reference to a high correlation of 0.5.		

Figure 1

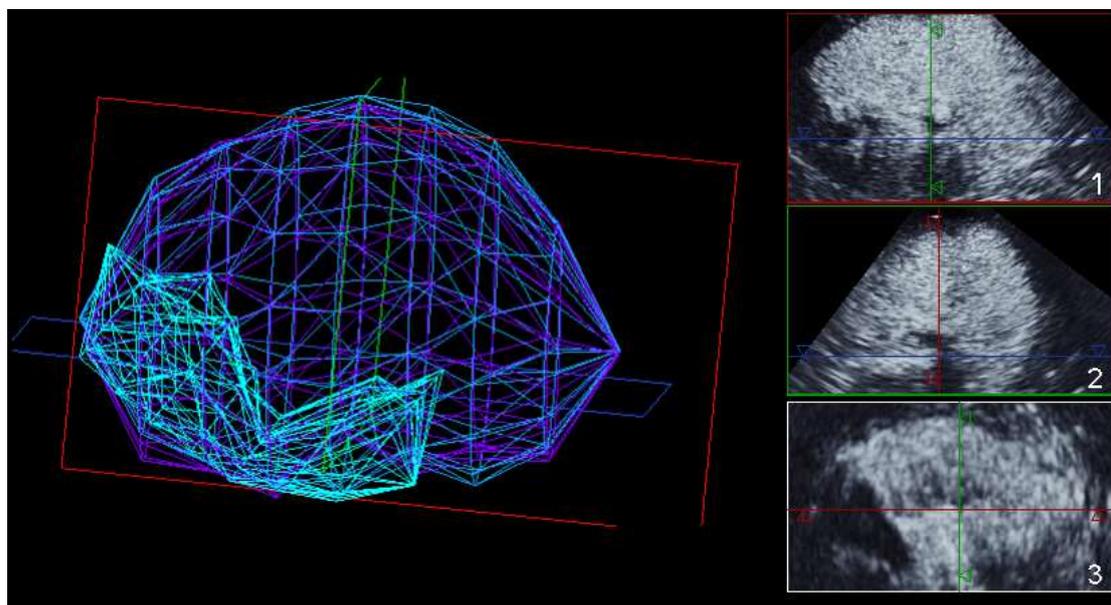


Figure 2

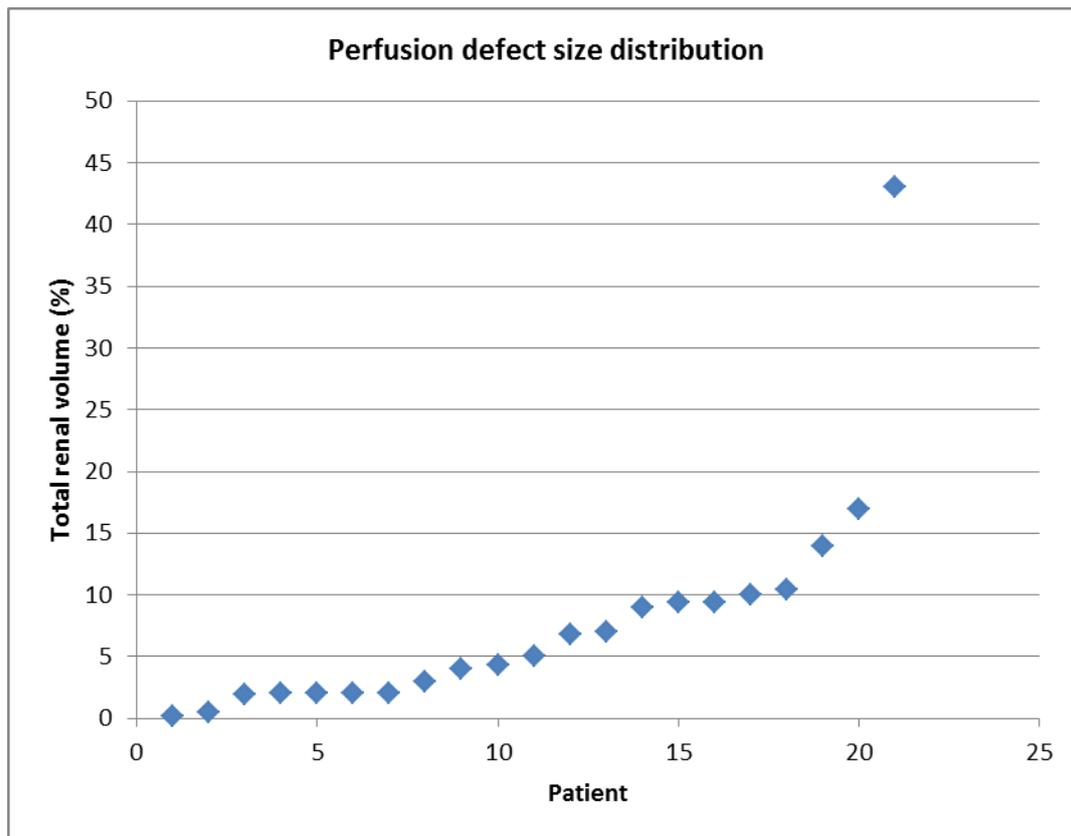


Figure 2: Distribution of perfusion defects seen in kidney transplants measured in percentage of total renal volume.