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3D Contrast enhanced ultrasound in perfusion studies of early renal transplants
£8230

Summary
Nuclear medicine, technetium 99m renogram (DTPA) is the primary investigation for perfusion defects post-transplantation. It is accurate (up to 99% sensitivity), but time consuming, expensive and has the innate risks of an examination using ionising radiation.
Contrast enhanced ultrasound (CEUS) is an emerging technology which may solve these issues, giving greater resolution and temporal information while having the potential to also add prognostic information from perfusion curves.
However, little research has been done to investigate whether CEUS has the ability to maintain the high sensitivity rates to replace DTPA as the primary investigation.
This project proposes a blinded controlled trial using 105 renal transplant patients examined with both DTPA and CEUS in the immediate post-surgical phase and reported independently of each other.
Reported vascular defects will be compared for association with Cohen's Kappa test and for difference with Chi2 test to determine equivalence between the two tests.
Any discrepancies in diagnosis between the tests will be reported as case studies to assess any change in clinical management.
The CEUS examinations will also be assessed with region of interest quantification software to assess any correlation with the haemodynamic parameters and long and short term graft viability.

Aims
To improve post surgical perfusion studies through three-dimensional contrast enhanced ultrasound

Research Objectives
Primary research question
Is 3D contrast enhanced ultrasound as accurate as nuclear medicine in the detection of renal transplant perfusion defects?

Secondary research question
Does the quantification of sub-total perfusion defects have an effect on the clinical management of the patient?

Tertiary research question
Can haemodynamic factors assessed with contrast enhanced ultrasound predict chronic graft dysfunction?

Introduction
All renal transplants undergo a perfusion study of the new graft as soon as possible after surgery to check for arterial or venous occlusion. Currently this is done using nuclear medicine with an injection of radio-isotope technetium 99m bound to diethylene triamine penta-acetic acid (DTPA) and a gamma camera to detect intensity of radio-isotope in the transplanted kidney. The examination takes around 30 minutes, must be done in the department on a special bed and carries with it the usual risk of using ionising radiation. The total amount of radiation involved is about the same amount as we receive from natural background radiation in the environment in about six months. This probably increases the risk of developing cancer by about 1in 10,000 (RCR, 2008) (average dose 2.5mSv). The trust figure for a transplant renogram is £244 pounds. The national figure is around £304.
Each patient also has a b-mode ultrasound to assess any structural abnormality such as peri-renal collection and the vasculature is also assessed, i.e. patency of main renal artery, main renal vein and the resistance index (RI) in the interlobar arteries.

Unlike b-mode ultrasound and nuclear medicine, contrast enhanced ultrasound can offer high resolution three-dimensional assessment of true cortical perfusion giving quantifiable percentage of cortex perfused. This could also detect shunting at inter-lobar level as well as other vascular defects, previously unseen using nuclear medicine. This has the potential to change patient management i.e. anti-coagulation.
We would also be able to assess factors, which may help to predict graft long-term viability. These would include assessing the perfusion rates of the cortex in comparison to the main or inter-lobar arteries and peri-nephric tissue or assessing the significance of small perfusion defects and comparing them to 3 month transplant success.
Contrast enhanced ultrasound can be performed for approximately £120 (£80 is standard ultrasound cost, £40 for the cost of the contrast). Considering ultrasound is already routinely performed post-transplantation the cost could be considered as low as £40 per examination for the contrast. This trust is funded for 100 transplants a year and each one will undergo a perfusion study post surgery. A single examination saving of £124 would translate to a saving of at least £12,400 annually. In 2006 there were 1800 kidney transplants (NKF, 2010) this would be a national saving of at least £223,200 per annum.
As well as being cheaper, contrast enhanced ultrasound does not use ionising radiation, only takes about 5 minutes to perform and can also be done at the bedside if the patient was too unwell to be moved (ITU, etc.).
Contrast enhanced ultrasound is a relatively new technique, developed within the last 10 years, which uses tiny bubbles of an inert gas to act as a blood pool agent and increase echo signal strength. Previously ultrasound has used Doppler signal to demonstrate blood flow. This relies on the relatively weak signals returned from moving blood cells to demonstrate a shift in frequency that can be interpreted as movement. Blood which is moving parallel to the probe or at too low a velocity to detect a frequency shift will not be seen on Doppler ultrasound. This means ultrasound has only been able to demonstrate flow in reasonably large vessels and not at the micro-vascular or capillary level. Therefore, true perfusion cannot be demonstrated using Doppler ultrasound.
Because micro-bubble contrast media is a strong reflector of sound, the signal return is much greater, therefore, unlike Doppler ultrasound, it can also show stationary blood pooling or very low flow such as capillaries. The bubbles range in size but have an average diameter of 2.5μm, similar in size to a red blood cell. This allows the micro-bubbles to penetrate capillaries and show true perfusion. When the micro-bubbles dissipate, the phosphor-lipid shell is absorbed and the sulphur hexafluoride gas is expelled by the pulmonary system. A typical dose of 2.4mls of contrast will result in an exhaled volume of approximately 19 μl of gas.

Methodology

Given the lack of empirical research in this area, this problem statement and hypotheses are best addressed using an empirical investigation.
An experimental blinded controlled, crossover design was selected with a prospective cohort sample.
The crossover study design would ensure comparability between the two arms of the study as they will be acting as their own control and eliminate population bias. Both examinations will be performed as close to each other as possible which should minimise any order effect.
For the equivalence part of this study, nuclear medicine has been selected as the gold standard with which to compare the contrast enhanced ultrasound as it is the frontline investigation and has high sensitivity and specificity, in regards to renal transplant perfusion abnormalities where the sensitivity has been quoted as high as 87-100% (Sanches et al, 2003).
For the long term prognostic predictive value of the CEUS this will be compared against 3 month graft viability in terms of blood values (Creatnine, urea, eGFR etc.), requirements for intervention (surgical, medication, etc.) and general function of the graft.
The study size and objectives are suitable given the timeframe stipulated by the University and the constraints in terms of population numbers.

Each participant will be given a patient information leaflet and written consent will be obtained. At this point any contraindications for the contrast media will be checked with the patient.
If the patient is too unwell to come down to the radiology department, the examination will be done at the bedside on the ward.
The patient will then undergo the standard ultrasound examination using an iU22 ultrasound machine (Philips, USA) with a 5-1 MHz. curvilinear abdominal probe, assessing the size and structure of the transplant, peri-renal collections, hydronephrosis and the vasculature within

the graft. This involves checking patency of the main renal artery and vein and using spectral Doppler to assess the waveform of the inter-lobar arteries.
A new patient file is then opened with an anonomised, unique identifier. The patient will then have a baseline 3D volume of the transplant kidney, encompassing the entire organ. This is done using a volume probe.
The machine is then set up for a contrast examination using a low MI preset (MI=0.06) and side by side tissue/contrast imaging using the same probe. The patient receives a 2.4mls bolus intravenous injection of Sonovue ultrasound contrast media (Bracco, Italy), made up as per manufacturer's instructions. This is the standard adult dose as recommended by the manufacturer. Simultaneously, the timer is started on the machine and capture is pressed to store the acquired. The patient will then be scanned continuously for 60 seconds and the data stored. At this point, a 3D volume of the graft will be acquired in the contrast setting.
The operator will then report the standard examination in the usual fashion.
The contrast examination will be assessed using a dedicated work station by the operator.

The patient will also undergo a DTPA nuclear medicine examination. This is done within the medical physics department. An intravenous injection of Technetium 99m is administered and the patient is then scanned over the abdomen with a gamma camera. This lasts for around 15 minutes and the patient is then returned to the ward. The images acquired by the gamma camera will then be reported by a consultant medical physicist and double read by a consultant Radiologist.

For any cases where there is a discrepancy between the results from the DTPA and the contrast ultrasound, the clinicians will be asked to state the clinical management plan for that patient prior to receiving the results from the CEUS and then again on receiving the CEUS results. This will document any change in management brought about by the information supplied by the CEUS.

The 3D US volume will be assessed in MPR format to look for perfusion defects. Any defects found will be quantified using stacked contour volume compared to b-mode kidney volume to give a percentage of viable kidney parenchyma.
This will be calculated as:-

$$\frac{\text{volume of perfused kidney}}{\text{volume of whole kidney}} \times 100 = \%\ \text{of kidney perfused}$$

This will be compared with the result from the DTPA. Firstly in terms of; is there a perfusion defect? And if so, what is the severity?
This study will use Cohen's Kappa to test for levels of agreement. This is an adjusted measure of agreement which, will not give a P value but will give a level of agreement from poor to excellent. The level of departure from the expected outcome or significant difference between the examinations will be calculated using Chi^2. Nuclear medicine will be the expected result and contrast ultrasound the observed result, using a Chi squared test to 3 degrees of freedom (one for each outcome) will provide a value on the divergence of any results with a P value of significance. It will not, however, state which examination is better if any divergence is found.
Relative sensitivity and specificity rates and positive and negative predictive values compared to nuclear medicine can be calculated from a positive/false positive table. The false negative rate (ß) (1–sensitivity) can also be used to calculate the power of the result (1–ß).

The data obtained from the clinicians regarding clinical management before and after the contrast enhanced ultrasound results will be presented as case studies.

The 60 seconds of contrast filling will be assessed using an ultrasound quantification package. Regions of interest will be placed on the medulla, cortex, iliac and renal arteries and perfusion gradients will be produced. Time to peak, area under curve and maximum intensity will be compared with any bloods, biopsy results and graft function up to and including the 3 month review, as most acute complications occur within the first 3 months. These groups will be compared for correlation and any predictive information.

The methodology and background have been discussed with the trust transplant team (both surgery and Nephrology) and they are in full support of the project. The project was also

discussed with the Tyneside Kidney Patient Association, who felt it was a useful tool at a difficult stage in the recuperative process.

Potential Impact
If proved successful this technique would have a significant local impact and potential national impact in kidney transplant after care. We would move away from a long, static investigation using ionizing radiation and use a safer, quicker, cheaper and portable alternative with higher temporal and spatial resolution.

Outcomes
The expected outcome is that contrast-enhanced ultrasound will prove to be at least as accurate as DTPA in perfusion defects in the early post-operative phase of kidney transplants. From the few studies previously done we can expect possible correlations with the contrast uptake and longer term kidney viability.

Evaluation and dissemination Strategy
This study has the potential to change practice in this hospital and possibly nationally.
The findings will be initially written up as a doctorate thesis for the University of Northumbria.
The findings from this study will be disseminated at the local ultrasound department weekly meetings and also, for wider dissemination, it will be presented to the Trust Radiology department via the lecture programme.
The paper will be submitted for publication in a relevant journal.
The study will also be submitted to a relevant conference such as the British Medical Ultrasound Society (BMUS) annual scientific meeting as a paper presentation.

Timetable
Source funding by July/August 2010
Submission to NRES- July 2010
Submission to university ethics- Sept.2010
Start data collection – Oct./Nov. 2010
Collect data until June 2012
Writing up stage until first draft submission- December 2012
Final thesis Submission June 2013