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Using Laser Speckle Contrast Imaging to Quantify Perfusion Quality in Kidney and Pancreas Grafts on Vascular Reperfusion: A Proof-of-Principle Study

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Introduction. The accuracy of intraoperative graft perfusion assessment still remains subjective, with doppler examination being the only objective adjunct. Laser speckle contrast imaging (LSCI) has been used to assess intraoperative blood flow in neurosurgery and in various surgical specialties. Despite its ability to accurately quantify perfusion at the microvascular level, it has not been clinically evaluated in kidney/kidney-pancreas transplantation for perfusion characterization. We aimed to evaluate the utility of LSCI and identify objective parameters that can be quantified at reperfusion. **Methods.** This study was registered in ClinicalTrials.gov (NCT04202237). The Moor FLPI-2 blood flow imager was used in 4 patients (1 Simultaneous Pancreas and Kidney, 2 deceased, and 1 living donor kidney transplants) during reperfusion to capture reperfusion data. The following parameters were measured: flux (average speed × concentration of moving red blood cells in the sample volume), doppler centroid, total and valid pixels, valid rate, and total and valid area. Flux data were analyzed with Moor FLPI analysis software. **Results.** The perfusion characteristics and flux images correlated with initial graft function. **Conclusions.** LSCI is a safe, noncontact imaging modality that provides real-time, accurate, high-resolution, full field blood flow images and a wide range of flux data to objectively quantify organ reperfusion intraoperatively in kidney/kidney-pancreas transplantation. This modality could be used to develop a robust numerical quantification system for the evaluation and reporting of intraoperative organ perfusion, and aid intraoperative decision-making. Perfusion data could be combined with biomarkers and immunological parameters to more accurately predict graft outcomes.

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Solid organ transplantation is now established as a routine therapeutic intervention for eligible patients with advanced organ dysfunction and failure across several organ systems. The immediate outcomes after transplantation are critically dependent on adequate vascular perfusion of the transplanted organ. There are several other determinants that have an

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impact on outcomes including the type of donation (living donation, donation after brain death [DBD] or donation after circulatory death [DCD]), ischemic times, age, and comorbidities of the donor and recipient. The hemodynamics and quality of organ perfusion at implantation are critical to engraftment and subsequent function of the transplanted organ. The assessment of graft perfusion is, by and large, visual and subjective in most solid organ transplants. Intraoperative Doppler evaluation is currently the only method of objectively quantifying the flow in vessels and grafts, mainly in kidney, pancreas, and liver transplantation. Laser speckle contrast imaging (LSCI) is a noninvasive imaging technique to assess vascular flow, including that of the microvasculature, which can be used intraoperatively.[1-3] This has been trialed in research settings, in neurosurgery as well as other areas in surgery. It has not been reported in transplantation.

Intraoperative imaging using the laser speckle contrast technique delivers real-time blood flow images along with numerical parametric flux data. The strength of the technique compared to the standard Doppler is in its ability to measure blood flow over areas of interest or the whole transplanted organ with accurate numerical quantification of blood flow at every point measured. Laser speckle has been used in a wide range of preclinical and clinical research applications. It has, however, not yet been used clinically for the assessment of perfusion in kidney and pancreas transplantation. This proof-of-principle study reports the use of the Moor FLPI-2 blood flow imager in capturing parameters of perfusion in 3 kidney and 1 simultaneous pancreas and kidney transplant recipients at the time of graft reperfusion.

MATERIALS AND METHODS

The study was given Research Ethics clearance by the North West–Greater Manchester West Research Ethics Committee. Research Ethics Committee Reference number 19/NW/0212. It was also registered in ClinicalTrials.gov (NCT04202237). All patients provided informed consent for the intraoperative use of the laser speckle blood flow imager.

LSCI is based on the speckle (interference) pattern caused by scattering of light when tissue with moving blood cells is illuminated by laser light. The changing pattern becomes blurred when there is high flow of blood, thereby reducing the contrast in that region. Conversely, with a low flow state, there is increased contrast. The Laser Speckle Contrast Analysis camera positioned some distance away from the area of interest can provide an accurate and instantaneous map of blood flow parameters in real time. A major advantage is the ability to capture a large area of interest or the entire field and is also able to generate high-resolution images.

The Moor FLPI-2 (Moor Instruments Ltd, Axminster, UK) blood flow imager was used in 4 patients during reperfusion to capture reperfusion data. The dual complementary metal-oxide-semiconductor color imager, with 12× optical zoom with autofocus captures real-time, visual data of 4K imaging for blood vessels with a frame capture of 100 images per second. The region of interest can be set to a square, circle or polygon for on line or off line analysis.

In all the patients, the imager fixed on to a portable stand was moved approximately 30–40 cm over the transplanted kidney or pancreas before reperfusion, maintaining operative sterility. The speckle lens was aimed vertically and perpendicular to the graft surface. Zoom settings were adjusted to capture the entire kidney or pancreas within the imaging field which was approximately 15 cm × 15 cm. The overhead theater lights were dimmed so that it did not interfere with the laser light. A polarizing filter was used to eliminate glare from reflective tissue. Image capture and data recording was commenced just prior to clamp release so that all perfusion parameters were captured from the moment of clamp release for approximately 60 s. All data acquisition was through a laptop computer (Dell Computer Corporation, Round Rock, TX) via standard universal serial bus and FireWire (IEEE 1394) interfaces. The 4 cases included as part of the study were: 2 deceased donor kidney transplants, a living donor kidney transplant, and a simultaneous pancreas and kidney transplant. Both categorical and continuous data were captured. Full organ surface blood perfusion imaging was carried out for all 4 patients (3 kidneys and 1 pancreas). Recipient and donor demographics, graft details, intraoperative details, postoperative imaging and outcome data were collected.

Each image was made up of a large number of pixels (580 × 752), each pixel a quantifiable blood flow value. All flux data captured were analyzed with moor FLPI analysis software package (moor FLPI Review V4.0, Moor instruments, Devon UK), that included data exportation to generic formats. The following parameters were measured: flux, Doppler centroid (DC), total pixels, valid pixels, valid rate, total area, and valid area. Flux is the product of average speed and concentration of moving red blood cells in the tissue sample volume, and DC indicates the intensity of the backscattered laser light. Valid pixel is number of pixels with a blood flow value. If there is an insufficient level of reflected light (DC) to give a validated reading, then this number will be lower than the total number of pixels in the region of interest. Valid rate is the percentage of valid pixels in the sample area/region of interest. Valid area is the valid pixels expressed in square millimeter.

RESULTS

Case 1

The recipient was a 59-y-old male on hemodialysis for end-stage renal failure (ESRF) secondary to type 2 diabetes, who received a kidney transplant from a 48-y-old DBD donor. The donor's left kidney was implanted into the recipient's right external iliac vessels and the cold ischemia time was 24 h. There were 2 arteries (main renal artery and an additional smaller lower polar artery), 1 vein and 1 ureter. Reperfusion was described as brisk and uniform with a good pulse in the hilum. The upper and lower polar arteries were anastomosed to the proximal and distal external iliac artery, respectively, and laser speckle Doppler imaging was performed at reperfusion. Perfusion parameters are described in Table 1. The flux values were uniform throughout the kidney with a high valid rate. Figure 1 depicts an ischemia–reperfusion pattern at the time of reperfusion and the initial fluctuations in blood flow. The microvascular perfusion stabilizes after 40 s after reperfusion. A postoperative day 1 radioisotope renogram was performed as per the institutional practice for suspected delayed graft function (DGF), and that showed 6.4% peak uptake and 11.6% total removal at 30 min, leading to an overall impression of a moderately functioning transplanted kidney. The recipient had initial DGF that resolved and had a functioning

TABLE 1.

Perfusion parameters over a region of interest at the time of reperfusion

Case	Region	Flux mean	Flux median	DC mean	DC median	Total pixels	Valid pixels	Valid rate (%)	Total area, mm²	Valid area, mm²
1	Kidney lower pole	90.2	79	64.0	65	375 624	367 444	97.8	8347.3	8165.5
1	Kidney hilum	90.8	79	63.5	65	402 080	389 950	97.0	8935.2	8665.6
2	Kidney upper pole	151.6	75	60.4	58	375 060	230 314	61.4	24 495.9	15 042.3
2	Kidney hilum	158.2	84	61.0	60	402 080	246 122	61.2	26 260.6	16 074.7
2	Kidney lower pole	52.8	18	58.5	57	402 080	289 276	71.9	27 475.0	19 766.9
2	Ureter	78.6	30	110.6	118	402 080	369 293	91.8	15 614.4	14 341.1
3	Kidney upper pole	463.7	320	80.1	73	5372	5372	100	158.6	158.6
3	Kidney mid pole	1953	1948	148.6	148	34 475	33 913	98.4	1017.8	1001.2
4	Pancreas body	249.2	195	75.7	70	375 060	246 771	65.8	21 590.6	14 205.6
4	Pancreas tail	240.5	188	75.8	70	402 080	253 140	63.0	23 146.0	14 572.2
4	Duodenal cuff	272.5	165	105.3	103	375 060	354 463	94.5	21 590.6	20 404.9

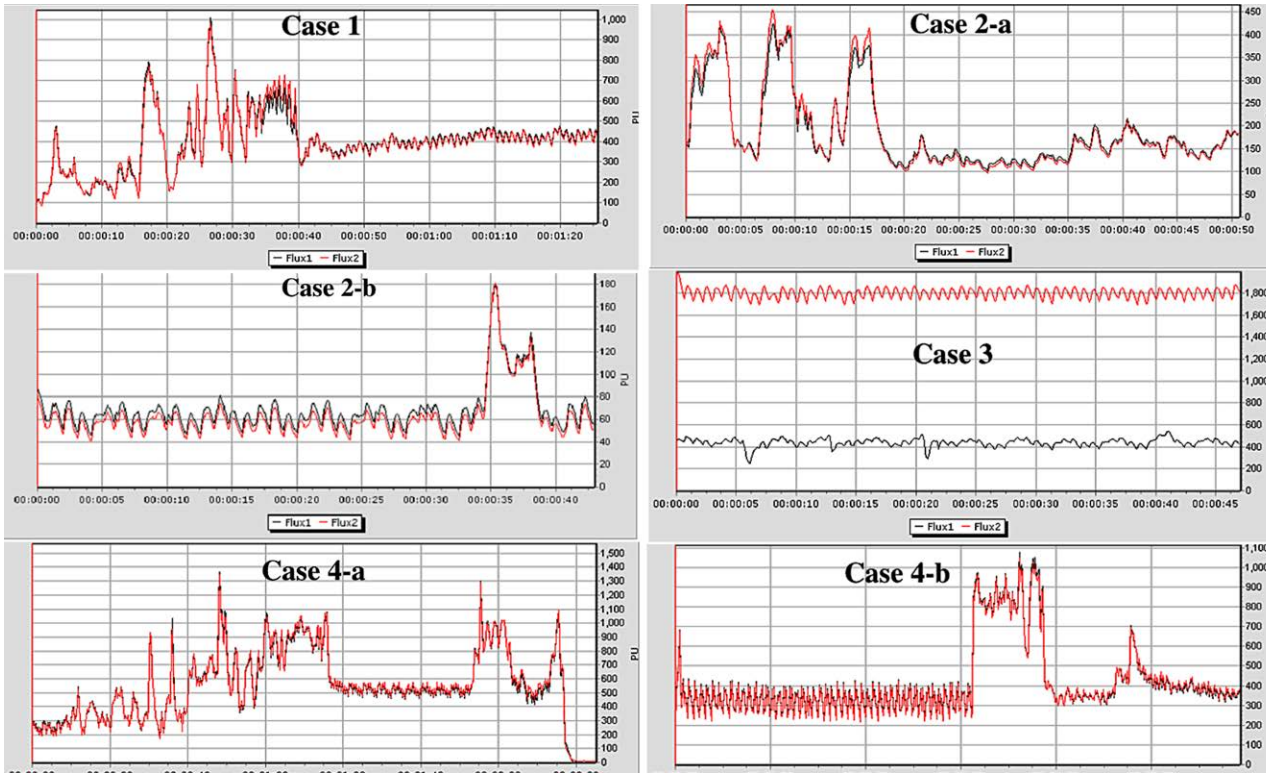

FIGURE 1. x-axis denotes time in seconds and y-axis denotes perfusion units. In case 1, flux 1 depicts perfusion in the hilum, and flux 2 depicts perfusion in the lower pole. In case 2, "a" depicts perfusion in the upper pole and hilum, "b" depicts perfusion in the ureter, and fluxes 1 and 2 are 2 adjacent points. In case 3, flux 1 is the perfusion recorded in a poorly perfused area and flux 2 is perfusion recorded in the well perfused hilar region. In case 4, "a" depicts perfusion in the body and tail of the pancreas, "b" depicts perfusion in the donor duodenal cuff, and fluxes 1 and 2 are 2 adjacent points.

graft with a creatinine of 131 μmol/L until he succumbed to sepsis 1 y posttransplant. Although the intraoperative LSCI perfusion parameters were satisfactory, DGF was likely due to prolonged cold ischemia time.

Case 2

The recipient was a 67-y-old female on hemodialysis for ESRF secondary to type 2 diabetes and hypertension, who received a kidney transplant from a 62-y-old DCD donor. The donor's left kidney was implanted into the recipient's right external iliac vessels. The cold ischemic time was 14 h and 58 min. The flux values as described in Table 1 was not uniform throughout the kidney. The flux values were better at the hilum and upper pole when compared to case 1, but the valid rate was low. Figure 1 depicts the ischemia–reperfusion pattern and subsequent stabilization of blood flow. The laser speckle perfusion units were much less when compared to the other 2 kidney transplants. A renogram performed on postoperative day 4 showed reduced tracer uptake seen in the upper pole of the kidney, the peak uptake was 1.3% and the total removal was 1.4% at 30 min, leading to an overall impression of very poor function. A subsequent renogram at day 8 showed poor function (4.2% overall removal). There was DGF for 2 wks and the recipient was discharged with

an improving kidney function. The poor intraoperative perfusion parameters corresponded to the subsequent renogram findings and initial clinical course (DGF). At 6 mon posttransplant, an ultrasound scan performed for declining kidney function showed an increase in echogenicity of the cortex suggesting underlying rejection and the estimated glomerular filtration rate dropped to 10. A biopsy was performed, which was inconclusive for rejection and also showed changes of acute tubular necrosis. The patient was empirically treated for rejection based on clinical suspicion. The transplanted kidney failed 7 mo after transplant because of presumed rejection and the patient recommenced hemodialysis. As the cause of graft failure was unclear, it is difficult to make conclusions based on the intraoperative LSCI parameters.

Case 3

The recipient was a 56-y-old female, predialysis, with ESRF secondary to type 2 diabetes, who received a kidney transplant from a 34-y-old living-related donor. The donor's left kidney was implanted into the recipient's right external iliac vessels. The cold ischemic time was 4 h and 50 min. A small upper polar artery was sacrificed. Reperfusion was brisk with a good pulse in the hilum. Approximately 2% of the area supplied by the upper polar artery was poorly perfused. Reperfusion parameters recorded at reperfusion are described in Table 1. The flux values and the valid rate were much higher compared to cases 1 and 2. The difference in perfusion between the well perfused hilar region and the less well perfused upper pole is clearly seen in Figure 1. The laser speckle perfusion units were markedly higher compared to the other 2 deceased donor kidneys, a reflection of the overall quality of reperfusion. It also is imperative to acknowledge that there are no standard perfusion unit values. A protocol radioisotope renogram performed on postoperative day 1 showed good tracer uptake in the kidney, the peak uptake was 4.8%, and the total removal was 67.5% at 30 min, leading to an overall impression of a good function. There was immediate graft function, and the recipient was discharged with stable kidney function. The good intraoperative LSCI perfusion parameters corresponded with the renogram findings and the clinical course (primary graft function). The recipient was repatriated to their base hospital at 3-mo posttransplant with a creatinine of 80 and an estimated glomerular filtration rate of 71.

Case 4

The recipient was a 34-y-old, with ESRF secondary to type 1 diabetes, who underwent a pre-emptive simultaneous pancreas and kidney transplant from a 36-y-old DBD donor. There was no abnormal hepatic arterial anatomy in the donor and the arteries supplying the pancreas graft were reconstructed with a standard Y-graft using the donor's iliac artery. The pancreas was implanted intraperitoneally with arterial inflow from the right common iliac artery, systemic venous drainage to the IVC, and enteric exocrine drainage. The cold ischemia time for the pancreas was 8 h and 13 min. Laser speckle Doppler imaging was commenced from the point of clamp release/pancreas reperfusion and was repeated after exocrine drainage was completed. Perfusion parameters are described in Table 1. The flux values and valid rate were variable in different regions. Figure 1 depicts the ischemia–reperfusion pattern of trace in the pancreas body and tail. The flux images of the body and tail along with the duodenum are depicted in Figure 2. The higher flux values with a higher valid rate at the duodenal cuff correlates with the high blood flow noted in the flux image. There was primary pancreas graft function and the recipient remains insulin-independent. The intraoperative LSCI perfusion parameters were satisfactory and correlated well with the clinical course (insulin independence and no enteric anastomotic complications).

DISCUSSION

Reperfusion of all grafts during solid organ transplantation is associated with varying degrees of ischemia–reperfusion Injury (IRI). The magnitude and clinical consequences of IRI depends on several factors including the category of donation. IRI is of greater magnitude in DCD and extended criteria donor organs and in those with longer cold and warm ischemia times. IRI is also a major contributing factor for DGF and is intrinsically linked to the quality of organ reperfusion.[4-8] Most studies quantify IRI after transplantation by the quantification of organ function, biomarkers, and imaging of the main vessels.

This is of most relevance in kidney transplantation, which forms the largest volume of solid organ transplants. There is a clinical need to have objective parameters of graft perfusion that could be combined with other graft metrics and biomarkers to predict outcomes. The adequacy and quality of organ perfusion is of critical importance and is linked to the perioperative management, therapeutic strategies, and graft outcomes. Hence, an accurate objective assessment of global perfusion after transplantation is crucial.

Organ reperfusion has historically been assessed subjectively by the implanting surgical team, with standard terminology being good, patchy, or poor. There is no objective method for immediate and accurate quantification of the graft perfusion intraoperatively. Objective evaluation of perfusion is primarily dependent on standard Doppler evaluation of the main vascular pedicle or focused on specific areas of interest in the transplanted organ. Doppler provides data on flow characteristics in the main vascular pedicle and branch vessels in the organ, but not at a more microvasculature level. Additionally, local heterogeneities in perfusion are difficult to determine with duplex sonography. The main effects of variations in perfusion are, however, manifested at the microvasculature level. The measurement of perfusion characteristics of the microvasculature is challenging due to the limited tissue penetrance of most techniques used and the variability in perfusion over time. Although there are several different techniques used in clinical practice such as infrared thermography, imaging plethysmography, optical coherence tomography, and laser speckle perfusion imaging, they are primarily restricted to surface level imaging because of the limited tissue penetration and have not been used in clinical transplantation.[9] Fluorescence imaging using Indocyanine green has been recently emerging to quantify renal cortical microperfusion and correlate the outcomes. However, indocyanine green requires administration of a fluorescent dye that could not only cause surgical hindrance and logistical issues, but also it does not give an accurate representation of blood flow.[10-13]

Although LSCI is primarily considered a research tool, it is being increasingly used in various clinical settings such as in reconstructive surgery, neurosurgery, parathyroid surgery, liver surgery, and gastrointestinal surgery to assess blood flow and

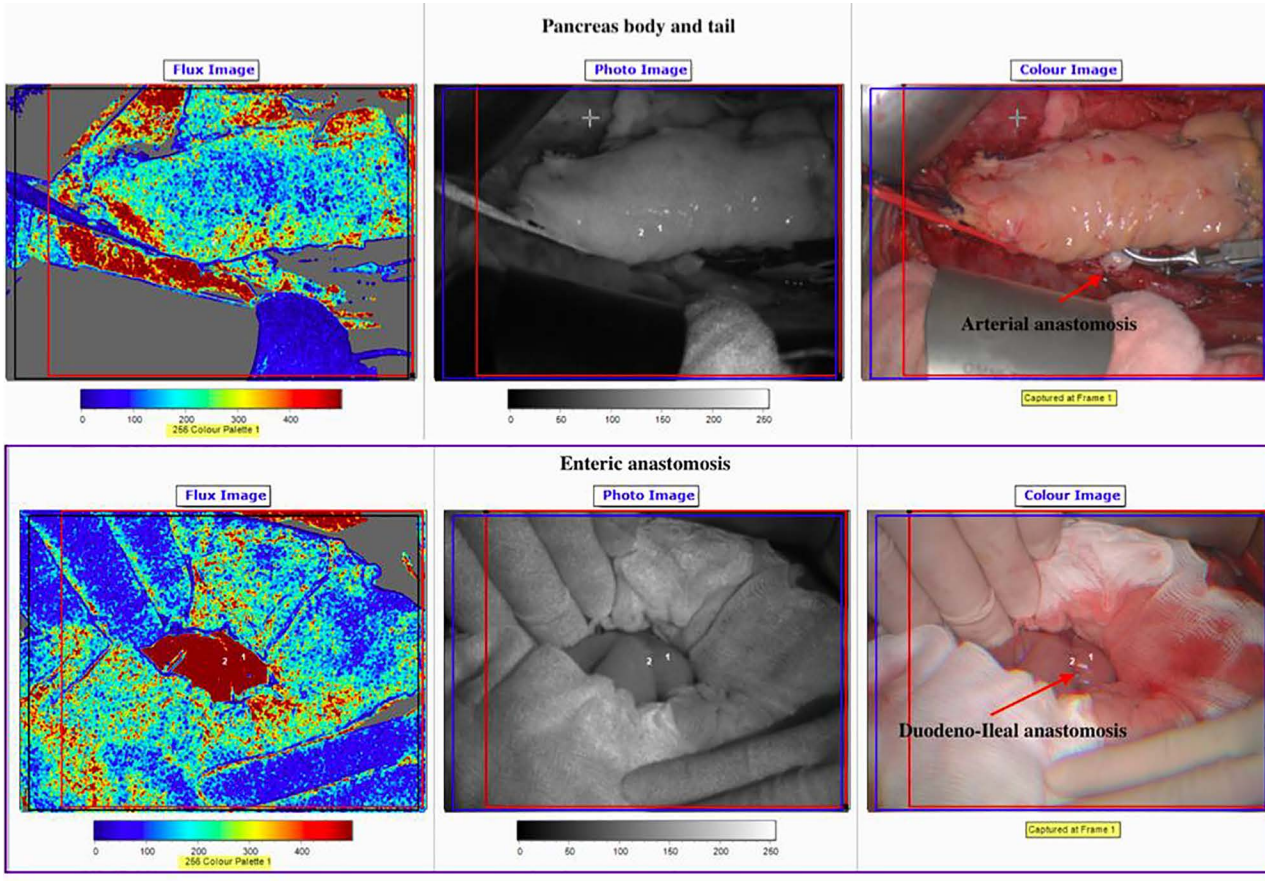

FIGURE 2. Intra-operative perfusion assessment. Flux image along with photo and color images of the pancreas body and tail after release of venous clamp (A) and the duodenal cuff after reperfusion and enteric anastomosis (B). The values in the color palette are laser speckle perfusion units. A, Due to low flow state corresponding to venous perfusion, there is an increased contrast in the flux image and the corresponding perfusion units are lower. B, the duodenoileal anastomosis appears blurred indicating high blood flow and the corresponding perfusion units are higher.

tissue perfusion intraoperatively.[14-22] In transplantation, laser speckle contrast analysis has been used to assess blood flow after uterine transplantation in animal models.[23] It has also been reported to be capable of imaging renal cortical microperfusion in porcine kidney transplantation.[24] Despite the accumulated research and clinical evidence, it has not translated to clinical use in kidney or kidney-pancreas transplantation so far.

After transplantation, evaluation of organ perfusion is carried out with a variety of methods. After kidney transplantation, radioisotope renograms can accurately quantify organ perfusion and function. They are expensive, however, and expose the transplant recipient to radiation. Other newer techniques such as contrast enhanced ultrasound have been used in both kidney and pancreas transplantation.[25] The choice of posttransplant imaging and their indications are based on the individual center practices. In pancreas transplantation, the enteric anastomosis has historically been the Achilles heel and, with the increasing utilization of DCD grafts, the importance of duodenal perfusion cannot be overemphasized. There has also been a resurgence in research on machine perfusion of the pancreas along with the assessment of IRI.[26] LSCI may be an ideal tool in objectively assessing perfusion in pancreas transplantation.

Incorporation of the intraoperative imaging with the laser speckle can provide a robust objective baseline of perfusion which can usefully contribute to therapeutic decision-making. Once accurate reperfusion and blood flow parameters are defined, it could potentially identify grafts at the risk of thrombosis or other morbidity and guide therapeutic strategies and interventions such as the use of dopamine (upregulates hemeoxygenase-1 activity thereby converting metalloproteins to inert byproducts and also improves renal blood flow) or anticoagulants or vascular smooth muscle relaxants such as calcium channel blockers in the post op period (to inhibit vasospasm). In cases 1 and 3, although the intraoperative LSCI data was reassuring, a radioisotope renogram was still performed due to institutional protocol. If robust flow characteristics can be defined, LSCI could obviate the need for routine protocol posttransplant imaging for perfusion. The one time capital investment needed for setting up this technology is 49 000 GBP, which includes the MOORFLPI2 imager, Laptop PC, software-MOORFLPI2-3VX, desk stand, commissioning, and 2-y warranty. Alternatively, operating leases are available for 2500 GBP/mo. All the consumables are reusable and hence thereafter, there is no additional cost associated for each case. An in-detailed cost benefit analysis comparing the various imaging modalities is beyond the scope of this article.

With the complexities of the organ systems and factors being measured, there will be several limitations that will restrict the use of LSCI, including movement artifacts and the need for zero flow areas to measure interpatient variability.[27,28] Despite being a safe and noncontact form of imaging, the limited tissue penetrance restricts the usage to only the intraoperative setting. The other limitation of this study is that the perfusion parameters were not correlated with other clinical parameters relating to ischemia and

reperfusion. Although it could be argued that in this study the perfusion parameters were not compared with a standard imaging technique, none of the currently available standard imaging techniques could be used intraoperatively to measure flow in the microvascular environment. Despite quantifying blood flow, it is difficult to convert flux units to absolute values of flow such as ml/gm/minute. Conversion factors are being developed and need to be standardized. Further studies with a much larger case series, likely including only patients with immediate graft function would allow for establishing reference values. Unlike the application of LSCI in other fields, the outcomes in transplantation are not binary but composite. Although perfusion is a crucial determinant for success of transplantation, there are other determinants as well. Hence, it is difficult to explicitly correlate the perfusion parameters with all the clinical parameters.

A further advancement of the technique that combines existing laser speckle contrast technology for perfusion imaging with reflectance spectroscopy for oxygenation change, provides blood flow and oxygen videos of any exposed tissue with color photo images which match blood flow and oxygenation. An optical zoom of up to 10 times enables imaging from an area of 5.6 mm × 7.5 mm up to 15 cm × 20 cm. Real-time video frames of up to 20 frames/s capture dynamic changes in flow and tissue oxygenation. Each frame combines 1× blood flow image, 1× oxyhemoglobin image, 1× deoxyhemoglobin image, and 1× color photograph. A spatial resolution of 10 μm per pixel provides a detailed morphology. An important facility is the ability to image multiple regions of interest to assess and quantify blood flow and oxygen changes in real time. Objective microcirculatory measurements obtained with the laser speckle and especially with the newer generation imaging could guide perioperative therapeutic interventions. Another potential area in transplantation is donor evaluation and management to evaluate perfusion in the settings of normothermic regional perfusion and normothermic machine perfusion and could potentially improve organ utilization. Combining objective blood flow metrics along with biochemical parameters could provide additional useful information on organ quality before implantation. Integration of the existing imaging system with machine learning and artificial intelligence would enable an automated evaluation of the acquired image data, and these are potential areas of future research of this imaging modality in transplantation.

CONCLUSION

This proof-of-principle study in 4 cases demonstrates that the LSCI provides a wide range of objective perfusion data quantifying organ reperfusion intraoperatively during kidney and kidney-pancreas transplantation. The technique is safe and avoids direct contact with the graft. Flow characteristics data captured from this method could be used to develop a robust numerical quantification system for the evaluation and reporting of intraoperative organ perfusion. These perfusion data could be combined with biomarkers and immunological parameters to more accurately predict graft outcomes in the future.

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