A new technique for assessing renal graft perfusion pre-operatively using contrast enhanced ultrasound (CEUS) - A porcine model viability study.

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Authors: S. Elliott, B. Stenberg, D. Talbot, A. Khurram, A. Kanwar, C. ray; Newcastle upon Tyne/UK  
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Purpose

Since the first case in 1950, renal transplantation has become a routine procedure and 65,500 operations are carried out annually worldwide [1]. Approximately 1800 of these are carried out per year in the UK [2]. The ability to store and transport the organs in cold perfusion has helped to revolutionise the transplant service.

One of the key indicators of the organ’s health while in storage is the perfusion level of the organ. This is demonstrated by flow and resistance through the renal artery. Despite the best efforts to refine the technique, such as the weight adjusted perfusion flow index [3], this only gives a general overview of the flow through the kidney; it does not take into account factors such as localised arterial occlusion, arterial-venous shunting or trauma which would allow reasonable perfusion resistance factors but would not reflect the true state of the organ.

The kidneys retrieved from a living donor or heart-beating donors tend to have fewer of these complications, however, non-heart-beating donors tend to be subjected to longer periods of warm ischemia to the organs prior to recovery, which is when the kidney can be most vulnerable to damage [4].

Contrast enhanced ultrasound (CEUS) is a relatively new technique which uses the properties of tiny gas bubbles to increase ultrasound signal return. Each bubble averages 2-3 microns in diameter, allowing them to pass through the capillary bed, but is not small enough for them to enter the interstitial space. For the first time this has allowed ultrasound to assess true perfusion, where velocities and spatial resolution are too low to be seen with Doppler ultrasound. This system has been used routinely in livers for up to 10 years and there have been several studies describing the use of CEUS after renal transplantation surgery [5,6,7]. However, no previous description of this or similar technique pre-transplantation could be found in a search of the relevant literature.

Three dimensional ultrasound (3DUS) is a fast and reliable way of acquiring volumes of data that can be assessed offline and generates a permanent record of the examination [8] making it useful for capturing the perfusion state of the kidney quickly in theatre where time is at a premium, allowing for subsequent analysis, such as while the kidney is having final preparation.

We hypothesise that pre-operative CEUS, particularly on non-beating heart donor kidneys could give valuable information regarding the state of the organ and its vascular anatomy, potentially saving a patient from an operation and graft which is not viable for transplantation.
Methods and Materials

Five kidneys were retrieved from adult pigs by the transplant surgeons and prepared using the protocol for a human transplant organ (Maastricht category II). To mimic non-heart-beating donation scenario the pigs were killed by anaesthetic overdose and we subjected the kidneys to a warm ischemia of 30 mins. The kidneys were then flushed with RSI solution (Aqix Ltd., UK).

The renal artery was cannulated and the kidney was attached to a Lifeport Kidney transporter (LKT-100-P) (Organ Recovery Systems, USA). This keeps the kidney perfused with KPS perfusion fluid (Organ Recovery Systems, USA) by means of a peristaltic pump and also maintains the kidney at a specified temperature using a water and ice cold-mass. In this case the temperature was held at around 2 degrees Celsius. The kidneys remained in cold storage for up to 24 hours prior to examination. Just prior to ultrasound study a tissue sample was obtained and this went for histological examination.

The maximum system pressure was set at 30mmHg. The perfusion pump is fitted with a wash valve which is a standard port for flushing the system. Through this we introduced 0.5mls of Sonovue contrast media (Bracco, Italy) as a bolus injection. Each kidney was subsequently scanned using an iU22 ultrasound machine (Philips Healthcare, Bothell, WA, USA) on a contrast specific preset using power modulated pulse inversion and side by side imaging. A sterile probe cover and standard sterile procedures were used to maintain an aseptic technique (image 2). The probe was kept stationary (Both X6-1 matrix 3D probe and C5-1 curvilinear probe were used (Philips, USA)) in longitudinal section for 30 seconds and the clip stored. The kidney was then scanned freely to assess for any focal areas of non-perfusion and a contrast specific 3D volume of the kidney was acquired.

The 30 second runs were then assessed using QLab (Philips, Bothell, WA, USA) CEUS quantification software. Standardised 5mm regions of interest were placed on the interlobar arteries, cortex and medulla and perfusion gradients were obtained for these areas.
Images for this section:

**Fig. 0**: Lifeport perfusion machine with reclaimed kidney (central chamber), the ice mass (left chamber) and peristaltic perfusion pump with injectable port (right side)

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**Fig. 0:** The kidney being scanned on IU22 and using the C5-1 probe (Philips Healthcare, Bothell, WA, USA) using a sterile probe cover and aseptic technique

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Results

At 30mmHG systemic pressure the flow within the kidneys ranged from 10-37 mls/min and the resistance to the flow ranged from 0.62-1.99 mmHg.

Kidneys 1, 3, 4 and 5 were well perfused with contrast flow seen throughout the majority of the kidney (image 1 and 2). Kidneys 1 and 3 had very small upper pole perfusion defects (image 3) that were clearly identified on the contrast specific ultrasound and three dimensional contrast ultrasound. Kidneys 4 and 5 had no perfusion defects identified.

Histology demonstrated that these kidneys had relatively well preserved glomeruli that show some early signs of mild damage, primarily in the form of effacement of the foot processes of the podocytes (image 4).

Despite an acceptable flow rate, kidney 2 demonstrated poor perfusion with flow seen in the arteries up to the inter-lobar level only with no significant cortical or medullary flow. The system appeared to be decompressing through a rupture in the medulla and capsule (Image 5).

In post-processing of the well perfused kidneys, the perfusion curves consistently demonstrated early and fast filling of the inter-lobar arteries, with subsequent filling of the cortex and then finally slower filling of the medullary pyramids. Gamma variate curves were applied to the data to demonstrate the perfusion trends (Images 6 and 7).
**Fig. 1:** Road map (MVI) of kidney showing sequential filling of the entire organ except the small defect in the upper pole

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Fig. 0: Road map (MVI) of kidney showing sequential filling of the entire organ except the small defect in the upper pole

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**Fig. 0:** Small perfusion defect seen at the upper pole of kidney 1 demonstrated using side by side contrast imaging (right side is fundamental ultrasound, left side is contrast specific)

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Fig. 0: Electron microscope slides demonstrating viable glomerular structures, that show some early signs of damage (secondary to the ischaemic-reperfusion-injury sequence) primarily 'effacement of the podocytes'. This change is present in both specimens and is simply a flattening and widening of the foot processes of the podocytes.

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Fig. 0: Arterial flow only seen at 40 seconds after injection in kidney 2 with the contrast media extravasating into the surrounding fluid (solid arrow)

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**Fig. 0:** Perfusion graph demonstrating mean echo intensity (dB) against time (s) for the interlobar arteries (purple and turquoise), cortex (red and blue) and medulla (yellow and orange) from the porcine kidney number 1

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Fig. 0: Perfusion graph demonstrating mean echo intensity (dB) against time (s) for the interlobar arteries (red), cortex (blue) and medulla (turquoise) from the porcine kidney number 5

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Conclusion

Discussion

This pilot study has demonstrated that not only is contrast-enhanced ultrasound possible on a cold perfused kidney transplant, it is a straightforward technique and can give a range of information on perfusion defects and flow dynamics the significance of which needs further investigation.

The technique is portable and can be performed using a sterile, aseptic technique allowing the examination to be performed within the theatre suite without removing the kidney from hypothermic perfusion, therefore, not requiring the graft to be subjected to a further period of warm ischaemia, potentially damaging the kidney.

A potential problem with the technique was the perfusion system on the machine. The Lifeport system has a disposable cartridge filter for solids which has a 20 micron nominal filtration size. The micro-bubble contrast media seemed to pass through this filter without obvious degradation of concentration as demonstrated by the equilibrium achieved in the perfusion graphs with no apparent reduction in mean intensity.

The ex vivo perfusion patterns seen in the well-perfused porcine models show similar curve distribution to those seen on post-operative studies of human transplants (image 1). This suggests that the flow dynamics form artery to cortex to medulla is largely uncompromised by using a perfusion pump.

The time-intensity curves have shown to give information regarding the state of the organ when used post-operatively [9], such as degree of ATN, and the significance of these pre-operative curves needs further investigation.

However, one difference in the perfusion curves is the diminishing intensity seen over time in vivo. This doesn't seem to occur ex vivo, presumably because nothing is destroying bubbles, which would be broken down by the arterial/venous shunting, cardiac cycle and the pulmonary bed in vivo [10].

In agreement with [11] we found 3D CEUS to be a good way of demonstrating the extent of any vascular defects present (image 2 and 3). It also allowed extremely fast acquisition time, keeping scan time to a minimum and allowing for reliable offline manipulation of the data afterwards. 3D CEUS would also provide a comprehensive baseline for vascular perfusion changes occurring pre-, intra- or post-operatively.
The gamma-variate curve was used to demonstrate the flow patterns as this has been shown to best show the curve of wash in and wash out seen in a perfusion study [13].

The lack of cortical and medullary flow in kidney 2 is particularly important as this demonstrates the limitations of just using the overall perfusion flow rates on the machine. Despite a flow of 10mls/minute, the kidney had no flow through the capillary bed and the flow was maintained by extravasation through the cortex and capsule into the surrounding fluid.

Sulphur hexafluoride micro-bubble contrast agents are generally very well tolerated in vivo (approximately 1:10,000 cases of hypersensitivity [14]). They do not pass out of the blood stream or over blood/brain barrier and therefore are not thought to cause neurological effects. They have also been shown not to cause renal damage in kidney perfusion examinations in animal models [15].

The use and characteristics of ultrasound contrast at low temperatures is interesting as this is likely to affect the resonant properties of the micro-bubbles, however, no subjective difference in signal strength was seen during the experiments and good signal returns were achieved from injected doses as small as 0.1mls of contrast media. No data could be found in the literature about the effects of temperature on ultrasound contrast media.
**Fig. 0:** Perfusion graph demonstrating mean echo intensity (dB) against time (s) for the interlobar arteries (turquoise), cortex (red) and medulla (yellow) from a 5 days post-operative renal transplant in a patient

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**Fig. 0:** Upper pole perfusion defect in kidney 3 demonstrated in 3 dimensions using a contrast specific volume acquisition and multi-planar reconstruction (MPR) software

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**Fig. 0:** Upper pole perfusion defect in kidney 3 demonstrated on normal side-by-side contrast imaging

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Personal Information

B. Stenberg MSc. PGCert.

Radiology,
Freeman Hospital,
Newcastle upon Tyne
NE7 7DN
UK

ben.stenberg@nuth.nhs.uk