MONASH UNIVERSITY

Department of Epidemiology and Preventive Medicine

"Renal Perfusion Evaluation with Contrast-Enhanced Ultrasonography"

PhD THESIS

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Melbourne 2014

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Notice 1

General Declaration

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes three original papers published in peer reviewed journals and three unpublished publications. The core theme of the thesis is the evaluation of renal perfusion with contrast-enhanced ultrasound in critically ill patients. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the department of Epidemiology and Preventive Medicine under the supervision of Rinaldo Bellomo and Michael Bailey.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research. The relative contributions of co-authors have been disclosed at the commencement of each results chapter in the "declaration for thesis chapter".

In the case of chapters 1-6 my contribution to the work involved the following:

Thesis chapter	Publication title	Status	Nature and extent of candidate's contribution
1	Measurement of kidney perfusion in critically ill patients	Published	Performed the literature review. Drafted and revised the manuscript
2	Bench-to-bedside review: Contrast enhanced ultrasonography -a promising technique to assess renal perfusion in the ICU	Published	Performed the literature review. Drafted and revised the manuscript
3	Contrast-enhanced ultrasound evaluation of renal microcirculation in sheep	Published	Participated in study design, performed CEUS studies, participated in data interpretation and drafted the manuscript
4	Contrast-enhanced ultrasound to evaluate changes in renal cortical perfusion around cardiac surgery: a pilot study	Published	Participated in study design, carried out participant recruitment, performed CEUS studies, participated in data interpretation and drafted the manuscript.
5	Contrast-enhanced ultrasound to evaluate changes in renal cortical microcirculation induced by noradrenaline: a pilot study	Published	Participated in study design, carried out participant recruitment, performed contrast-enhanced ultrasound studies, participated in date interpretation and drafted the manuscript
6	Contrast-enhanced ultrasound evaluation of renal microcirculation response to terlipressin in hepato-renal syndrome: A preliminary report	Published	Participated in study design, carried out participant recruitment, performed CEUS studies, participated in data interpretation and drafted the manuscript

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Acknowledgement

For all studies, Bracco (Milano, Italy) provided the contrast agent (Sonovue®) free of charge. Bracco Research (Geneva, Switzerland) provided the VueJect™ pump as well as Sonotumor Software free of charge. Both these companies were allowed to read draft of the manuscripts before submission but had no influence on their content or decision for submission.

Signed: Date: 15th November 2014

A. Schneider

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THESIS ABSTRACT

Background

Acute kidney injury (AKI) is a common and important complication of critical illness associated with increased morbidity, mortality and costs. Alterations in renal perfusion are thought to play a central role in its pathogenesis. This causal relationship remains, however, largely speculative as data on renal perfusion in AKI and critical illness are scarce. Indeed, methods enabling renal perfusion quantification are either invasive, expensive or inapplicable to critically ill patients.

Contrast-enhanced ultrasonography (CEUS) is a recent imaging modality which provides a unique means of visualizing tissue perfusion. Studies have suggested that CEUS could enable blood flow quantification. CEUS would be an ideal tool in the intensive care unit (ICU) where knowledge of renal perfusion could help prevent or manage AKI. However, CEUS was never used nor validated in the ICU and its clinical use remains to be established.

Hypothesis and specific aims

We hypothesized that evaluation of renal microcirculation with CEUS was feasible and could change medical management in the ICU.

To test this hypothesis, we have designed four studies with the following specific aims:

1. Validate preliminary results and improve technical measurements in a sheep model.
2. Demonstrate CEUS's feasibility and safety in the ICU. Obtain preliminary results of renal microcirculation modifications associated with elective cardiac surgery.
3. Evaluate the impact of CEUS measurements in two clinical situations:
    a. Circulatory failure requiring noradrenaline infusion
    b. Type-1 hepato-renal syndrome requiring terlipressin treatment

Overall methods and main results

1. In a sheep model in which renal blood flow could be modified pharmacologically and mechanically, we have compared CEUS-derived parameters to measurements of a an implanted flow probe. We found that CEUS derived parameters were highly heterogeneous and that they did not parallel changes in renal blood flow. Technical measurements were improved and simpler protocols designed for further studies.

2. We have performed CEUS studies before and after an elective cardiac surgery in twelve patients. This study was the first attempt to quantify renal microcirculation in critically ill patients and has demonstrated CEUS's safety and feasibility in ICU.

3. We have compared changes in CEUS-derived parameters under two levels of blood pressure (BP) in twelve patients with circulatory shock. We have established that microcirculatory response to an increase in BP as assessed by CEUS was highly heterogeneous and unpredictable. This finding is consistent with data from large trials and suggest that a CEUS-guided strategy to determine ideal target BP could be tested.

4. We have compared changes in CEUS-derived parameters before and after the administration of terlipressin in four patients with type-1 hepato-renal syndrome. We found dramatic changes in CEUS-derived parameters after terlipressin therapy and perfusion responses appeared variable. Larger studies are necessary to establish the sensitivity and specificity of CEUS to predict terlipressin responsiveness.

Conclusions

CEUS is safe and feasible in the intensive care unit. CEUS-derived perfusion indices might not fully correlate with renal blood flow as they are indicative of renal microcirculation changes. Further studies are required to establish the significance of CEUS-derived indices changes in clinical practice.

CHAPTER 1

INTRODUCTION: MEASUREMENT OF KIDNEY PERFUSION IN CRITICALLY-ILL PATIENTS

DECLARATION FOR THESIS CHAPTER 1

In the case of Chapter 1, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Performed the literature review. Drafted and revised the manuscript	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
M. Goodwin*	Critical review of manuscript	NA
R. Bellomo	Critical review of manuscript	NA

*: From the Department of Radiology, Austin Health, Heidelberg, Melbourne, Australia

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	Date: 15th November 2014
Main Supervisor's Signature	[redacted]	ate: 15th November 2014

1. INTRODUCTION: MEASUREMENT OF KIDNEY PERFUSION IN CRITICALLY ILL PATIENTS

1.1. INTRODUCTION

Acute kidney injury (AKI) is a major complication of critical illness [1] occurring in 30 to 40% of all critically ill patients and in its severe form requires renal replacement therapy (RRT), in approximately 5% of patients [2]. AKI has been shown to be an independent predictor for mortality [3] and is associated with invasive therapy and substantial costs [4].

Despite its importance, the pathophysiology of AKI is still poorly understood. AKI is most commonly associated with systemic diseases such as septic shock, major surgery and cardiogenic shock [1] but a specific mechanism causing AKI to develop in specific patients can rarely be determined. Since the early description of an "acute uremia syndrome" in crush injury victims during World War II [5,6] and its association with histopathological findings similar to those found in experimental renal artery ligation, ischemia or some form of alteration in renal blood flow (RBF) has been thought to play a pivotal role in the pathogenesis of AKI. This paradigm that essentially all AKI in critically ill patients is due to some form or degree of ischemia remains of continuing conceptual dominance to this day [7]. Despite such dominance, there are only very limited data supporting this concept. In a recent systematic review, Prowle et al [8] highlighted the extraordinary fact that RBF measurement, irrespective of the technique used, has only been reported in 46 critically ill patients (five studies) within the last sixty years. Thus, our knowledge, understanding, and theoretical constructs regarding global renal perfusion in critically ill patients with RRT-treated AKI (an estimated 5% of all ICU admissions for a total of approximately a quarter of a million such patients each year in developed countries alone) is, like an inverted pyramid, based on extremely weak evidence.

Furthermore, given the complex and heterogeneous nature of the renal vasculature, evaluating the flow in the main renal arteries (macro-circulation) might not provide sufficient information to adequately understand perfusion alterations in complex diseases such as septic or cardiogenic shock. Indeed, some pathophysiological processes might be associated with increased global RBF [9,10] despite loss of function. In such instances, there is experimental evidence that, at least in sepsis, this phenomenon might be caused by intra-renal shunting [11]. Hence, correlation between macroscopic RBF and function is far from being linear. Therefore, techniques allowing the study of microcirculatory parameters such as cortico-medullary perfusion ratio, regional tissue oxygenation measurement and assessment of their relative change over time might be more valuable in increasing our understanding of the pathophysiology of AKI.

In this state-of-the art review, we discuss the value, challenges, limitations, safety and feasibility of different techniques for the measurement of kidney perfusion in critically ill patients. The advantages of each technique will be weighed against its disadvantages in the context of the critically ill patient with AKI. Particular emphasis will be placed upon techniques enabling some evaluation of the microcirculation, because emerging experimental evidence suggests that these are the techniques that are the most likely to improve our understanding of the disease and help clinicians develop and apply physiologically logical interventions.

1.2. NON-IMAGING METHODS FOR RENAL BLOOD FLOW QUANTIFICATION

1.2.1. Microsphere deposition methods

Microsphere distribution methods 12-14 can provide very detailed information on regional perfusion between and within organs. After intra-vascular injection of radioactively labelled microspheres, their tissue deposition is proportional to blood supply. Several tracers can be

used to evaluate changes after pharmacological intervention. The tissue needs to be harvested and sectioned to allow measurement of radioactivity, which is proportional to the amount of microspheres deposited hence to the organ perfusion [15]. Results are typically reported in "flow per gram of whole tissue".

This method is obviously limited to animal research as it involves tissue collection. It is, however, very often used as a comparator in studies validating organ flow measurement.

1.2.2. Paraamino-hippurate (PAH) clearance

The classic physiological method to estimate renal plasma flow is by calculation of paraamino-hippurate (PAH) clearance. PAH is an amide derivative of the amino acid glycine and para-aminobenzoic acid. PAH is almost fully removed from the plasma during its first pass though the kidney. Its renal clearance can therefore be used as an estimate of the renal plasma flow (ERPF). Typically, this technique involves a bolus followed by a continuous infusion of PAH. PAH concentration is then measured in blood and urine samples. ERPF is then calculated using the classic clearance formula (RPF ~ $Cl_{PAH} = U_{PAH} \times V / P_{PAH}$).

Unfortunately, PAH is not used in clinical practice as the chemical analysis procedure is very cumbersome, not widely available and cannot provide information in "real time". Additionally, as they rely on urine concentration measurement, PAH clearance methods cannot be applied in oligo-anuric patients and its non-invasive use in AKI is therefore limited. On the other hand, PAH clearance can be used to measure ERPF for research purposes even in oligo-anuric patients. Such measurement, however, becomes invasive and requires renal vein cannulation and sampling.

1.2.3. Renal vein thermo-dilution methods

Renal thermo-dilution methods were first described in the 1970s [16,17], but gained some attention in critically ill patients more recently [18,19]. These methods involve the insertion of an

indwelling catheter into one of the renal veins. Renal blood flow calculation is based on measured changes in temperature of the renal vein blood after injection of a bolus of isotonic saline at room temperature [19]. Using such methods Ricksten et al. have conducted several elegant studies of global renal perfusion in cardiac surgery patients [20-22]. For instance, they have demonstrated [21] that restoring mean arterial pressure from 60 to 75 mmHg improved renal oxygen supply/demand relationship after cardiac surgery in patients with vasodilatory shock and AKI. This technique, however, remains highly invasive and, because of the risk of renal vein thrombosis, can only be applied for a limited period of time.

1.2.4. Xenon washout

Xenon washout techniques were used for research purposes in the seventies [23]. This invasive technique relies on intra-arterial injection of a radioactively marked tracer (Xe^{133}) and external counting with a scintillation probe. Mean renal blood flow is calculated from the initial slope of the disappearance curve.

This method was very useful to establish renal vasculature reactivity to different pharmacologic agents [24,25]. However, it has currently been replaced by less invasive, more precise imaging techniques.

1.2.5. Intravascular Doppler

Blood flow velocity can be evaluated invasively using an intra-arterial Doppler wire. This technique enables calculation of RBF provided that the diameter of the vessel can be estimated [26]. Accurate measurements can be made in small straight tubes (< 4.76mm) and if flow rate < 200 ml/min [27]. Its utility in humans, where typical RBF is in the range of 300 ml/min, is therefore limited. Similarly, this technique cannot account for the presence of collateral vessels and can therefore grossly underestimate flow.

Given these technical limitations and its invasiveness, intravascular Doppler is not currently applicable to critically ill patients even in the setting of a research protocol. It can be used as a comparator in animal study in order to validate newer methods.

1.3. NUCLEAR MEDICINE TECHNIQUES

1.3.1. Scintigraphy

Scintigraphy relies on the injection of radiolabeled isotopes and the capture of the emitted radiation by external gamma cameras generating 2D images. Using isotopes such as Iodine-131 (^{131}I) coupled to orthoiodohippurate (OIH), scintigraphy is able to accurately quantify RBF. This technique can be coupled with simultaneous measures of glomerular filtration rate (GFR) with the ability to determine relative function for each kidney.

Several isotopes have been used and although ^{131} I labeled OIH still represents the gold standard for RBF quantification, it is not used clinically as it is associated with high radiation exposure particularly in patients with renal failure. Therefore, 99mTc-mercapto-acetylglycyl-glycyl-glycine (^{99m}Tc-MAG3) is currently the marker of choice for this purpose [28,29]. This molecule is mainly excreted via tubular secretion at the distal part of proximal tubule with a very high first pass elimination [30]. Semi-quantitative measurement of RBF can be obtained by using the first-pass time-activity curve generated from a region of interest over the kidney [31]. Other techniques expressing RBF as a fraction of cardiac output have been proposed [32]. Because of its high and unpredictable (75-90%) plasma protein binding properties, ^{99m}Tc-MAG3 based techniques are not as accurate as those based on ^{131}I-OIH (75-90%) to estimate RBF [33]. In addition, ^{99m}Tc-MAG3 is not purely eliminated by the renal route and the percentage eliminated via the hepato-biliary pathway increases in renal dysfunction. Other technetium based molecules have been used [99mTc-Ethylene-l-dicysteine (99mTc-EC) or (99m)Tc-tricarbonyl nitrilotriacetic acid (99mTc(CO)3NTA)] [34] with lower hepato-biliary elimination and higher kidney to background ratio have been proposed.

Overall, 99mTc-diethylenetriaminepentaacetic acid (DTPA) is most commonly used for GFR measurement. It delivers slightly lower values as compared with standard inulin clearance but is easier to prepare and more readily available. However, the results generated by the so-called Gates methods [35] have not been shown to be superior to creatinine based formulas [36].

Renal scintigraphy finds most of its clinical application in renal transplantation medicine. In this context, it can help differentiate acute tubular necrosis from transplant rejection [31]. In AKI, renal scintigraphy is usually of little value for the identification of the mechanism of renal dysfunction, although some patterns can be identified (ureteric obstruction, ischemia) [37]. It can be used to determine the symmetry of the disease and provide information on organ size and overall perfusion. Unfortunately, it does not provide information on intra-renal flow distribution.

1.3.2. Positron emission tomography (PET)

Positron emission tomography (PET) scans rely on the injection of a positron emitting isotope tracer such as 18-Fluorine in the most commonly used PET tracer 18-F fluorodeoxyglucose (FDG). Many other isotopes emit positrons, though, including Rubidium-82, 11-Carbon and 15-O labeled H_2O. The emitted positron interacts with an electron, emitting a pair of high-energy photons in the annihilation process. These photons are detected by scintillators in the scanner generating 3D images. PET can be coupled with computed tomography (CT) and can then provide significantly better spatial resolution than scintigraphy alone.

Two fundamental approaches have been proposed to measure renal perfusion with PET: dynamic imaging following bolus injection and static imaging at steady state using an ultra-short-lived tracer. The first technique makes use of a highly extracted blood flow tracer such as rubidium-82 [38]. This method has been used in animal models of renal artery obstruction, occlusion and reperfusion [39]. The second technique makes use of ultra-short-lived tracers such as oxygen-15-labelled water (half life of 2 min) [40]. This was studied by Juillard et al [41], who

showed an excellent correlation with microsphere techniques in an animal model and by Alpert et al [42] who used O-15 water to measure RBF in healthy volunteers. Other potential applications include measurements of RBF in renovascular disease, in rejection or acute tubular necrosis of transplanted organs, in drug induced nephropathies, ureteral obstructions before and after revascularisation and before and after placement of ureteral stents [40].

Despite its theoretical large potential, there has been very little use of PET in functional imaging. More importantly, its use in critically ill patients is likely to be very limited even for research purposes. Indeed, PET protocols are long and require extensive mobilisation, which, in unstable patients is often labour intensive and can be associated with safety hazards. This adds to the general limitations associated with the difficulty of producing and handling non-standard tracers, their cost and the requirement for many of them for a nearby cyclotron (because of their extremely short half life). Finally, PET involves radioactivity exposure (although minimal), which might make acceptance by ethics committee or next of kin or ICU staff more difficult in the context of a clinical study.

1.4. MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging has gained immense popularity over the last decades as it allows generation of very high-resolution images including 3D reconstructions, without ionising radiation. Imaging is based on the imaging of protons, using measurement of a radiofrequency signal emitted by protons regaining their thermodynamic equilibrium after their spins have been aligned in a large magnetic field. Protons in different tissues return to their equilibrium state at different relaxation rates.

Within the numerous MRI techniques available, some enable RBF quantification and, importantly, some enable a degree of parametric mapping of intra-organ blood flow distribution or tissue oxygenation. These properties make MRI very appealing to study renal perfusion alterations in critical illness. Unfortunately, these approaches are likely to be limited

to research protocols as they involve a lengthy and potentially hazardous transfer, important costs and are limited by the availability of MRI machines.

1.4.1. Contrast-enhanced MRI modalities

MRI based perfusion studies have classically been based on contrast-enhancement by gadolinium-based solutions. Gadolinium agents produce contrast on MRI scans because gadolinium is a paramagnetic substance, which therefore has a marked local effect on the speed at which adjacent protons return to their thermodynamic equilibrium. Fast acquisition techniques allow sufficient temporal resolution to monitor intra-renal signal changes during first pass of the agent. Approaches have been described [43] enabling quantification of absolute cortical and medullary perfusion. This would make gadolinium based MRI technology of great interest for the assessment of renal perfusion.

Unfortunately, the discovery of nephrogenic systemic fibrosis and its probable association with gadolinium accumulation in renal failure (acute or chronic) [44] greatly limits its interest in AKI and critically ill patients. The warnings for gadolinium contrast agents have recently been updated and three agents are currently contra-indicated in patients with AKI or with chronic renal impairment and a GFR< 30 ml/min. The other agents do not have this contraindication although extensive precautions are still advised. [45].

Newer contrast agents based on ultra-small particles of iron oxide (USPIO) molecules have been presented and seem to be safe and potentially useful for RBF measurement [46]. However, their safety profile is not yet fully established and their clinical role is still to be established in particular since other MRI techniques, which do not need intravenous contrast agents, are now available.

1.4.2. Cine phase contrast MRI

Cine phase contrast MRI is an MR angiographic technique that allows measurement of renal flow in both renal arteries without a contrast agent. Central to the technique is the fact that protons that are moving along the direction of a magnetic field gradient receive a phase shift proportional to their velocity: static protons are unaffected and receive no phase shift, but moving protons will have their phase changed. The amplitude of this change is dependent of the velocity of the proton. Phase contrast MRI has been very well validated to measure aortic flow rate but less so for renal arteries because of their small size and issues related to respiratory movements [47-49] (Figure 1.1).

RBF measurements by cine phase contrast MR are well correlated with simultaneous PAH clearance measurement [50] and the results are reproducible [51]. King et al [52] used it as a tool to predict clinical response after percutaneous angioplasty in renal artery stenosis.

This technique has recently been used in critically ill patients to determine RBF in sepsis [53]. To the best of our knowledge, this study was the first to measure global RBF non-invasively in critically ill patients with sepsis associated AKI. Values for RBF varied markedly from 392 to 1337 ml/min (normal values range according to size and cardiac output from 800 to 1200ml/min). This study provided a clear demonstration that, in septic critically ill patients with AKI, RBF can range from low to supranormal and confirmed what animal studies had long suggested: septic AKI is not a uniform disease and is not reliably associated with decreased RBF (so-called ischemia). Even more importantly and in keeping with experimental observations, these values correlated well with the patient's cardiac index and renal vascular resistance but not with the patient's GFR. This clear dissociation between global perfusion and global function is important because it implies changes in microvascular perfusion (shunting or changes in intra-glomerular pressure dynamics or both).

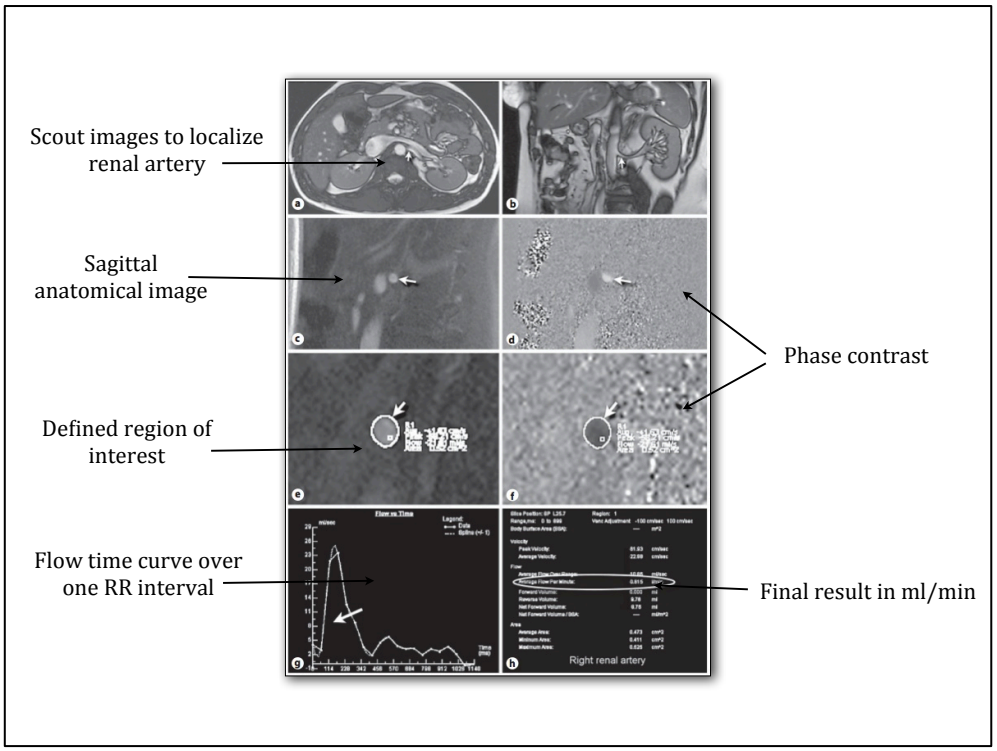


Figure 1.1: Cine phase contrast imaging. (A) and (B): initial acquisition of scout images enabling localization of renal arteries (white arrows). (C): sagittal image of the renal artery on phase compensated image (white arrow) (D): phase contrast image of the renal artery (white arrow) at the same level. (E)&(F): magnified views of (C) and (D) where the investigator has placed a region of interest over the renal artery. (G): time intensity curve for one RR interval H: the area under the curve multiplied by the heart rate approximates the renal blood flow.
Adapted with permission from Prowle JR, Molan MP, Hornsey E, Bellomo R: Cine phase-contrast magnetic resonance imaging for the measurement of renal blood flow. Contrib Nephrol 2010, 165:329-336.

1.4.3. Arterial spin labelling

Arterial spin labelling (ASL) is a MRI modality typically used for cerebral perfusion studies [54]. In an analogy with cine phase contrast techniques, ASL uses blood as an endogenous contrast agent. Blood flowing towards a tissue is selectively labeled to have an opposite magnetization compared to this tissue. A perfusion-weighted image can be produced by subtracting an image in which inflowing spins have been labelled from an image in which spin labelling has not been used (Figure 1.2). [55].

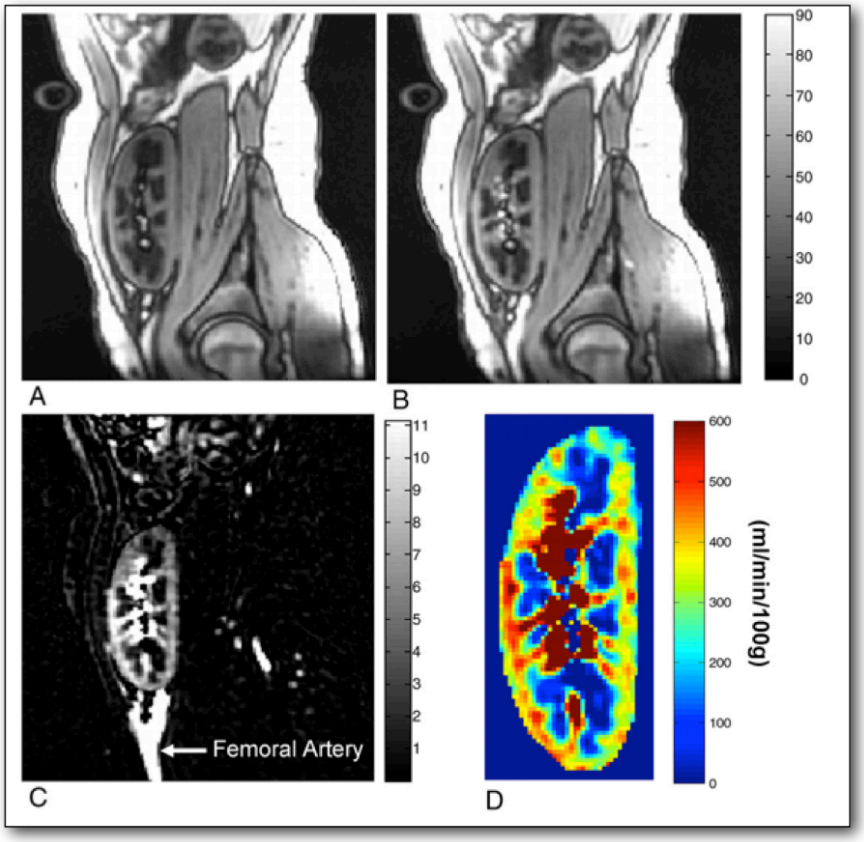


Fig 1.2: Arterial spin labelling in a transplant kidney: Tagged image: moving spins have been labeled (A), control image B) and difference between A and B representing perfusion weighted image (C) and resulting perfusion map (D) shown in units of ml/min per 100 g. (adapted with permission from Artz et al: Arterial spin labeling MRI for assessment of perfusion in native and transplanted kidneys. Magnetic resonance imaging 2011, 29(1):74-82).

ASL allows imaging of the renal arteries despite their complex orientation [56]. A fairly good correlation of ASL with PAH clearance has been reported [57] and some applications after renal transplantation or in renal artery stenosis [55,58] have been proposed. One of the main interests of ASL is its ability to draw parametric maps of relative perfusion (Figure 1.2) allowing clinicians to study geographical intra-organ differences in perfusion as opposed to overall organ blood flow. All pixels in a specific tissue can be averaged to provide mean perfusion. Such maps could enable the study of differential perfusion between cortex and medulla in critical illness and to identify ischemic and hyperaemic areas within the kidney.

1.4.4. Blood Oxygen Level Dependent (BOLD) MRI

BOLD MRI takes advantage of the different magnetic properties between oxygenated and deoxygenated haemoglobin. Oxyhaemoglobin (the principal form found in arterial blood) has no major magnetic properties, but deoxyhaemoglobin is strongly paramagnetic, generating local magnetic field inhomogeneities corresponding to an increase in relaxivity defined as R2*. The amount of deoxyhaemoglobin functions as a biologic contrast agent and can be related to the strength of R2* weighted pulse sequences. BOLD MRI generates images whose signal intensity is a reflection of tissue metabolism representing balance between O2 consumption and delivery. Relatively low spatial resolution is a problem inherent to the technique.

BOLD enables the generation of parametric maps (Figure 3) of oxygenation in the kidney as illustrated by Textor et al [59] in kidneys with renal artery stenosis. This technique has been used by Warner et al [60] to demonstrate an increase in tissue oxygenation after administration of diuretics particularly in the medullary areas and confirmed by implanted O2 probes. Similarly, Prujim et al [61], showed an increase in medullary oxygenation in healthy volunteers after a decrease in their salt intake.

Although not directly a measure of RBF, BOLD MRI might deliver valuable information as it delivers data integrating O2 delivery and consumption.

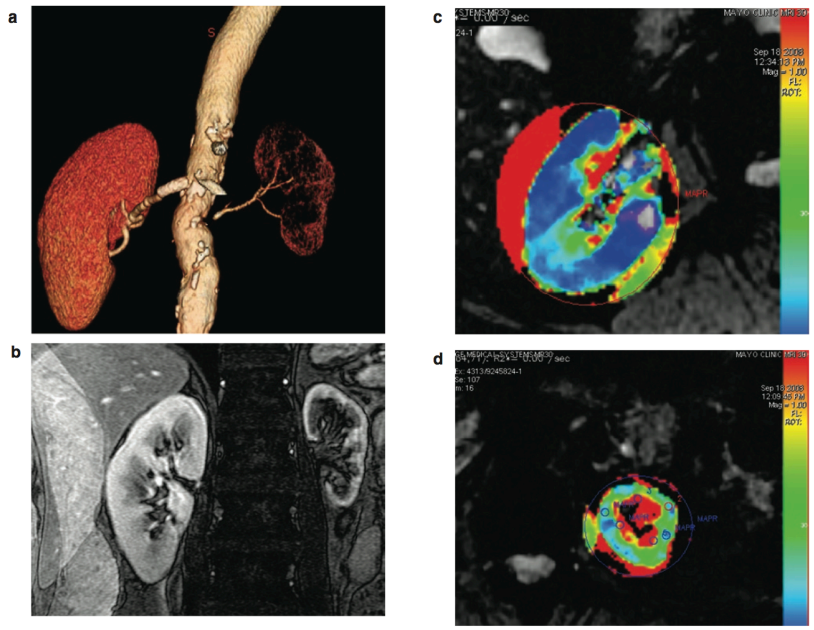


Figure 3: BOLD MRI in severe occlusive disease of the left kidney:
T2 imaging by CT angiogram (A) and T2 MR (B) demonstrating severe renal-artery stenosis (L kidney) and presence of a stent in the contralateral kidney. (C) and (D) :Parametric maps of R2* (reflecting the dexyhemoglobin level) from BOLD MRI in the same patient. In the right kidney (normal, well-perfused), most of the cortex shows low R2* signal (blue) and there are some small areas with high R2* signal in the medulla (red). Conversely, in the left kidney (vascular compromise), there are higher levels of cortical R2* indicating the presence of more deoxygenated blood and a large, deep area with high R2* signal (deoxygenated haemoglobin) in the medulla (with permission from Textor et al Renovascular hypertension and ischemic nephropathy: state of the art. Am J Hypertens. 2010;23:1159–69).

Although the various MRI-based techniques discussed above offer promise in our ability to investigate changes in renal perfusion in critically ill patients, it is difficult to imagine how they could be widely applied at this stage. The MRI environment is hostile to the critically ill and carries some significant safety concerns during transport and during a prolonged period in the magnet. In addition, obtaining high quality MRI scans in the critically ill is also often very challenging. The high cost adds a further degree of difficulty, which makes repeated assessment logistically very difficult.

1.5. ULTRASONOGRAPHY

Ultrasonography is the most commonly used imaging modality in the initial evaluation of patients with acute or chronic kidney diseases. It is widely available, easy to use, free of complication and can be performed at the bedside.

Standard US provides information on kidney size (a small kidney suggests possible atrophy in the context of CKD, a large kidney might suggest the presence of infiltrative disease), cortical thickness and echogenicity and enables imaging of the excretory tract to diagnose outflow obstruction. In AKI, however, standard US examination is normal most of the time. Assessment of renal perfusion by ultrasound can be approached by Doppler techniques or with microbubble-based contrast agents (contrast enhanced ultrasound).

1.5.1. Doppler ultrasound

Conventional US can be enhanced by using the Doppler effect. The Doppler effect occurs because the frequency of a reflected sound wave changes on whether it is moving towards the ultrasound probe or away from it. The speed and direction of flow in a specific scanned volume can be calculated. Doppler ultrasound enables the generation of time-velocity curves from which peak systolic and end-diastolic velocities can be obtained. Based on these values different indices can be calculated and associated with a measurement of the renal artery diameter RBF can be estimated. The most commonly reported index is called resistive index (RI). RI is calculated according to the formula:

RI= (peak systolic velocity – lowest diastolic velocity)/ peak systolic velocity.

These measurements, however, have several limitations: measurements are sensitive to numerous parameters such as vessel stiffness, heart rate (increased rate over-estimated end diastolic velocity, heart rhythm (difficult to obtain reliable values during atrial fibrillation), external compression by transducer (in particular in a transplanted kidney) or Valsalva manoeuvres that can decrease flow velocity [62]. These indices were poorly correlated with invasive measurement of RBF in a sheep model [63]. However, Lerolle et al have demonstrated

that Doppler indices on admission could predict AKI in critically ill patients [64]. Unfortunately, because of the way it is calculated, an increased RI may indicate the presence of increased renal vascular resistance with decreased flow or the presence of normal renal resistance with increased flow or even the presence of decreased resistance with markedly increased flow. Ureteric obstruction also significantly affects measurement of RI. The RI alone is therefore easily confounding.

US-Doppler studies are routinely performed during the follow-up of renal transplant where the absence of a decrease of RI could be a sign of early rejection [65].

Altogether, US-Doppler has many advantages, it is non-invasive, can be performed at the bedside and can be repeated to evaluate changes after an intervention. However, Doppler US is inherently both patient and operator-dependent. Its overall reliability and the relationship between derived indices and renal perfusion require further investigation.

1.5.2. Contrast-enhanced ultrasound

Gases are ideal contrast agents for ultrasonography as they are highly compressible and their density is 1000 less than blood. Embedded within a shell they can be made to form microbubbles [66], which are extremely potent ultrasound reflectors. Microbubbles change shape when they interact with ultrasound waves resulting in the generation of non-linear signals. Microbubbles can be obtained rapidly by agitating saline. Such microbubbles are used to diagnose right to left shunt during cardiac echocardiography. However, these bubbles are very heterogeneous in size and shape and their half-life is very short and they can be associated with cerebral ischemic events [67].

In the last decade, commercial preparations of contrast agents for ultrasounds have become available. These agents demonstrate increased stability and have uniform sizes. Microbubbles found in commercial preparations of ultrasound contrast agents (UCA) have very uniform sizes about that of a red blood cell. This property enables the bubbles to circulate through the

pulmonary capillaries, hence to be visualised in arterial beds. Although some initial concerns were raised, UCA can now be considered safe after post-marketing experience from over 1 million patients has been reported [68,69].

Blood flow quantification using CEUS has been first described by Wei et al [70] in a canine model. The same technique was used by Kishimoto et al to measure RBF demonstrating a good correlation with changes in RBF as estimated by PAH clearance [71]. Schwenger [72] and Benozzi [73] et al demonstrated that CEUS was able to distinguish acute rejection from acute tubular necrosis. Another study, in healthy volunteers demonstrated [74] that CEUS was able to detect a 20% decrease in renal blood flow as induced by an angiotensin II infusion. Above all, CEUS can provide real time visualisation of renal microcirculation. Since it is very well tolerated and can be applied on the bedside, it could in theory be used to determine changes in microcirculation after therapeutic interventions. This would enable us to better understand the intra-renal microcirculatory changes following our common interventions and potentially drive our practice in patients at risk of AKI. As an example, as illustrated in figure 4, CEUS was able to confirm a strong microcirculatory response to terlipressin in a patient with hepato-renal syndrome.

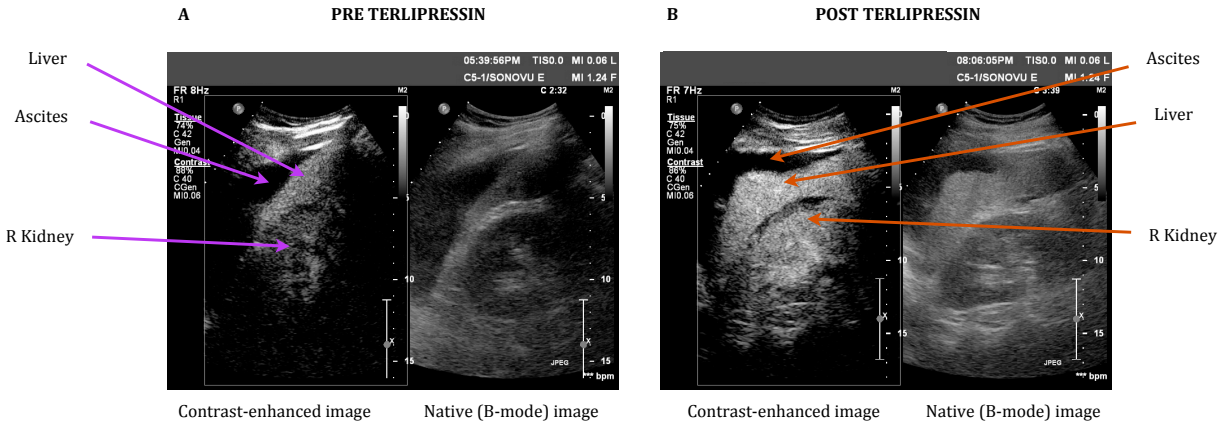


Figure 4: Example of microcirculatory changes as seen with contrast-enhanced ultrasound Images taken during a contrast-enhanced ultrasound study performed on a patient with chronic liver disease and hepato renal syndrome. The image is centered on the patient's right kidney. Two images are shown, the first (A) was taken just before the intravenous administration of 1mg of terlipressin and the second (B) 2 hours after. This study demonstrates increased renal perfusion in response to the drug administration, as indicated by brighter signal within the renal cortex on the right image.

CEUS although in its early stages of validation, seems to be a promising technique to evaluate renal perfusion in critical illness. Indeed, it can be performed at the bedside, is minimally invasive and safe. CEUS provides information on microcirculation and potentially could improve our understanding of flow alterations in critical illness associated AKI.

1.6. CONCLUSIONS

Assessment of RBF is important but difficult in AKI. Most techniques are not applicable at the bedside and require extensive patient manipulation, which, in the critically ill patient greatly reduces the practical applicability of any given technique. Furthermore, most techniques only enable global organ flow estimation while information on the microcirculation is perhaps more likely to be useful in achieving an understanding the pathogenesis of AKI. CEUS is the first technique to overcome most of these limitations. CEUS may soon play a significant role in our ability to investigate microcirculatory changes in AKI.

CHAPTER 2

CONTRAST-ENHANCED ULTRASONOGRAPHY: A PROMISING TECHNIQUE TO ASSESS RENAL PERFUSION IN THE ICU

DECLARATION FOR THESIS CHAPTER 2

In the case of Chapter 2, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Performed the literature review. Drafted and revised the manuscript	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Lynne Johnson*	Critically reviewed the manuscript	NA
Mark Goodwin*	Critically reviewed the manuscript	NA
Anthony Schelleman*	Critically reviewed the manuscript	NA
Rinaldo Bellomo[3]	Critically reviewed the manuscript	NA

* From the Radiology department, Austin Hospital, Heidelberg, Victoria, Australia

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	Date: 15th November 2014
Main Supervisor's Signature	[redacted]	Date: 15th November 2014

2. CONTRAST-ENHANCED ULTRASONOGRAPHY: A PROMISING TECHNIQUE TO ASSESS RENAL PERFUSION IN THE ICU

Acknowledgments:

The authors want to thank Michel Schneider, Marcel Arditi and Nicolas Rognin from Bracco Research Geneva SpA for their precious help and collaboration.

2.1. INTRODUCTION

Acute kidney injury (AKI) is common and severe in critically ill patients with a reported incidence between 1 and 30% and mortality between 28 and 90%, depending on the definition used. Uchino et al [1] in a recent multicenter study involving nearly 30'000 critically-ill patients reported an incidence of 5.7% and a mortality of 60.3%. In patients with multiple organ failure, acute kidney injury is an independent predictor of mortality [3,75].

The pathophysiology of AKI remains poorly understood. However, as renal function is intimately linked to organ blood flow, alterations in renal perfusion are considered key to the pathophysiology of AKI. Little, however, is known about renal perfusion in critically ill patient [8] or about its association with AKI. This is because methods currently available to assess and monitor renal perfusion are either inaccurate or not rapidly applicable in routine ICU patients. The physiological gold standard to estimate renal plasma flow is the calculation of Para-Amino-Hippurate (PAH) clearance. Unfortunately, this technique is inaccurate in the presence of oliguria [8,76]. Doppler ultrasound (US) studies have been demonstrated to be inaccurate in estimating renal blood flow [63] and only provide information about flow in main arteries. In addition, all methods only give information about global organ perfusion and not about the renal microcirculation or on the intra-renal distribution of blood flow.

Imaging methods such as scintigraphy or magnetic resonance imaging (MRI) are much more accurate and can provide valuable information on kidney perfusion.[49,77] However, their use in the ICU is limited by equipment availability, costs and their requirement for extensive and prolonged patient manipulation, which is associated with risk and major logistic challenges. These techniques can be used in research protocols but are not suitable for routine use in most ICU patients and cannot be repeated several times within the same day.

A method allowing reproducible renal perfusion quantification that would be applicable at the bedside, and would be minimally invasive or even non-invasive would be ideal in the ICU. Such a method might increase our knowledge of the correlation between renal perfusion alterations and AKI. It could also potentially help clinicians detect patients at risk of renal failure and adapt treatment early to prevent AKI.

2.2. CONTRAST ENHANCED ULTRASOUND (CEUS)

2.2.1 Ultrasonography

Ultrasonography is an ultrasound-based diagnostic imaging technique. It relies on the property of sound waves to reflect at interfaces of media of different densities as they travel through them, the greater the difference in density (acoustic impedance), the more echogenic the interface [78,79].

Modern ultrasound equipment is portable, reasonably cheap and allows bedside examination without requiring patient manipulation. Standard ultrasound examinations are already performed in the ICU for diagnostic reasons (abdominal or liver studies, cardiac echography) and to guide interventions (central venous line placement, pleural effusion drainage). Such equipment is becoming widely available and echography is now a standard technique within most modern intensive care units [80].

To evaluate circulation and blood flow, Doppler studies can be performed with standard echography equipment. The clinical use of Doppler studies is however limited by the lower

limit of detection, its inability to detect slow flow velocity and limited accuracy in quantifying renal blood flow. Microbubble based contrast agents have lowered the detection threshold and now allow detection of blood in vessels as small as capillaries. Together with appropriate imaging modes and modern software, CEUS allows organ blood flow quantification.

2.2.2. Microbubbles based contrast agents

Gases are ideal contrast agents for echography since they are highly compressible (17'000 times more than water) and since their density is 1'000 times less than the density of blood [81]. Embedded within a shell, gases can be made to form microbubbles, which are extremely potent ultrasound reflectors, nine orders of magnitude higher than a solid article of the same size. These microbubbles change shape when they interact with ultrasound waves, contracting during the compression (high pressure) phase and expanding during the rarefaction (low pressure) phase. At low-intermediate acoustic pressure, these microbubbles oscillations result in the generation of non-linear signals [82].

The first reported echographic contrast agent was based on air microbubbles created by agitating saline, which was then rapidly injected intravenously. It was used to examine the right heart and detect right-to-left shunts. The clinical use of agitated saline is however very limited due to the very short life (a few seconds) of air microbubbles and their inability to traverse the pulmonary circulation. Safety concerns were also recently raised, cerebral ischemic events being reported after use of agitated saline, probably related to the broad and uncontrolled size distribution of such bubbles [67].

Technological improvements have resulted in the development of small-sized shell-stabilized microbubbles with adequate half-life and the capacity to cross the pulmonary circulation (Figure 2.1 and 2.2).

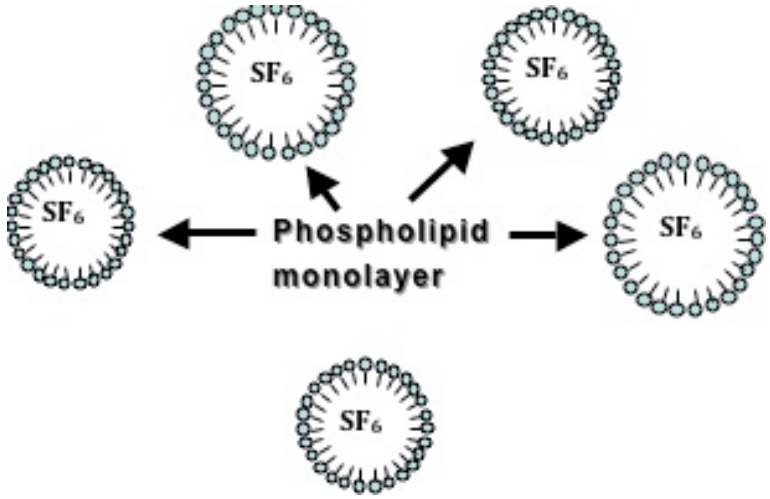


Figure 2.1: Schematic illustration of a microbubble contrast agent: Sonovue (courtesy of Bracco SpA)

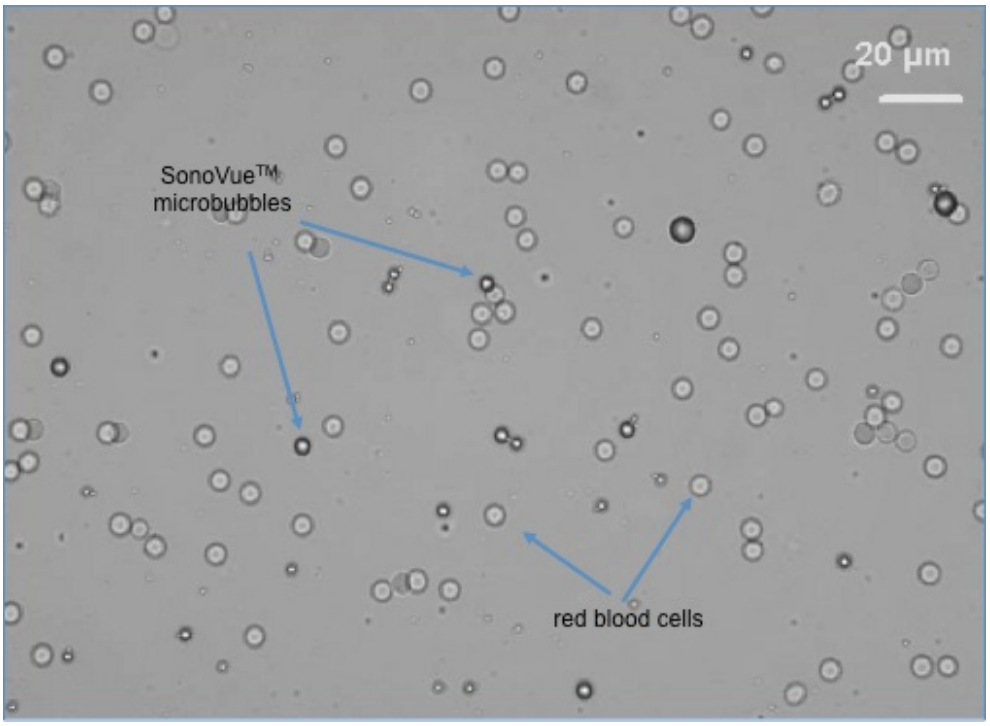


Figure 2.2: Optical microscopic view of microbubbles in rabbit blood (courtesy of Bracco SpA)

The first commercially available US contrast agent was Albunex® (Molecular Biosystems Inc, USA). It was obtained by sonicating a 5% human albumin solution in the presence of air resulting in the formation of air microbubbles stabilized by a thin denaturated albumin shell. [83]. Current (second) generation microbubble contrast agents make use of inert, poorly soluble perfluorinated gases and are stabilized by phospholipids or albumin [84].Their half-life in the circulation is a few minutes and the perfluorinated gas present in the microbubbles is totally excreted by the lungs [85].

After intravenous injection, microbubbles behave as pure blood agents as their size (1-6 µm) prevents them from diffusing through the endothelium. They allow vascular bed opacification and have opened the way to detecting the microcirculation [86].The different commercially available US contrast agents and their composition are detailed in Table 1.

CONTRAST AGENT	SHELL	GAS	REGISTERED IN
Optison	Human albumin	Perfluoropropane	USA, Canada
Definity (Luminity)	Phospholipids	Perfluoropropane	USA, Europe, Canada
Sonovue	Phospholipids	Sulfur hexafluoride	Canada, Europe, China, India, Korea
Sonazoid	Phospholipids	Perfluorobutane	Japan

Table 1: Commercially available ultrasound contrast agents

2.2.3. Optimal US Equipment settings

Parallel to the development of efficient contrast agents, novel imaging modes have been introduced. Harmonic B mode, phase or pulse inversion, power modulation, power pulse inversion, and coherent pulse sequencing are some of the contrast-specific imaging modes (also designated as “nonlinear” imaging modes) available in modern US equipment [87]. Based on the non-linear properties of microbubbles submitted to acoustic pressure, these imaging

modes use a combination of changes in pulse phase and amplitude to selectively minimize tissue echoes and enhance ultrasound contrast agent echoes. They all make use of low mechanical index (MI) imaging or intermittent imaging or both, since microbubbles can be destroyed when subjected to high acoustic pressures (e.g. MI > 0.7). This unique property is used for perfusion quantification in the so-called destruction (flash) refilling approach.

Today, contrast-specific modes are available on most mid- to high-end US equipment.

2.3. SAFETY

As for any other drug, ultrasound contrast agents have been submitted to extensive clinical investigations both for safety and efficacy, before approval by national health authorities.

Since microbubbles of gas are injected into the circulation, legitimate concerns about tissue embolism can be raised, especially since, as discussed earlier, initial attempts by agitated saline may have been associated with embolic events [67]. However, the microbubbles in the commercial US contrast agents have a much smaller and uniform size than those produced by agitating saline. They are also much more stable and do not coalesce. Therefore, they have a very low potential for embolization. This has been confirmed by intravital microscopy in the cremaster or spinotrapezius muscle with different contrast agents [88-90]. These studies show that the microvascular rheology of the US contrast agents are similar to that of red blood cells and that microbubble entrapment within the capillaries is negligible and transient.

Initial post-marketing surveillance over five years and more than 1 million patient studies have demonstrated no medically significant risk other than allergic events, which appear to occur at a rate of approximately 1 per 10,000 [91]. Central nervous system reactions have also been rarely reported and may or may not be associated with hypersensitivity reactions. Reported adverse events are generally infrequent and mild and may include headache, fatigue, palpitations, nausea, dizziness, dry mouth, altered sense of smell or taste, dyspnea, urticaria, pruritus, back pain, chest pain or rash.

In October 2007, following 4 deaths in patients with severe underlying conditions one to twelve hours after injection of Definity®, a "black box warning" stating that use of these agents was contraindicated in unstable patients was released by the American FDA. This initiated an intense controversy. Many clinicians still remained convinced that ultrasound contrast agents were safe.

Since then, many studies including one in a very large number of patients have been published establishing CEUS as a safe procedure. For example, Kusnetzky [92] demonstrated that the background mortality of patients undergoing contrast echography was not significantly higher than that of patients undergoing non-contrast echocardiography. Dolan [69] studied 42,408 patients from three centers in the US that had received contrast agents for either resting or stress echocardiography and did not find any difference in mortality or adverse events compared to matched controls,. A multicenter registry including 58,254 hospitalized patients that underwent echocardiography published by Main [68] actually showed a decrease in acute mortality as compared with patients not receiving contrast agent. Wei and al [93] reported a rate of severe reactions of 0.01% and no death in 78,383 patients including 10,000 acutely ill patients (either in the ICU or with acute chest pain of possible cardiac origin) who had received US contrast agents.

Today, many investigators believe that US contrast agents can be considered safe even in unstable patients, even though the FDA has not yet withdrawn the black box warning. As for any drug or contrast agent, the risk of anaphylactic reaction remains present and the use of these products in unstable patients should be restricted to centers with full resuscitation capacities.

As discussed in the next paragraph, the blood flow quantification requires use of high mechanical index US for very short period of time (flashes). Some concerns have been raised about the safety of this procedure. Jimenez [94], showed in a porcine model that repeated insonification of the kidney at high MI did not produce any histological change neither

immediately after the procedure nor 4 hours later. There was in particular no signs of inflammatory response and no signs of extravasation of erythrocyte from the capillary system.

2.4. BLOOD FLOW QUANTIFICATION BY CEUS

Since microbubbles remain confined to the intravascular space, and have a rheology similar to that of red blood cells, contrast uptake as a function of time can be used to estimate quantitative perfusion parameters, such as regional blood volume or blood flow.

2.4.1. Theories and methods

Perfusion quantification by CEUS may be performed with a microbubble-destruction technique, introduced in 1998 by Wei et al [70,95]. This technique was validated in a canine model with intermittent imaging with destructive frames at increasing imaging frame rates, microbubbles being continuously infused by means of a syringe pump. This allowed investigators to build a curve representing replenishment kinetics, from a series of clips at different frame rates. Fitting of this curve allows derivation of two relative parameters representing perfusion in the tissue: the regional blood volume (plateau value) and blood velocity (initial slope of the replenishment curve).

The local blood flow, *F*, is thus the product of microbubble velocity by regional blood volume.

$$F \propto A \cdot \beta$$

Where A corresponds to the plateau signal intensity and β is the initial slope of the replenishment curve. Although these data were derived from the myocardium, the authors stated that this method was applicable to any tissue accessible to ultrasound.

This technique was later extended to non-destructive imaging at low Mechanical Index (MI) with the application of a few destructive frames at higher MI to completely destroy the microbubbles in the scan plane [96]. The reperfusion (or replenishment) of microbubbles in the scan plane at low MI is recorded as a single clip, and analyzed using the Wei et al. model to derive perfusion parameters.

Another formal approach to the estimation of flow, developed by Arditi et al. [97] for the low MI imaging approach, allows improved perfusion estimates by considering an echo power signal and taking into account the ultrasound beam geometry. This approach was recently implemented in the form of a prototype software (Bracco Research SpA, Geneva, Switzerland) for off-line processing of refilling sequences. Using this software, video data are first linearized to compute an echo-power signal whose amplitude is proportional to the local contrast agent concentration. As described in the approach by Tiemann et al. [96], fitting of these signals after destruction allows perfusion quantification. Here, the perfusion parameters considered are: relative blood volume (rBV), mean transit time (mTT), and blood flow (rBV/mTT) (Fig. 2.3). The software further allows the generation of parametric maps, showing the spatial distribution of perfusion parameters at the pixel level. This approach establishes a basis for extracting information on the perfusion of vascular beds in vivo, and allows relative quantification between selected areas of interest (AOIs), provided that appropriate instrument calibration is implemented for the data linearization phase.

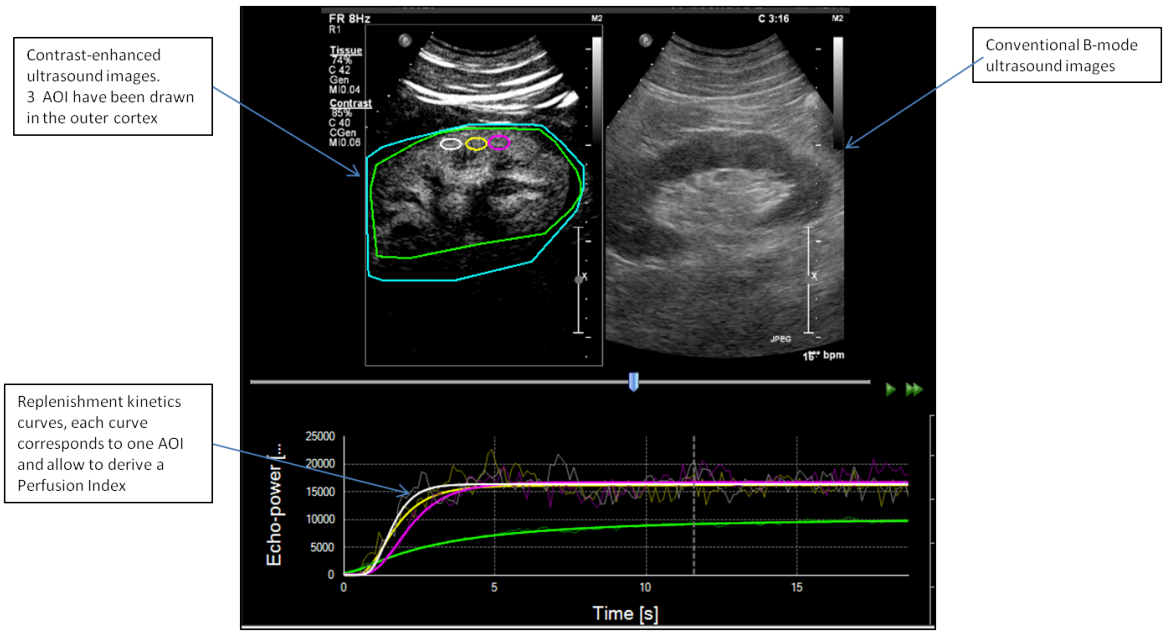


Figure 2.3: Renal perfusion index measurement using dedicated quantification software: Screenshot of Sonotumor™, shown as an example of software allowing perfusion quantification in CEUS sequences. The upper segments show the contrast-enhanced images (left) as well as the conventional US images (right). This is where the reader can draw area of interest (AOI) that will be analysed by the software. A replenishment curves (lower segment) is then generated for each AOI. These curves represent the intensity of the echo-power as a function of time after the flash. Bold lines are fitted curves of the actual measured data represented by the clear lines. The fitted curves allow the derivation of a perfusion index (PI) for each AOI.

2.4.2. Clinical applications

Several authors have subsequently used these methods and a general good agreement has been reported in several organs and tissues.

For example, Rim and al. [98] were able to quantify cerebral blood flow in dogs at baseline and after cerebral blood flow alteration through hypo or hypercapnia. A good correlation was found between A x β derived parameters and cerebral blood flow as measured by an accepted reference method (radiolabeled microspheres).

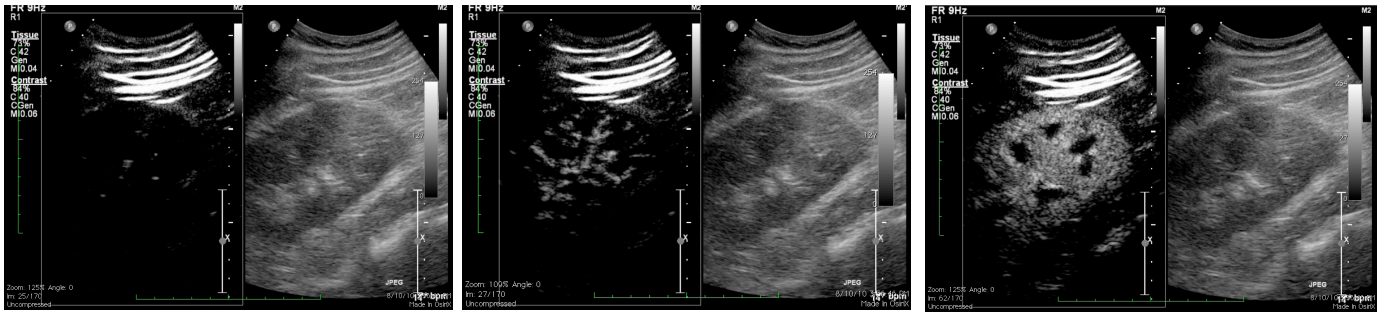
Vogel and al. [99] were able to quantify myocardial perfusion in humans. In their study, a linear correlation was found between myocardial CEUS derived parameters and PET perfusion data in healthy volunteers at rest (N=15) and during adenosine-induced hyperemia (N=5). These investigators also compared CEUS with intracoronary Doppler measurements before and

during intracoronary adenosine injection in patients undergoing coronary angiograms and found good agreement with coronary flow velocity reserve.

As far as renal perfusion is concerned, Kishimoto and al. [71] found a congruent modification of microcirculation with an infusion of dopamine in 9 healthy subjects. They subsequently used the same technique [100] to study to effect of Valsartan on renal perfusion in 7 healthy volunteers and found a significant increase in microbubble velocity after oral administration of Valsartan which correlated well with the increase in total renal blood flow determined by PAH clearance ($p < 0.05$). In a recently published study [101], Kalantarinia and Wei tested the utility of CEUS to monitor the expected increase in renal blood flow after a high protein meal in healthy adults. They found a statistically significant increase (by 42.8%) in renal blood flow ($A \times \beta$ parameter) compared with baseline ($p=0.002$).

We recently performed a study in 10 healthy volunteers evaluating changes in the perfusion index (PI) a variable which is proportional to blood flow) seen during intravenous infusion of angiotensin II and after oral captopril. We found a statistically significant and dose dependant decrease in PI during increasing doses of ATII as compared to baseline. The decreases in PI were already detectable when the renal plasma flow (as estimated by PAH clearance) decreased by 15% [102].

To further illustrate the feasibility of CEUS in ICU, we present the example of a 66 year-old-man recently studied in our center. This patient had a past medical history of non-insulin dependent diabetes mellitus, hypertension and hypercholesterolemia. He was complaining of chest pain and an angiogram revealed a severe triple vessel disease. He was scheduled for an elective CABG. We performed a renal CEUS before and after the cardiac surgery. The contrast agent injections were perfectly tolerated. The images could be acquired in less than 15 minutes including the post-operative study, which was performed within one hour of admission to ICU. Figure 2.4 presents some still images illustrating the destruction-refilling sequences in that patient.



4a 4b 4c

Figure 2.4: Example of destruction refilling sequences obtained in a 60 year old male patient, 1 hour after coronary artery bypass surgery: Each part of the figure is divided in two; the left parts show contrast specific images and the right parts standard B mode images. After the destruction flash (4a left), no signal is detectable in the contrast specific image (ie all the microbubbles have been destroyed). 5 seconds after destruction (4b left), partial replenishement of the main arteries with contrast can be noticed. 10 seconds post destruction, the kidney is fully replenished with contrast (4c left). No significant changes are observed in B-mode images (4a-c right)

Interestingly, we noted a decrease in the perfusion indices from a baseline of 10523 (before the surgery) to 7786 (-26.0%, table 2). Clinically, a transient period of oliguria was noted around 12 hours post surgery and the plasma creatinine concentration increased from 79 mcmol/l to 155 (RIFLE F). With adequate fluid resuscitation and furosemide administration, the urinary output finally normalized but the plasma creatinine concentration remained elevated on hospital discharge (121 mcm/l).

		Pre-op		Post-op		Difference
			Average		Average	
Mean transit time (mTT) (seconds)	AOI 1	1.51		2.06		
	AOI 2	1.86	1.91	2.03	2.09	+ 9.5%
	AOI 3	2.35		2.18		
Relative blood volume (rBV)(a.u)	AOI 1	20’073		16’896		
	AOI 2	19’309	20’100	16’574	16’239	- 19.2%
	AOI 3	20’920		15’248		
Perfusion index (PI) (=RBV/mTT)(a.u)	AOI 1	13’293		8’202		
	AOI 2	10’381	10’523	8’164	7786	- 26.0%
	AOI 3	8’902		6’994		

Table 2 : CEUS derived parameters in a 60 year old patient before and after CABG surgery complicated by AKI stage I: The mean transit time (or the « speed » of replenishment) increased by close to 10% after surgery as compared to baseline. In the same time, the relative blood volume (or echo density) decreased by almost 20%. Altogether, the resulting perfusion index dropped by 26%, This indicates diminished renal perfusion. RBV is a measure of pixel luminance and is proportional to local contrast agent concentration. This value does not have physical unit (a.u.= arbitrary units), hence is not comparable from one US machine to another, but its modifications are proportional to changes in contrast agent concentration. The software applies a linearization of the video data to generate a number proportional to the concentration of contrast. This operation aims to reverse the log compression applied by all US machines and is based on the pixel luminance. The luminance observed in each pixel is transformed by a program that attributes a value between 0 and 255 to each pixel. This value is then squared to reflect signal power. Possible values are between 0 and 255^2. These values don't have physical units but are proportional to the local concentration of contrast agent. In terms of absolute number, the alues are not comparable between different systems (US machines) or different settings of a same system, but these values’ kinetics are respected.

2.4.3. Experience in transplant medicine

In renal transplant medicine, a detailed evaluation of blood flow in the sub-capsular capillaries is highly desirable since the latter are primarily involved in acute rejection. Fisher and al. [103] examined 32 patients, 5 to 7 days after kidney transplantation and were able to show that a temporal difference in the contrast agent arrival slopes between two main territories allowed

the differentiation of acute graft rejection from an normal clinical course (where the slopes were uniform).

In 26 transplant patients, Schwenger et al. [72] reported a highly significant correlation (p=0.0004) between renal blood flow as estimated by CEUS and serum creatinine, In the same study, these investigators found that the determination of renal blood flow by CEUS reached a higher sensitivity (91 vs. 82%) specificity (82% vs. 64%) and accuracy (85 vs 73%) for the diagnosis of Chronic Allograft Nephropathy than conventional Doppler US. These findings where confirmed by Benozzi et al [73] who performed CEUS in 39 kidney recipients at 5, 25 and 30 days after grafting. These researchers were able to show that some CEUS derived parameters allowed the distinction between acute tubular necrosis and acute rejection episodes (the cortico-medullar ratio of the RBV and mTT were lower in the acute tubular necrosis group as opposed to the control group, while another parameter, the time to peak (TTP) was higher than control in acute rejection events).

2.5. PERSPECTIVES: CEUS IN AKI

CEUS is able to determine and quantify changes in renal perfusion and these changes play a role in the clinical outcome as illustrated by the important findings in transplant medicine. In the critically ill patient, alterations in renal perfusion are expected to be found in various situations such as septic shock, low cardiac output states, hepato-renal syndrome, and hypovolemia. However, it is still unclear whether these alterations are significant and related to renal outcome, whether they assist in diagnosis and early intervention or whether they represent the consequence rather than the cause of tubular injury. Given such uncertainty and the need to make rapid and repeated measurements early in the course of a patient's illness, CEUS could become an important tool to evaluate and quantify renal perfusion alterations in these different pathologic conditions. More importantly, CEUS might enable the study of severity, timing, and change over time of renal perfusion as well as the intra-renal distribution of perfusion abnormalities. In a second step, these observations might help draw a link

between perfusion abnormalities as shown by CEUS derived parameters and AKI and, and establish therapeutic targets and surrogate markers of adequate renal resuscitation. Finally, CEUS could be used to evaluate the renal perfusion effect of several of the hemodynamic interventions applied to patient care within the ICU.

However, in order to develop and validate this CEUS approach (as done in transplant medicine), specifically designed studies need to be performed in the setting of the ICU to investigate the various CEUS derived parameters in the main syndromes known to be associated with AKI and to develop and validate CEUS derived indices as it was done in transplant medicine. The characteristics of CEUS appear to make this approach uniquely possible.

2.6. CEUS IN ICU

CEUS is particularly well designed for use in the ICU. It combines the advantages of being fast, safe, non-invasive and repeatedly applicable at the bedside. Second generation ultrasound contrast agents are safe and well tolerated. This should allow repeated scans throughout the day to monitor the evolution of renal perfusion and response to treatment. The kidney is relatively easy to scan in the supine position and obtaining good quality images is relatively easy in most ICU patients. The technology allowing renal perfusion evaluation is still under improvement but our initial experience shows that CEUS can detect a 15% decrease in renal blood flow. This level of sensitivity is probably well above the value of changes expected to occur during the major hemodynamic events/syndrome known to be associated with AKI [8]. Thus, it seems unlikely that false negative examinations would occur with this technology.

2.7. CONCLUSION

CEUS is a safe, non-invasive and reliable technique. In many ways, it is ideally designed to monitor renal blood flow in ICU patients. Studies in renal transplant patients have shown its potential utility in clinical practice. Similar studies should now be performed in ICU patients to

determine whether CEUS parameters predict or facilitate the early diagnosis of AKI and whether they can help assess the impact of therapeutic interventions in real time. CEUS would then logically help identify patients at risk of AKI at an earlier time and allow clinicians to adapt therapy to optimize renal perfusion and perhaps prevent AKI.

CHAPTER 3

CONTRAST-ENHANCED ULTRASOUND EVALUATION OF RENAL MICROCIRCULATION IN SHEEP

DECLARATION FOR THESIS CHAPTER 3

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution
Participated in study design, performed contrast-enhanced ultrasound studies together with TH and TS, participated in data interpretation and drafted the manuscript.	60%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution
Paolo Calzavacca[1]	participated in study design performed the animal preparation together with CM, was in charge of anaesthesia maintenance and study drugs administration. Drafted the relevant section in the method section of the manuscript and critically reviewed the manuscript.	NA
Anthony Schelleman[2]	participated in study design, performed contrast-enhanced ultrasound studies together with AS and TH and critically reviewed the manuscript.	NA
Tim Huynh[2]	participated in study design, performed contrast-enhanced ultrasound studies together with AS and TS critically reviewed the manuscript.	NA
Michael Bailey	participated in study design, performed statistical analyses and critically reviewed the manuscript	NA
Clive May[1]	participated in study design, performed the animal preparation together with PC and critically reviewed the manuscript	NA
Rinaldo Bellomo	participated to the study design, data interpretation and critically reviewed the manuscript.	NA

1. Florey Institute of Neuroscience and Mental Health, University of Melbourne, VIC, Australia.
2. 4: Radiology department, Austin Health, Heidelberg, Victoria, Australia

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature		Date: 15th November 2014

Main Supervisor's Signature		ate: 15th November 2014

In chapter one and two, we have reviewed the different technologies currently available to evaluate and quantify renal blood flow and presented CEUS as a promising tool for this purpose in critically ill patients.

The degree of validation of CEUS for renal perfusion evaluation remains low and new studies are required to establish its role in the intensive care unit. CEUS is commonly performed by radiologists to characterise focal liver nodules and sometimes by cardiologists to improve echocardiographic image quality in otherwise non-echogenic patients. These indications do not involve blood flow quantification. In addition, clinical experience with CEUS is limited in Australia in particular because ultrasound contrast agents are only available in limited quantities.

Therefore, before moving on to clinical studies involving critically ill patients, we felt that a preliminary animal study would be necessary. This study should involve a model in which renal blood flow could be easily and accurately evaluated in order to offer a strong gold standard to which CEUS measurements could be compared to. Perfect correlation between the two measurements was not expected as CEUS would provide a measurement of micro-circulation and not of macrocirculation. However, such study would provide an occasion to refine technical measurements and improve our understanding and mastering of the technique.

The study presented in chapter 3 has been designed and conducted in collaboration with the Howard Florey institute. It involves a sheep model that was developed there and previously used in numerous studies. In this model, a Doppler flow probe is implanted around the renal artery. Such probe provides measurement of renal artery blood flow in real time and with high accuracy. We have used pharmacological agents as well as mechanical means (peri-arterial occluder) to increase or decrease renal blood flow as measured with the flow probe. CEUS was then performed under these different flow conditions.

3. CONTRAST-ENHANCED ULTRASOUND EVALUATION OF RENAL MICROCIRCULATION IN SHEEP

Acknowledgements

Bracco (Milano, Italy) provided the contrast agent (Sonovue®) free of charge.
Bracco Research (Geneva, Switzerland) provided the VueJect™ pump as well as Vuebox® Software free of charge. Both these companies were allowed to read draft of the manuscript before submission but had no influence on its content or decision for submission.

Sources of Support

This work was supported by National Health and Medical Research Council of Australia (NHMRC) (Project Grant 1009280) and the Victorian Government's Operational Infrastructure Support Program. C.N. May was supported by a NHMRC Research Fellowship.

3.1. INTRODUCTION

Acute kidney injury (AKI) is a common and important complication of critical illness associated with increased morbidity, mortality and costs [1,104]. Alterations in renal perfusion are thought to play a central role in its pathogenesis. This causal relationship remains, however, largely speculative as data on renal perfusion in AKI and critical illness are scarce [8]. Moreover, methods enabling renal perfusion quantification are either invasive, very expensive or difficult to apply in critically ill patients.

Contrast-enhanced ultrasonography (CEUS) is a recent imaging modality which provides a unique means of visualizing tissue perfusion. Several studies have suggested that CEUS could enable the quantification of blood flow in an organ [70,95]. These techniques have been used in the brain [98], myocardium [99] and, to some degree, in the kidney [71,105,106]. CEUS would be an ideal tool in the intensive care unit because it is safe [68], and applicable on the bedside [107].

Indeed, knowledge of renal perfusion changes after an intervention has the potential to greatly influence medical management in critical illness. However, challenges remain before renal perfusion quantification with CEUS can be used in clinical practice and further studies are required to validate its measurements.

In this study we aimed to estimate renal cortical microcirculatory changes by CEUS in during pharmacologically or mechanical induced modifications of renal blood flow (RBF).

3.2. METHODS

3.2.1. Animal preparation

Experiments were conducted on 6 adult (1 to 2 years of age) Merino ewes. The experimental procedures were approved by the animal experimental ethics committee of the Florey Institute of Neuroscience under guidelines laid down by the National Health and Medical Research Council of Australia.

All animals underwent two sterile procedures under general anaesthesia at two weeks intervals and a similar two weeks recovery period was allowed before the experiment was undertaken. For all procedures, anaesthesia was induced with intravenous sodium thiopentone (10–15 mg/kg) for intubation followed by maintenance with oxygen/air/isoflurane (end-tidal isoflurane, 1.6–2.0%), peri-procedural antibiotic prophylaxis (procaine penicillin, Troy Laboratories Ptd Ltd, Smithfield, NSW, Australia or Mavlab, Qld, Australia) was administered and post-surgical analgesia maintained with intramuscular injection of flunixin meglumine (1 mg/kg) (Troy Laboratories or Mavlab, Qld, Australia) before and 24h following surgery. Of note, the last exposure to NSAID was at least two weeks prior to the experiment.

During the first procedure, a carotid arterial loop was created to facilitate subsequent arterial cannulation and a transit-time flow probe (20mm, Transonics Systems, Ithaca, NY, USA) was

implanted in the pulmonary artery through left side thoracotomy. During the second procedure, a transit time flow probe (4 mm) and a renal artery occluder (4 mm, IVM, In Vivo Metric, Healdsburg, California, USA) were placed around the left renal artery.

The day before the experiment, cannulae were inserted into the carotid arterial loop for continuous arterial pressure monitoring and into a jugular vein for drugs infusions. Analog signals (mean arterial pressure (MAP), heart rate, cardiac index (CI) and RBF)) were collected on computer using a customized data-acquisition system (Spike2; CED; Cambridge, UK). The data were continuously recorded at 100 Hz and averaged every minute during experiments. To prevent major interferences with ultrasound equipment, RBF acquisition was interrupted during CEUS scans.

3.2.2. Experimental protocol

To prevent pseudo-anaphylaxis and secondary pulmonary hypertension in response to ultrasound contrast agents (UCA) administration which was observed in other species [108,109], pre-medication with dexamethasone (DBL®, Hospira, Wasserburg, Germany) 0.5 mg per kg was administered two hours before the administration of the UCA.

General anaesthesia was induced and maintained as described above. At least 15 minutes were allowed to stabilization of all parameters before RBF manipulations were started. Each intervention was preceded by 5 minutes of baseline haemodynamic data collection. After each intervention, a recovery period of at least 15 minutes was allowed.

Three interventions were performed to manipulate RBF. RBF was decreased using a continuous infusion of angiotensin II (AngII, Hypertensin Ciba™, Ciba-Geigy, Basel, Switzerland) with rates set to target a 25% (AngII low) and 50% (AngII high) decrease in RBF. Second, a mechanical decrease of RBF was achieved using the renal artery vascular occluder. The level of inflation of the occluding device was titrated to aim for a 25% (Occl

25%) and a 50% (Occl 50%) decrease in RBF as measured in real time by the transit-time flow probe. Finally, 25 mg of captopril (Captopril, E.R. Squibb & Sons, Princeton, New Jersey, USA) was given as a bolus with the intent to increase RBF. The two interventions aiming at decreasing RBF (AngII administration and occlusion) were performed in a random order by the investigator in charge of anaesthesia maintenance (PC). Captopril administration was always performed last.

At each study time, once hemodynamic parameters (RBF, MAP and CI) had stabilized, a CEUS scan of the left kidney (detailed procedure infra) was performed.

3.2.3. CEUS procedure

For this study, we used Sonovue® (Bracco, Milano, Italy) as an UCA. The UCA was infused into a central vein using a dedicated syringe pump (VueJect®, Bracco Research, Geneva, Switzerland). Low mechanical index (MI=0.06) ultrasound of the left kidney was performed with a Philips IU22® ultrasound machine and a C5-1® 5 MHz probe. A long axis view of the kidney was obtained by placing the transducer probe over the lower back of the animal. Once adequate images of the kidney were obtained, UCA infusion was started at 1 ml/min. Image depth, focus, gain and frame rate were optimized at the beginning of each experiment and held constant during the study. After a two minutes period required to obtain a steady state, five consecutive destruction/refilling sequences (with 15s refilling time) were obtained [66,110]. Destruction was obtained by applying a flash of increased ultrasound intensity (5 pulses with high mechanical index (> 1.0).

3.2.4. Sequence analyses

Ultrasound data sets were exported in a digital imaging and communication in medicine (DICOM) format and analysed offline using VueBox® (Bracco Research, Geneva, Switzerland), a dedicated software package. An example of offline analysis is presented in Figure 1. Suboptimal sequences with inadequate contrast enhancement or excessive (or off

plane) movement artefact were excluded. For each sequence, one region of interest (ROI) was drawn. In order to minimize the influence of local perfusion heterogeneities, this ROI was drawn so that it enclosed the largest area of visible renal cortex on the surface of the kidney closest to the ultrasound probe. When parametric map depicted high heterogeneity in the area, the ROI was adapted to exclude non representative areas. For instance, cortical areas that were only intermittently visible because of breathing or other artefacts and areas representing blood vessels transections were not included in the ROI.

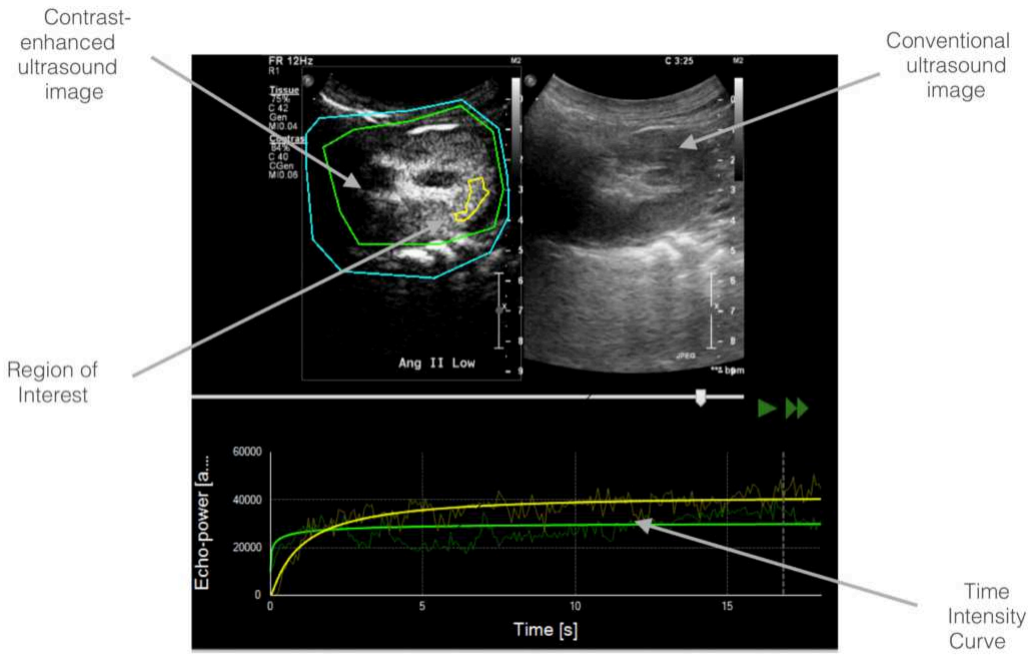


Figure 3.1: Software analysis data: Top left panel shows contrast-enhanced ultrasound images with region of interest drawn (yellow line). Top right panel shows conventional (B mode) imaging for anatomical localisation. Bottom panel shows linearised time intensity curve for the region of interest (yellow curve) and overall image (green curve_not relevant for perfusion quantification).

The software generates linearized time intensity curves from which mean transit time (mTT) and relative blood volume (RBV) parameters are computed. These parameters have been described in detail elsewhere [66,97]. In brief, RBV is proportional to the local fractional blood volume as well as to the contrast agent concentration and is expressed in arbitrary units (AU). The mTT is a measure of time needed to replenish the imaging plane with fresh bubbles

following destruction by ultrasonic flash, and is inversely proportional to blood flow velocity ; mTT is expressed in seconds. A perfusion index (PI) is obtained by calculating the ratio RBV / mTT. PIs are thought to be proportional to perfusion within a region of interest and are expressed in AU.

For each subject and study time, the median value for interpretable measurements was considered for analysis. Results from CEUS examination are reported as mean value and as percentage changes from nearest baseline for RBV, mTT and PI. Given the expected inter-observation variability and based on previous research [105], a change of more than 25% between two measurements was considered to be significant.

3.2.5. Repeat baseline measurement

As per study protocol, CEUS baseline measurements were obtained at the start of the experiment and were only repeated in case of significant modification of RBF or other hemodynamic parameter as compared with baseline values. Therefore, a baseline measurement was not repeated before each series of occlusions and before captopril administration. In such cases, and in cases where baseline measurement were discarded for poor quality, changes in CEUS derived parameters were compared to the nearest baseline available.

3.2.6. Statistical analysis

RBV, mTT and PI are reported as median (interquartile range). In addition, as baseline values are known to be highly heterogeneous due to inter-subject variability (organ depth, subcutaneous thickness and composition), we report changes in those values as median percentage change (range) from nearest baseline, each animal being its own control. Analyses were performed using SPSS® version 21 (IBM, Armonk, NY, USA). All outcomes were assessed for normality and as RBV, mTT and PI were all well approximated by log-normal distributions, each was log-transformed prior to analysis. RBF measurements were found to

be normally distributed. A two-sided p-value of 0.05 was considered to be statistically significant

3.3. RESULTS

3.3.1. Renal blood flow

As shown in Figure 3.2, both pharmacological and mechanical interventions were associated with the expected, proportional and significant changes in renal blood flow (RBF).

Target reductions in RBF were obtained in all animals with AngII infusion. Overall, RBF decreased from a baseline of 147 (± 29) ml/min to 112 (±19) (AngII low, $p<0.001$)) and to 96 (±22) ml/min (AngII high, $p<0.01$).

Mechanical occlusion of the renal artery induced a 25% reduction in RBF in all animals and a 50% reduction in 4/6 animals. Overall, such occlusion was associated with a decrease in RBF, from a baseline of 177 (± 34.9) ml/min to 128 (± 25.5) ml/min (Occl 25%, $p<0.001$) and to 83 (± 17.2) ml/min (Occl 50%, $p<0.001$). Finally, the administration of captopril was associated with an increase in RBF from 172 (± 44) ml/min to 206 (± 50.9) ml/min ($p<0.05$).

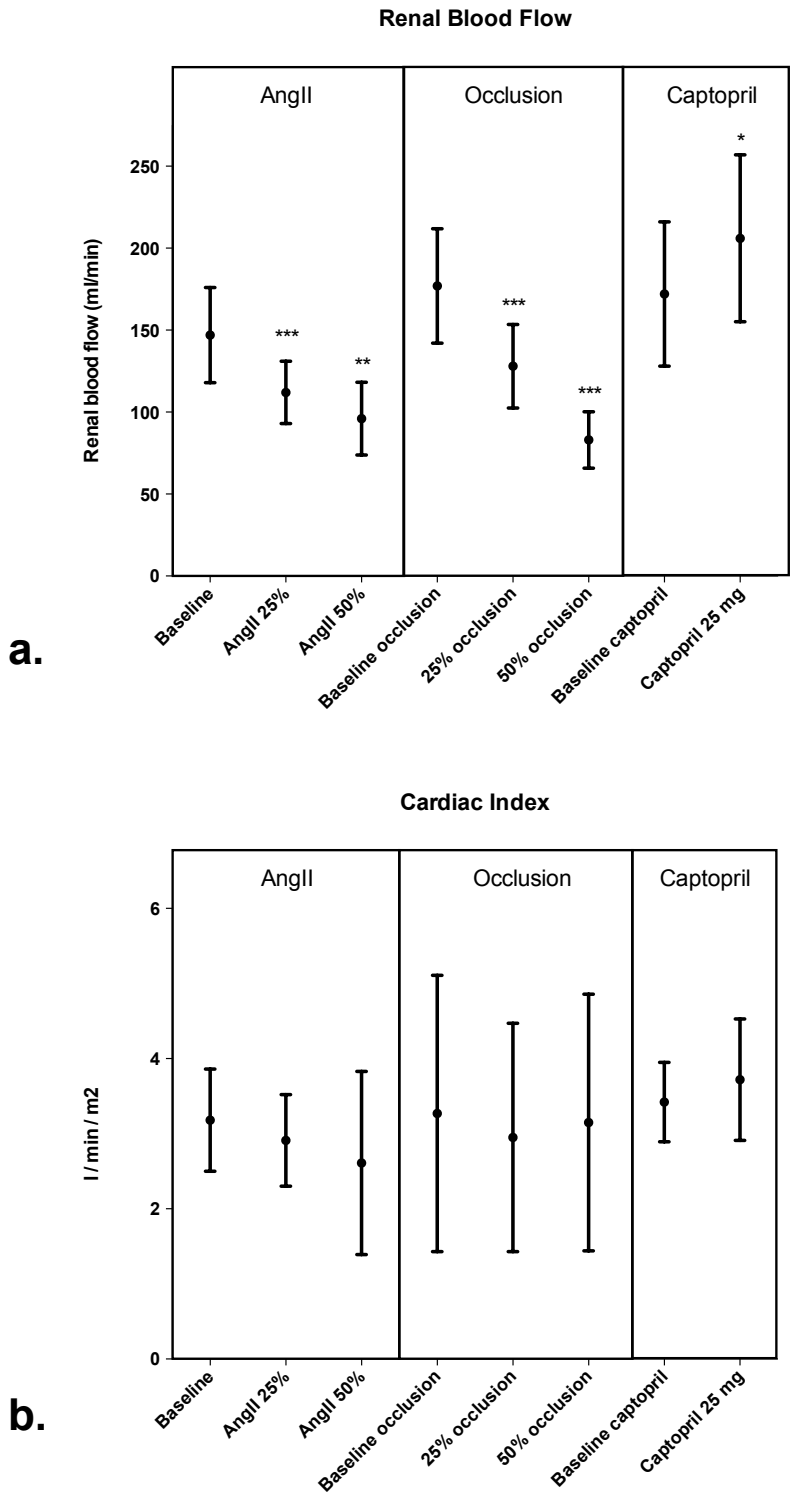


Figure 3.2: Changes in renal blood flow as measured by implanted transit-time Flow probes

Renal blood flow as measured by implanted transit-time flow probes. Cardiac index as measured by pulmonary artery catheter. Presented values are means +/- standard deviation (error bars).

AngII: Angiotensine II. NB: 50% occlusion was obtained in 4/6 animals

* p value <0.05 ** = p value < 0.01 *** = p value < 0.001

3.3.2. Pooled CEUS derived parameters

Median CEUS derived parameters are reported in Figure 3.3.

Overall, most differences were found to be non-significant. The only two changes in CEUS derived parameters which reached statistical significance were a decrease in RBV associated with a 25% reduction of RBF with mechanical occlusion and an increase in mTT associated with the high dose of AngII that caused a 50% reduction in RBF.

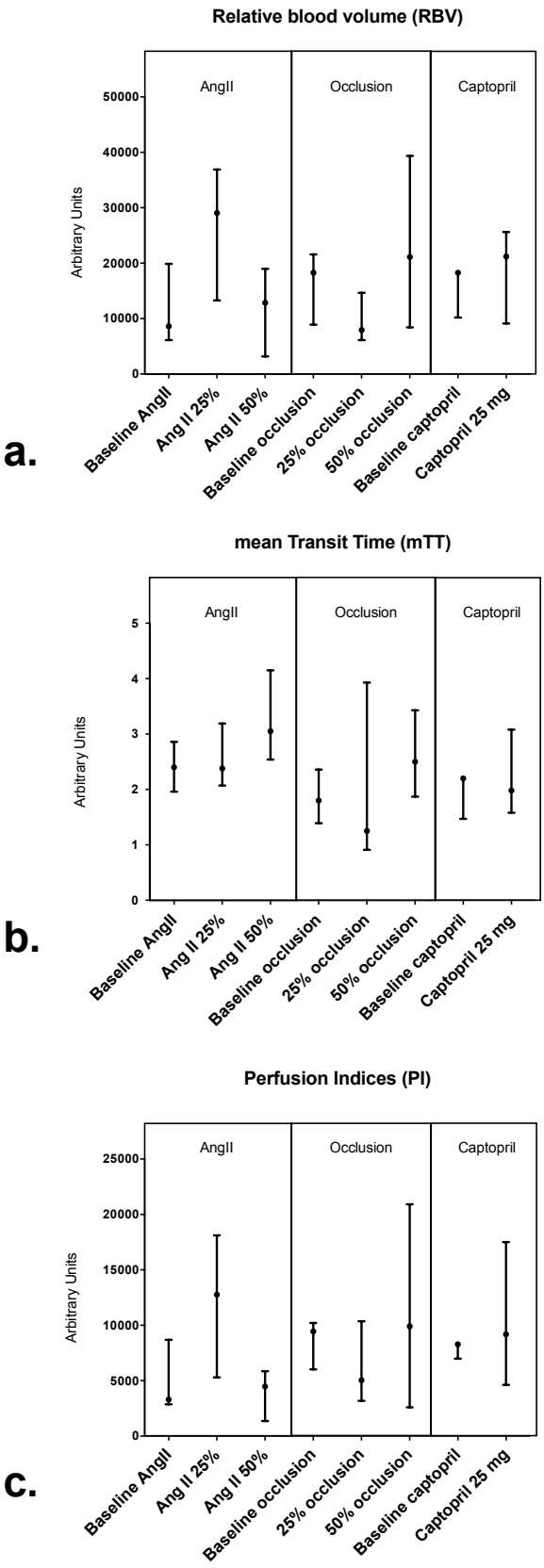


Figure 3.3: Mean CEUS derived parameters: a. Relative blood volume (RBV), b. mean Transit Time (mTT), c. Perfusion Indices (PI) Presented values are median and interquartile range (error bars) NB: Interpretable baseline measurement available in 4/6 (Occlusion and AngII) and 4/6 (captopril) animals. AngII: Angiotensine II * p value <0.05

3.3.3. Values expressed as percentage change from the nearest baseline

Values of PI expressed as a percentage change from nearest baseline (using each individual animal as its own control) are presented in Figure 3.4.

Angiotensin II infusion

A 25% reduction in RBF as induced by AngII infusion was associated with a median 89% increase in RBV (range: 56% decrease to a 152% increase), a 19% median increase in mTT (range: 6% decrease to 32% increase) resulting in an overall 62% median increase (range: 68 decrease to a 167 increase) in PI.

A 50% reduction in RBF as induced by AngII infusion was associated with a median 47% increase in RBV (range: 84% decrease to 119% increase), a 43% (range: 40 to 99%) increase in mTT resulting in an overall 5% increase in PI (range: 92% decrease to a 53% increase).

Mechanical occlusion

A 25% reduction in RBF as induced by occluder inflation was associated with a median 32% decrease in RBV (range: 31% to 47%), a 33% decrease (range: 45% decrease to a 18% increase) in mTT resulting in an overall 2% decrease (range: 43% decrease to 2% increase) in PI.

A 50% reduction in RBF as induced by occluder inflation was associated with a median 48% increase in RBV (range: -53% decrease to a 178% increase), a 25% increase in mTT (range: 10% decrease to a 55% increase) resulting in an overall 67% increase in PI (range: 63% decrease to a 120% increase)

Captopril

The administration of captopril was associated with a median 5% increase in RBV (range: 7% decrease to a 67% increase), a 7% increase in mTT (range: 16% decrease to a 26% increase) resulting in an overall 8% decrease in PI (range: 25% decrease to a 101% increase).

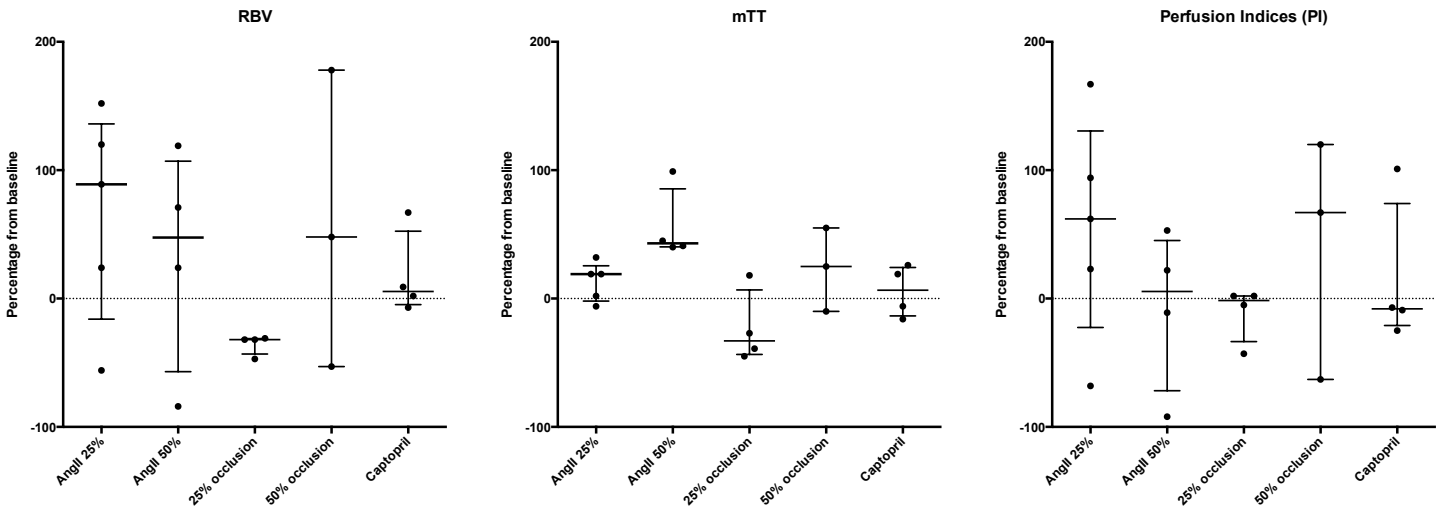


Figure 3.4: Changes in perfusion indices expressed as percentage of nearest baseline
Each datapoint corresponds to one subject.
Horizontal line bars correspond to median values and error bars to interquartile range.

3.3.4. Data consistency

Missing values

Four baseline values were not repeated (as per study protocol) and a 50% reduction of RBF could not be obtained in two situations. In addition, CEUS data were judged as not interpretable at four time points (two baselines, one during AngII low dose infusion and one during 25% occlusion). Hence, altogether, valid results were obtained in 38/48 (79.9%) of study time points. Most (5/10) missing values occurred during the occlusion phase of the experiment.

In-between animals consistency

Individual animal data are presented in Figure 3.5. There was poor consistency for derived CEUS-PI's between animals and heterogeneous response to RBF manipulations.

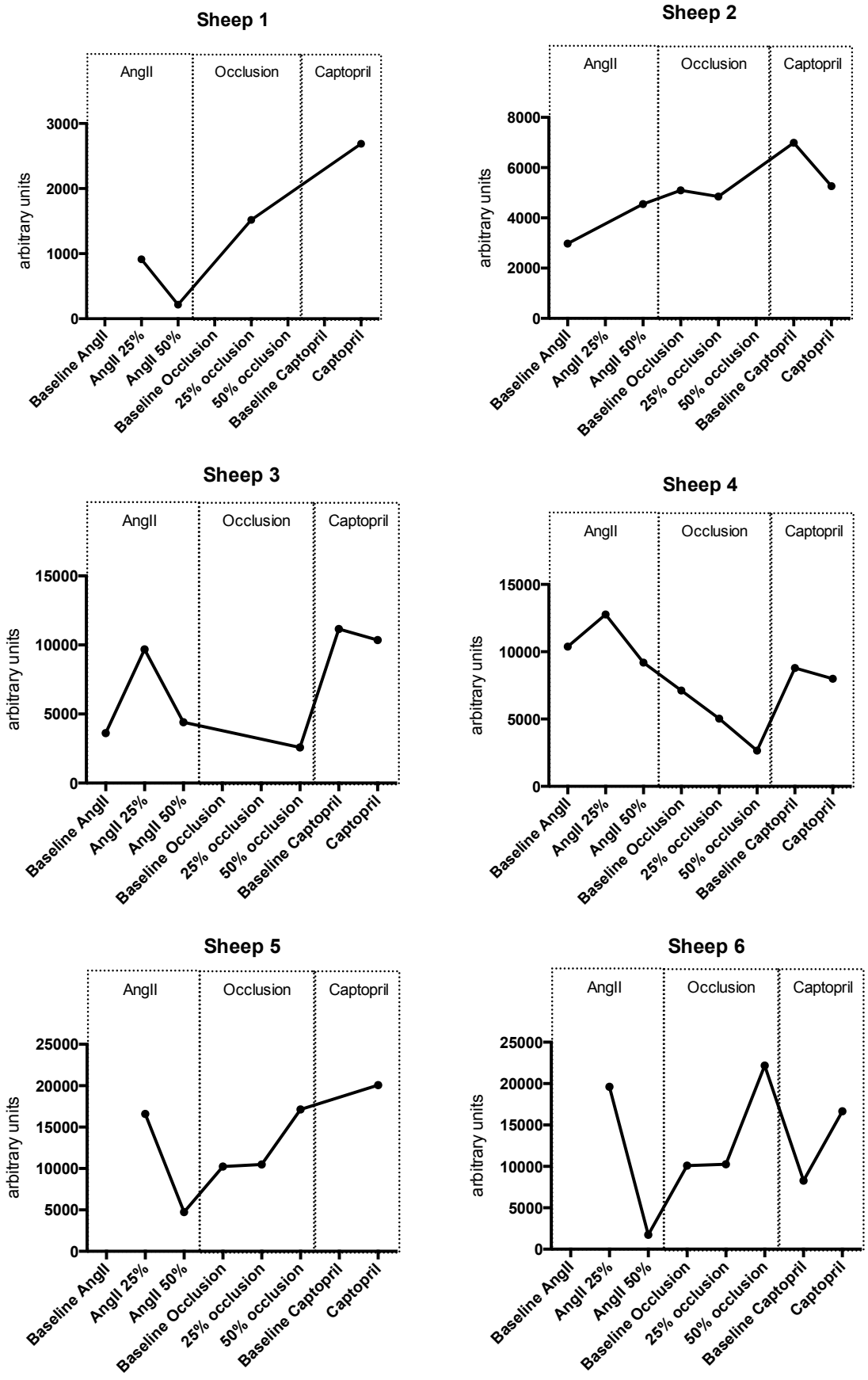


Figure 3.5: Individual data (Perfusion Indices) (AngII: Angiotensine II)

3.4. DISCUSSION

3.4.1. Summary of key findings

We performed an observational study in six merino sheep to evaluate changes in renal cortical microcirculation as evaluated by CEUS in response to pharmacological and mechanical modifications of renal blood flow (RBF). We found that appropriate reductions in RBF could be obtained in all animals with an infusion of AngII and in most of them with an implanted renal artery occluder and that a 20% increase in RBF could be obtained with administration of 25 mg of captopril. However, we found that obtaining high quality images in this sheep model was challenging. We also found that, within the limitations of our model, CEUS derived perfusion indices did not parallel changes in RBF and that findings were highly heterogeneous. We found that a similar decrease in RBF was associated with unpredictable and divergent changes in perfusions indices according to whether they were obtained with a mechanical occlusion of the artery or the administration of AngII.

3.4.2. Comparison with previous studies

A previous human study has used CEUS to compare changes in renal microcirculation in response to changes in renal perfusion as induced by AngII and Captopril [105]. In this study, AngII administration was associated with marked and statistically significant decreases in PI, even when renal plasma flow was only reduced by 15%.

A recent animal study [106] used CEUS to measure renal microcirculation parameters in rats. Similar to our protocol, the investigators used AngII to decrease RBF. This study compared two methods of blood flow quantification and demonstrated an excellent correlation between CEUS derived parameters and blood flow. We failed to replicate these data in our sheep model. This might in part be due in part to our decision to use parasagittal images, as the findings from the above study suggested that coronal images had better sensitivity.

In addition, our results were associated with important heterogeneity. Heterogeneity among subject and measurements has been previously reported, however to a much lesser degree than in the current study. Potential reasons for increased heterogeneity might involve the model used and our study design and are discussed in the limitations section.

A moderate (25%) reduction in RBF as induced by AngII was associated with an increase in PI, while a larger (50%) reduction was associated with a decrease in PI. This was essentially linked to a proportional increase in mTT (indicative of delayed replenishment) which reached statistical significance during 50% occlusion associated with a persistent increase in RBV (indicative of increased UCA concentration).

These findings seemed quite robust as such pattern was exhibited in similar magnitude in all but one animal where a baseline was available. This could be explained by the specific effect of AngII on renal microcirculation. Indeed, both afferent (AA) and efferent (EA) arteries respond to AngII with a dose-dependent vasoconstriction [111]. Such response is significantly more pronounced at the EA level with vasoconstriction occurring at lower concentration [112,113]. Therefore, a proportional increase in transit time with increased AngII induced RBF reduction could be expected. The associated increase in UCA concentration (RBV) remains to be investigated and might well be artefactual (see limitations section).

To the best of our knowledge, no study has evaluated changes in renal cortical microcirculation in response to renal artery occlusion. The influence of a mechanical occlusion on renal function was described using a similar model of sheep [114]. In this study, neither an acute (30 minutes) 25, 50 or 75% nor a prolonged (80% for 2 hours) reduction in RBF as induced by an implanted occluder were associated with sustained loss of kidney function. In this study, occlusion phases were associated with transient increases in MAP with returns to baseline within 2 hrs.

In comparison, we found that a similar decrease in RBF was associated with a modest (+11% with Occl25%) or marked (+41% Occl50%) *increase* in renal cortical microcirculation. These changes were associated with no (Occl25%) or a modest (+14 mmHg, Occl50%) increase in

mean arterial pressure but no change in cardiac output. This could be consistent with modest activation of the renin-angiotensin-aldosterone system (RAS) [115,116]. Hence, such progressive activation might explain the paradoxical increase in perfusion indices in the same fashion as observed with low dose AngII (predominant EA vasoconstriction). However, given the small number of observations such conclusions remain speculative.

3.4.3. Strengths an limitations

Heterogeneity of measurements is a common limitation of all attempts to use CEUS for organ perfusion quantification. Sources for this heterogeneity have been reviewed in details by Tang et al [117]. In order to limit the influence of the most important factors associated with heterogeneity, we kept mechanical index, dynamic range, frequency and gain constant throughout the experiment.

Ensuring the reproducibility of anatomical location of ROI proved challenging in our experimental animals. For adequate perfusion quantification, focus depth and insonification angle need to be kept constant. In sheep, kidney position seems to be greatly altered by respiration and peristaltic activity. To overcome this limitation, we have used anatomical landmarks and compared live images with previously acquired baseline images. In addition, several sequences were obtained at each study point and only similar looking areas were retained for data analyses. Finally, we tried to locate ROI's at similar depth and distance from the focal depth to ensure a more uniform acoustic field as recommended by Averkiou et al [118]. Motion artefact was partially tackled by the selection of a probe angle limiting this motion and by the use of an advanced image stabilisation algorithm in the VueBox® software. Unfortunately, such compensation can only deal with in-plane motion and perhaps further studies should make use of 3D probes [119]. Further attenuation of signal was associated with transient interposition of air fluid cavities possibly related to the sheep large four-compartment stomach. This issue limited the number of usable sequences.

Further heterogeneity might have been due to protocol-related changes in blood pressure. Indeed, such changes are known to directly affect the mean size of the bubbles and their resonant frequency. Moravi et al [120] observed an approximate 20% decrease in video intensity for albumin-coated bubbles during systole compared with diastole. However, changes in blood pressure observed in our protocol are of lesser intensity and their influence on the results might be limited.

Changes in the inhaled fraction of anaesthetic gas might have impacted the intensity of CEUS signal [121]. Indeed, inhaled gas concentrations might alter microbubbles size and dynamically change their response to the ultrasound beam and be associated with a decrease in their blood concentration as larger bubbles tend to be filtered in the lung. To overcome this limitation, only minor and strictly mandatory changes in inspired fractions of gases were allowed during the experiment. In addition, anaesthetic gas might have decreased RBF through its effects on the activation of the RAS [122]. However, this is unlikely to have influenced our results as study interventions were targeted based on the readings of a flow meter.

The increase in intensity sometimes observed during the second administration of contrast agent in the same subject has been related to the saturation of pulmonary macrophages by the first injection, leading to increased signals from the second injection [117]. The importance of this effect in sheep and its impact on our results is unknown.

The administration of dexamethasone as a pre-medication to prevent pseudo-anaphylaxis was necessary. However, such medication might influence the circulation and perhaps explain the shift in RBF seen throughout the study. However, as repeated baseline measurements were taken, such shift is unlikely to have biased our results.

Finally, our study protocol might not have allowed enough time between interventions to enable establishment of a new steady state in the microcirculation. This is suggested by the fact that the "baseline" value for RBF did not systematically return to their "original" baseline and presented a trend to an upward shift. This limitation was partially attenuated by the repetition of baseline measurements. Unfortunately, such measurements were not performed before each series of occlusions.

3.4.4. Implications for clinicians and further studies

Renal perfusion quantification with CEUS remains a promising yet non-validated tool.

Indeed, the lack of a gold standard for the quantification of microcirculatory perfusion and the large heterogeneity of the results (and its associated potential for major error) limits the application of this technology in a clinical setting for the time being.

Most of the aforementioned sources of variability are likely to primarily influence the intensity of the enhancement by the UCA, therefore the RBV parameter is that most likely to be associated with measurement errors.

Further studies are required to improve technical measurements in order to limit heterogeneity and better validate this technique. These studies should aim at determining ideal conditions to limit measurements heterogeneity.

3.5. CONCLUSIONS

CEUS derived parameters were highly heterogeneous in this sheep model. The current protocol and model did not allow the evaluation of the correlation between macro and micro-circulation assessment by CEUS. Further studies should include other animal models and simpler protocols and possibly use of a 3D probe.

CHAPTER 4

CEUS TO EVALUATE CHANGES IN RENAL CORTICAL PERFUSION AROUND CARDIAC SURGERY: A PILOT STUDY

DECLARATION FOR THESIS CHAPTER 4

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Participated in study design, carried out participant recruitment, performed contrast-enhanced ultrasound studies, participated in date interpretation and drafted the manuscript.	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%)**
Mark D. Goodwin[1]	participated in study design, performed data analyses using dedicated software	NA
Anthony Schelleman[1]	participated in study design, performed data analyses using dedicated software	NA
Michael Bailey	participated in study design, performed statistical analyses	NA
Lynne Johnson[1]	participated in study design, contributed to contrast-enhanced ultrasound studies	NA
Rinaldo Bellomo	participated to the study design, data interpretation and critically reviewed the manuscript	NA

1= From the radiology department, Austin Health, Heidelberg, Victoria, Australia

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	Date: 15th November 2014
Main Supervisor's Signature	[redacted]	Date: 15th November 2014

In chapter 3, we have presented our animal study. By performing this experiments, we have improved our understanding of the technology and technical measurements: ultrasound probe positioning and handling, contrast infusion, region of interest determination, sequences selection… It highlighted numerous limitations of the technique and forced us to try and understand reasons underlying data heterogeneity. This knowledge enabled us to design strong and reliable protocols for CEUS measurements aiming at limiting data heterogeneity.

As this stage, we felt we were ready for a first in man study! Indeed, to the best of our knowledge, renal perfusion quantification with CEUS was never performed in a critically ill patient before. Our first human study was therefore designed as a feasibility and safety study.

For this purpose, patients undergoing elective cardiac surgery represent an ideal population. Indeed, such major surgery is associated with an transient period of cardio-pulmonary instability and all patients are routinely admitted to the intensive care. They therefore represent "elective critically ill patients". CEUS measurement can therefore be performed before the operation while they are still in a stable condition. This ensures obtention of high quality baseline measurement and rules out patient specific anaphylaxis to ultrasound contrast agent.

The second major advantage of this population is the presence of invasive monitoring in the immediate post-operative period. Indeed, catheters enabling continuous arterial blood pressure, pulmonary artery pressure and cardiac output monitoring are typically inserted in in the immediate pre-operative period. In addition, they benefit from standard ICU monitoring (including three leads electrocardiography, pulsoxymetry), regular blood gases analyses and one to one nursing surveillance. Overall, this setting provides a unique and extremely sensitive arsenal for the detection of adverse events. The following CEUS measurements would therefore be performed during this immediate post-operative period, when cardio-pulmonary instability is at its peak and full monitoring available.

Both these characteristics make this trial unique and particularly important to determine the safety of ultrasound contrast agents. In addition, the evaluation of data such as the time between admission and CEUS can could provides interesting real life data of feasibility of such technology in a busy unit and unstable patients requiring immediate medical attention.

4. CEUS TO EVALUATE CHANGES IN RENAL CORTICAL PERFUSION AROUND CARDIAC SURGERY: A PILOT STUDY

Acknowledgements

Bracco (Milano, Italy) provided the contrast agent (Sonovue®) free of charge.

Bracco Research (Geneva, Switzerland) provided the VueJect™ pump as well as Sonotumor Software free of charge.

Both these companies were allowed to read draft of the manuscript before submission but had no influence on its content or decision for submission.

4.1. INTRODUCTION

Acute kidney injury (AKI) is a frequent complication of cardiac surgery and renal replacement therapy (RRT) is required in 1 to 4% of the cases [123-128]. Such severe AKI has been shown to be independently associated with increased in-hospital mortality [129].

The pathophysiology of cardiac surgery associated AKI is still poorly understood. A decrease in renal blood flow (RBF) is believed to play a pivotal role in its pathogenesis [7,130]. There are, however, only very limited human data supporting this concept. Indeed, RBF [8] measurement, irrespective of the technique used, has only been reported in 46 critically ill patients (five studies) within the last sixty years. Thus, our knowledge, understanding, and theoretical constructs regarding renal perfusion in critically ill patients are based on extremely weak direct evidence.

Furthermore, given the complex and heterogeneous nature of the renal vasculature, some pathophysiological processes might be associated with increased global RBF [9,10] despite loss of function suggesting intra-renal shunting [11]. Therefore, techniques allowing the study of microcirculatory parameters might be more valuable in increasing our understanding of the pathophysiology of AKI. Such parameters can be evaluated by a relatively recent imaging

technique: renal contrast-enhanced ultrasonography (CEUS). However, CEUS is still used infrequently in clinical practice. In particular, there are no data on the feasibility, safety, reproducibility and diagnostic value of CEUS in critically ill patients.

We hypothesized that CEUS would be safe and feasible and allow quantification of changes in the renal cortical microcirculation in patients undergoing cardiac surgery.

4.2. METHODS

The study was approved by the Austin Health Research Ethics Committee (H2010/03798).

4.2.1. Study protocol

We approached 12 patients planned for elective cardiac surgery and obtained informed consent. We restricted inclusion to patients deemed at high risk of AKI [131,132]. We therefore included only patient fulfilling one or more of the following criteria: age above 70 years, preexisting renal impairment (preoperative plasma creatinine concentration > 120 μmol/l), NYHA class III/IV or impaired left ventricular function (LVEF<35%), valvular surgery, redo cardiac surgery or insulin-dependent type 2 diabetes mellitus.

We excluded patients with intolerance to Sonovue® (Bracco, Milano, Italy) or any other ultrasound contrast agent, end stage renal disease (plasma creatinine concentration > 300 μmol/l or on haemodialysis, emergency cardiac surgery, planned off-pump cardiac surgery, known blood borne infectious diseases, inability to obtain informed consent or enrollment in conflicting research study.

For each study patient, we performed renal CEUS on three occasions: before the surgical procedure (baseline), on ICU admission and the day after the operation.

4.2.2. Ultrasound equipment and settings

We performed all measurements using an IU22® ultrasound system (Philips, Amsterdam, Netherlands) with a C5-1 probe (1-5 MHz). We used contrast specific mode with low

mechanical index (MI: 0.06) (R1). We set gain and depth for each patient during baseline and kept them constant for all further measurements.

We used Sonovue® (Bracco, Milan, Italy) as the contrast agent. The agent was administered as continuous infusion at a rate of 1 ml/min using VueJect™ syringe pump (Bracco Research, Geneva, Switzerland). After the start of the infusion, we allowed a two-minutes equilibration period and then performed and recorded five destruction-reperfusion sequences. We achieved contrast microbubble destruction by applying five pulses at high MI (flash: MI 1.24) and observed refilling at low MI (15 seconds total refilling time). We ascertained full destruction of contrast agent in the scan plane before performing destruction-reperfusion sequences.

For further examinations, to ensure that a similar portion of the renal cortex was examined, we used anatomical landmarks and visually compared the image with previously acquired sequences.

4.2.3. Data analyses

We exported destruction-reperfusion sequences in DICOM format and analysed them using dedicated software (Sonotumor™, Bracco Research, Geneva, Switzerland). These analyses were performed by two independent radiologists (MG and TS) blinded to patient and time. In order to compensate for minor breathing artifacts, all sequences were applied with a motion compensation prior to the start of the analyses.

For each sequence, one region of interest (ROI) was drawn. In order to minimize the influence of local perfusion heterogeneities, this ROI was drawn so that it enclosed all the visible renal cortex on the surface of the kidney closest to the ultrasound probe. Cortex that was only intermittently visible because of breathing or other artefacts was not included in the ROI.

The software generates a perfusion index (PI), which is thought to be proportional to perfusion within a region of interest. Such PI is calculated by dividing the relative blood volume (RBV) by the mean transit time (mTT) and is expressed in arbitrary units (a.u.). These parameters have been described in detail elsewhere [66,97]. In brief, the RBV is a measure of pixel luminance

and is proportional to contrast agent concentration within a region of interest (increases with higher level of perfusion). The mTT is a measure of the time to replenishment after flash destruction of the contrast agent (decreases with higher level of perfusion).

For each patient and study time, the median value for the five measurements was considered for analysis. Suboptimal sequences with inadequate insonification or excessive breathing artefact were excluded as evaluated by both readers. In case of disagreement on sequence exclusion, the two readers reviewed the sequences simultaneously and consensus was reached.

4.2.4. Safety parameters

For baseline studies, we performed non-invasive hemodynamic (cardiac rhythm, non-invasive blood pressure and pulse oximetry) monitoring during contrast-agent infusion and for the subsequent 30 minutes.

For post-operative studies full hemodynamic monitoring including invasive arterial blood pressure, pulmonary artery pressures, and cardiac index (via pulmonary artery catheter) was available for all patients.

4.2.5. Statistical analysis

Analysis was performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). All outcomes were assessed for normality and as RBV, mTT and PI were all well approximated by log-normal distributions, each was log-transformed prior to analysis. Inter-observer agreement was determined using correlation analyses as well as intra-class correlation[133]. For both tests, values ranged from 0 to 1 (0: absence of correlation, 1: perfect correlation). They were reported using a Bland-Altman plot with 95% limits of agreement.

Descriptive results are reported as means (standard deviation) for normally distributed data and median (interquartile range) otherwise. Longitudinal analysis determining changes from baseline was performed using mixed linear modelling, with each patient treated as a random effect. Multivariable models were constructed considering all available hemodynamic and

biological parameters (mean arterial pressure, cardiac index, vasoconstrictor infusion, lactate serum level, arterial pH, and serum haemoglobin level) with statistically significant variables included in the final models.. A two-sided p-value of 0.05 was considered to be statistically significant.

4.3. RESULTS

4.3.1. Patients description and outcomes

Characteristics of the twelve patients are presented in Table 1. The procedure was coronary artery graft surgery (CAGS) in six, valvular replacement surgery (including a double valve surgery) in three and combination of CAGS and valvular surgery in three patients. Median hospital length of stay was 11 (8-16.5) days. All patients survived to hospital discharge.

	Body Weight (Kg)	Operation	CPB duration	LVEF	APACHE III score	VasoC	Diuretics	Baseline GFR (ml/min)*	RIFLE score	Hospital LOS
1	123	CABG	79	>60%	48	N	Y	142	I	8
2	101	CABG	104	>60%	36	Y	N	87	0	7
3	107	AVR+CABG	207	40%	45	Y	N	152	R	7
4	80	MVR	130	>60%	38	Y	N	98	0	32
5	76	AVR+CABG	226	20%	68	Y	Y	47	R	62
6	83	MVR+TVR	119	>60%	59	Y	Y	47	0	8
7	81	CABG	86	>60%	40	N	N	59	0	8
8	78	AVR	114	40%	32	N	N	120	0	15
9	81	CABG	69	40%	71	Y	N	59	0	15
10	78	CABG	No CBP	>60%	54	N	N	113	0	11
11	79	CABG	90	>60%	56	Y	N	52	0	11
12	106	AVR+CABG	151	>60%	74	Y	Y	86	R	18

Table 1 patients characteristics
CPB= Cardiopulmonary bypass; CAGB= Coronary arteries graft bypass; AVR= Aortic valve replacement; MVR= Mitral valve replacement; TVR= Tricuspid valve replacement; LVEF= Left ventricular ejection fraction; LOS= Length of stay (days) RIFLE: Risk, Injury, Failure, Loss and End-Stage Renal Failure. VasoC: Vasoconstrictors GFR: Glomerular filtration rate
* Calculated with the Cockroft-Gault equation (ml/min)

4.3.2. Feasibility

Although some of the scans were performed on hemodynamically unstable patients, all of them could be performed as per protocol. As an example, the "ICU admission scan" was completed at a mean of 110 (SD 42) minutes after ICU admission. All scans were performed in less than half an hour (effective infusion time <8 minutes for all patients).

Because of increased subcutaneous fluid and drains after the operation, adequate visualisation of the kidney was sometimes challenging. However adequate contrast enhancement with contrast agent was obtained for all patients at all time points. At least one sequence for each study time-point was judged by the two readers to be of adequate quality for interpretation. An illustration of destruction-refilling sequences obtained during the study is presented in Figure 4.1.

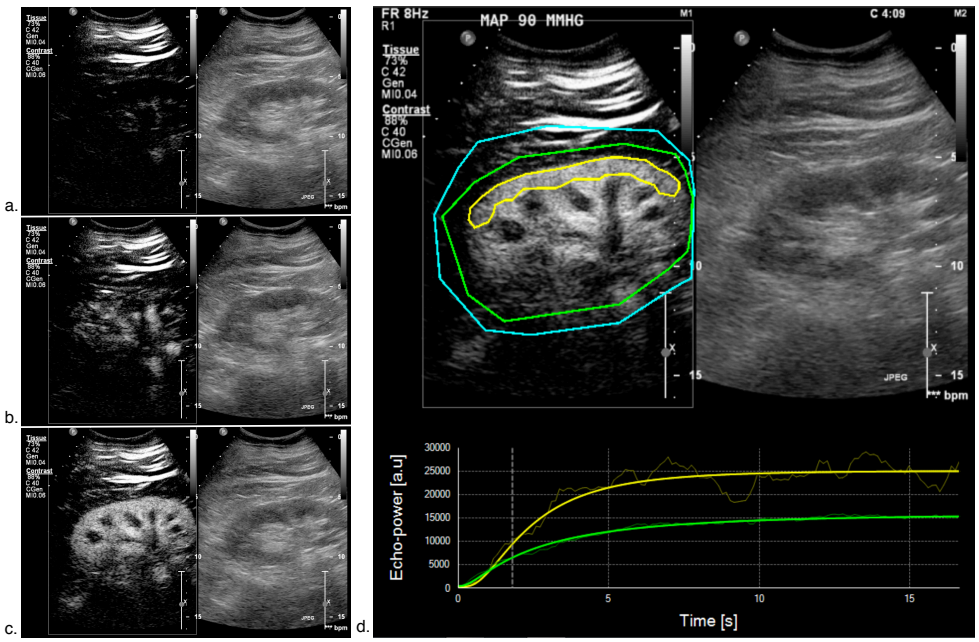


Figure 4.1: Illustration of destruction-reperfusion sequence: During continuous infusion of the contrast agent, microbubbles destruction is obtained by applying pulses at high mechanical index (high ultrasound intensity). Microcirculation replenishment is then observed. All images represent renal contrast-enhanced ultrasonography, the left part of the image shows contrast-image mode imaging and the right part the standard (B-mode) image.
1a: just after the flash.
1b: during replenishment (2 seconds after flash)
1c: at full replenishment (6 seconds after flash)
1d: Sequence analysis with Sonotumor®: a region of interest was drawn (yellow line) in the largest possible area of renal cortex closer to the ultrasound probe. The software generates a time intensity curve. This curve is used to generate CEUS derived parameters.

4.3.3. Tolerance

Overall 36 contrast-enhanced ultrasounds were performed using a total of 72 vials of Sonovue® (10 ml per scan). Of these, 24 were performed in the intensive care unit including nine in patients requiring vasoconstrictors for severe hypotension. No adverse effect was noted. Haemodynamic characteristics of patients before and after CEUS are presented in Table 2.

Table 2: Safety data (Mean (SD))

	2hrs pre	1hr pre	CEUS	1hr post	2hrs post	p value
Cardiac index	2.87 (0.62)	2.96 (0.46)	2.9 (0.45)	2.9 (0.58)	2.92 (0.48)	0.99
Heart rate (beat per minute)	84.67 (15.56)	83.83 (15.01)	84 (12.48)	84.65 (14.62)	84.88 (15.08)	0.69
Lactate (mmol/l)	1.73 (1.05)	1.56 (0.84)	1.43 (0.85)	1.81 (0.87)	1.62 (1.09)	0.72
Mean arterial pressure (mmHg)	80.17 (15.24)	79.96 (10.14)	78.46 (9.62)	77.17 (11.24)	76.42 (10.4)	0.43
Systolic pulmonary arterial pressure (mmHg)	29.28 (15.13)	28.8 (14.17)	27.85 (14.12)	28.67 (14.74)	28.71 (15.47)	0.98
Noradrenaline infusion rate (mcg/min)	1.75 (2.83)	1.88 (2.83)	1.92 (3.59)	2.5 (4.6)	2.42 (4.6)	0.94
Respiratory rate (Breath per minute)	15.26 (4.69)	14.96 (4.65)	16.04 (4.54)	14.83 (3.75)	15.14 (3.87)	0.39

4.3.4. Inter-observer agreement

Correlation between readers was excellent for PI (R^2=0.96, p<0.0001) as well as RBV (R^2=0.94, p<0.001) but only moderate for mTT (0.51, p<0.0001). Intraclass correlation was 0.69 (95% CI 0.5-0.84) for PI, 0.68 (95% CI 0.48- 0.83) for RBV and 0.43 (95% CI 0.25-0.63) for mTT.

As presented in Figure 4.2, agreement between readers was good. Mean bias for PI was -412 a.u. (6.9% of mean value) with limits of agreement from -4065 to 3243 a.u. (± 61% of mean value). For RBV, mean bias was -1320 (9.2% of mean value) with limits of agreements form -

5670 to 3030 (±30.4% of mean value). Similarly, for mTT the mean bias was -0.39 (9.1% of mean value) with limits of agreements -7.8 to 7.1 (± 172% of mean value).

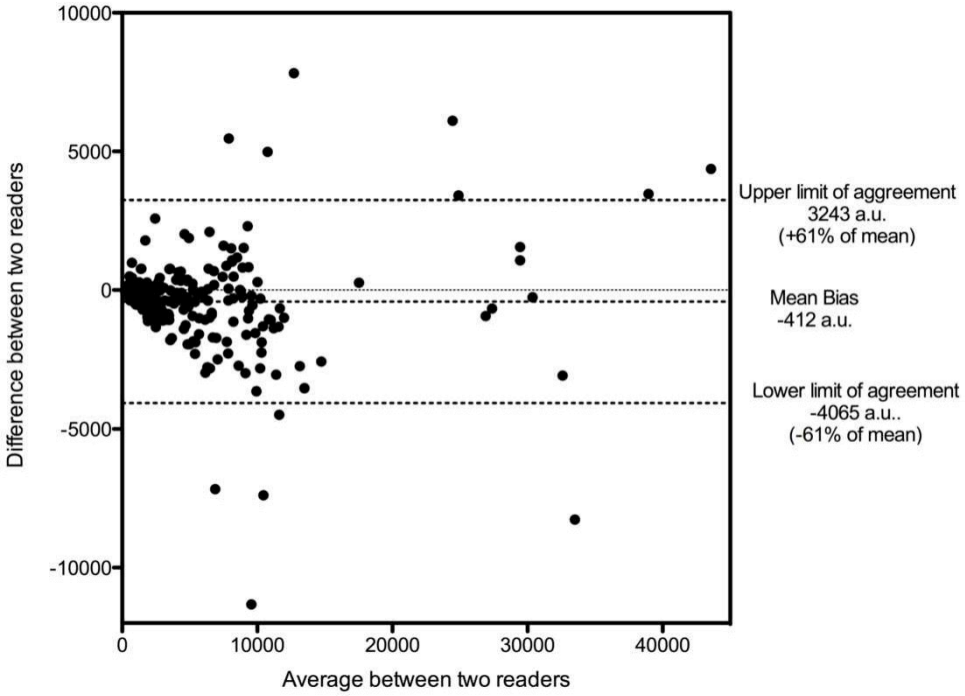


Figure 4.2: Bland-Altman plot for inter-observer agreement (PI=perfusion indices)
a.u.= arbitrary units. SD= standard deviation

4.3.5. CEUS derived parameters

Perfusion indices (PI)

Baseline PI values ranged from 1069 to 29446 arbitrary units (a.u.). Changes in such values indexed to baseline are presented in Figure 3. Compared with baseline, PI values decreased 24 hours after admission in nine patients. Pooled values for PI are presented in Figure 4.

PI decreased from a baseline median value of 6750 (2042-8263) to 3936 (1645-6004) on ICU admission (-42%, p=0.33) and to 3308 (1243-4573) 24 hours later (-51%, p<0.01). After adjustment for mean arterial pressure, inotrope infusion and haemoglobin, there was no

difference in PI between baseline and ICU admission (p=0.70) but there was a significant decrease in PI 24 hrs after admission (p=0.03).

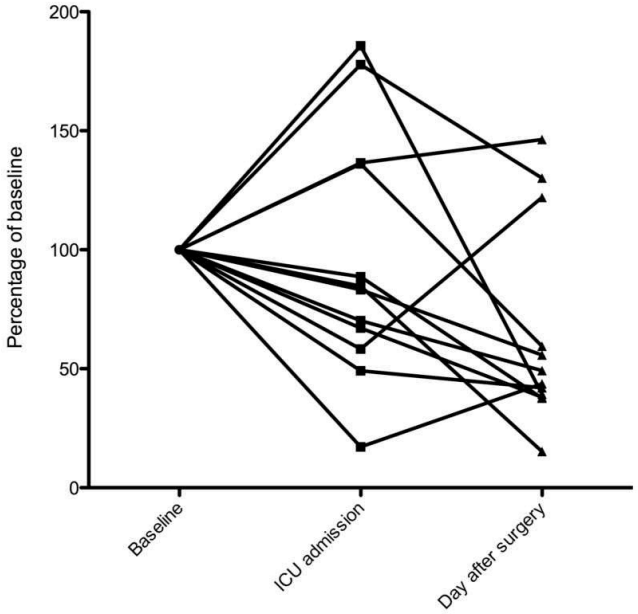


Figure 4.3: Perfusion indices: individual patients results indexed

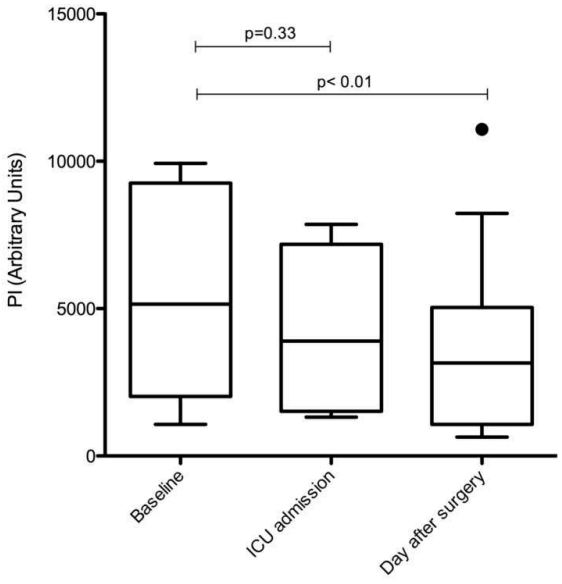


Figure 4.4: Overall results perfusion indices

Relative blood volume (RBV)

Baseline RBV values ranged from 4846 to 29958 a.u.. Compared with baseline, nine patients had a lower RBV 24hrs after surgery.

Pooled RBV decreased from a value of 15342 a.u. (IQR 7862-20490) on baseline to 13113 a.u. (IQR 8952-17310) on ICU admission (-14%, p=0.31) and to 11929 a.u. (IQR 6312-15904) 24hrs after admission (-22%, p=0.09). After adjustment for mean arterial pressure, inotrope infusion and haemoglobin, there was no difference in RBV between baseline and ICU admission (p=0.29) and a trend for a decrease 24 hrs after admission (p=0.11).

Mean transit time (mTT)

Baseline mTT values ranged from 1.0 to 7.7 seconds. Compared with baseline, most (10/12) patients experienced an increase in their mTT 24 hrs after the admission.

Pooled mTT increased from a median value of 2.9 seconds (IQR 2.1-3.3) on baseline to 3.3 (IQR 2.0-4.2) on ICU admission (+14%, p=0.73) and to 4.3 (IQR 2.8-4.7) 24 hrs later (+48%, p=0.04). After adjustment for mean arterial pressure, inotrope infusion and haemoglobin, there was no difference in mTT between baseline and ICU admission (p=0.15) and 24 hrs after admission (p=0.37).

4.3.6. Correlation with changes in creatinine levels

Four patients developed acute kidney injury (RIFLE-R in three and RIFLE-I in one) but none required renal replacement therapy.

There was no correlation between changes in perfusion indices in the first 24hrs after cardiac surgery and changes in serum creatinine levels.

4.4. DISCUSSION

4.4.1. Key findings

Using contrast-enhanced ultrasonography, we were able to quantify changes in the microcirculation of the renal cortex before and after cardiac surgery in 12 patients deemed at risk of AKI. In these patients, we performed 36 CEUS scans, including 24 in the intensive care unit. Such studies were all performed in less than half an hour and did not interfere with clinical management. Tolerance was excellent and no adverse effect was noted. When compared with baseline, we found no overall difference in CEUS derived parameters (PI, RBV and mTT) on ICU admission. However, 24 hours after the operation, there was an overall 50% decrease in the perfusion index, suggestive of decreased renal cortical perfusion.

4.4.2. Comparison with previous studies

The general safety of CEUS has been demonstrated in several large retrospective studies [68,92,93], one of which included critically ill patients [93]. The reported rate of adverse events including potentially severe "anaphylactoid" reactions or complement activation-related pseudo-allergy (CARPA) [134] are in the range of 1 per 10,000 administrations. There is, however, no detailed data on CEUS safety after cardio-pulmonary bypass, or during mechanical ventilation or vasoconstrictor administration. Our study provides such information. In addition, we were able to report the absence of changes in systolic pulmonary pressure after contrast agent administration. This is consistent with previous findings [135] in patients undergoing right heart catheterization with Definity® as a contrast agent. Our data confirm the absence of measurable physiological changes even in hemodynamically unstable patients during administration of Sonovue® for CEUS.

In a previous study [74], we evaluated the ability of CEUS to detect changes in renal cortical perfusion in healthy volunteers. We found that CEUS was able to detect a 15% change in renal flow as induced by angiotensin II or captopril administration. These results were consistent

with those found using a different technique by Kishimoto et al. [71]. However, to the best of our knowledge, there has not been any previous attempt to use CEUS for renal perfusion quantification in patients undergoing cardiac surgery, hence our findings are novel and cannot be compared with previous data. Estimated of global renal blood flow have been obtained with the measurement of para-immuno hippurate clearance corrected by renal vein sampling [19]. This technique requires the insertion of an 8-Fr catheter into the left renal vein under fluoroscopic guidance. Using this invasive strategy, authors have demonstrated, in similar patients, the potential of noradrenaline to improve oxygen delivery, GFR and renal oxygen supply/demand relationship in cardiac surgery patients with vasodilatory shock and AKI [136], an increase in RBF induced by mannitol [137] or dopamine [138].

The lack of correlation between estimate of flow and function is consistent with data obtained with cine-phase contrast magnetic resonance imaging [53].

4.4.3. Clinical significance

Our pilot study supports the feasibility and safety of CEUS at the bedside in the intensive care unit, in particular in the period around cardiac surgery even in patients deemed at risk of AKI. Despite some relative hemodynamic instability, adequate visualisation of the kidney was feasible in all cases. The findings of decreased cortical perfusion at 24 hours are plausible and consistent with previous findings [53]. This study, together with larger safety studies, establishes CEUS as a fast, safe and feasible procedure in critically ill patients.

Larger studies are required to confirm or refute the decrease in renal cortical perfusion after cardiac surgery suggested by our results.

4.4.4. Strength and limitations

To the best of our knowledge, this study is the first to report non-invasive, real time measurement of renal cortical microcirculation in humans before and after cardiac surgery. In addition, it has several strengths. Extensive invasive monitoring was available for all patients

in the post-operative period enabling detection of subclinical adverse event. All scans were performed by a single operator (AS) and were analysed by two senior radiologists blinded to patient and time. This enabled inter-observer agreement evaluation. Despite important baseline heterogeneity, a statistically significant decrease in renal cortical perfusion was detected with a sample size as small as 12 patients.

On the other hand, this study has several limitations. First, CEUS parameters could not be correlated to a comparator/gold standard. Indeed, such measurements of the microcirculation are novel and no gold standard has emerged. Comparison with macrocirculation using techniques such as PAH clearance or MRI would have been theoretically possible but logistically very complicated or invasive and could not occur simultaneously. In addition, such comparison would be informative at best, but could also potentially be misleading, as the correlation between macro and microcirculation is not necessarily linear.

There was important heterogeneity in individual results and baseline perfusion indices. However, such heterogeneity was not likely to arise from interpretation errors as illustrated by the good agreement between the readers. Similar heterogeneity in baseline measurements was found in our previous study in healthy subjects [74]. It is more likely to be associated with different patient properties such as depth of organ, thickness of subcutaneous tissue and renal capsule or other properties influencing ultrasound beam attenuation. This is consistent with the greater variability observed in the RBV component, which is more sensitive to attenuation (as it is a measure of the intensity of the ultrasound signal). The mTT component, which is the time to replenishment, could be a more robust measure. However, this heterogeneity should not have influenced the validity of our results, as only changes in values from baseline in identical patients were considered.

The decrease in renal perfusion was not found in all patients. However, patients had different clinical courses and heterogeneity would be expected given the differences in age, type of operation and duration of bypass.

We did not find a correlation between CEUS indices and impairment of renal function; however, given the small number of patients, this findings needs to be confirmed in larger studies.

Finally, we were not able to report on medullary perfusion. Indeed, such a parameter together with cortico-medullary perfusion ratios of CEUS parameters would be of great interest. However, medullary perfusion, with our current technology, was not measurable with adequate reproducibility.

4.4.5. Future studies

Further studies are required to confirm or refute these results. In particular, due to the limited number of patients included in this study, we were not able to draw any conclusion regarding correlation of changes in CEUS-derived indices and clinical outcomes such as AKI, need for renal replacement therapy and mortality.

4.5. CONCLUSIONS

Contrast-enhanced ultrasonography is feasible and well tolerated in patients undergoing cardiac surgery in particular immediately after ICU admission. CEUS derived-parameters suggest a decrease in renal perfusion occurring within 24 hours of surgery. Further studies with larger sample size are required to establish whether there is a correlation between changes in microvascular cortical flow and markers of renal function.

CHAPTER 5

CEUS TO EVALUATE CHANGES IN RENAL CORTICAL MICROCIRCULATION INDUCED BY NORADRENALINE: A PILOT STUDY

DECLARATION FOR THESIS CHAPTER 5

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Participated in study design, carried out participant recruitment, performed contrast-enhanced ultrasound studies, participated in date interpretation and drafted the manuscript.	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%)**
Mark D. Goodwin[1]	participated in study design and critically reviewed the manuscript	NA
Anthony Schelleman[1]	participated in study design and critically reviewed the manuscript	NA
Michael Bailey	participated in study design, performed statistical analyses	NA
Lynne Johnson[1]	participated in study design, contributed to contrast-enhanced ultrasound studies	NA
Rinaldo Bellomo	participated to the study design, data interpretation and critically reviewed the manuscript	NA

1= From the radiology department, Austin Health, Heidelberg, Victoria, Australia

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature		Date: 15th November 2014
Main Supervisor's Signature		Date: 15th November 2014

After the two preliminary studies (described in chapter 3 and 4), we sought to evaluate the role of CEUS in two clinical situations in which, knowledge of renal perfusion could be of particular importance: circulatory shock (described in chap. 5) and hepato-renal syndrome (described in chap. 6).

Shock, is a clinical situation where the circulatory system is not able to deliver oxygen to the tissues resulting in organ failure potentially leading to death. Shock is one of the most common cause of admission in the ICU. Sepsis (septic shock), heart failure (cardiogenic shock) and bleeding (hypovolemic shock) are the main causes of shock. Shock is generally associated with severe hypotension. Medical management, in addition to the treatment of specific underlying cause (antibiotics and source control ; inotropic or mechanic support of the heart ; volume administration and bleeding source control) is usually supportive and aims at normalising cardio-respiratory parameters. Among those, systemic blood pressure is typically increased using vasoconstrictor drugs such as noradrenaline. However, the level to which this blood pressure needs to be increased is unknown and recent literature suggests that such level might vary from patient to patient.

An imaging modality enabling to determine whether an increase in blood pressure is associated with an increase in organ perfusion could help rationalise the use of vasopressors and determine early in their clinical course which patients are likely to respond to a higher blood pressure.

We sought to use CEUS to determine whether an increase in blood pressure was associated with an increase in renal perfusion in patients with shock.

5. CEUS TO EVALUATE CHANGES IN RENAL CORTICAL MICROCIRCULATION INDUCED BY NORADRENALINE: A PILOT STUDY

ACKNOWLEDGEMENTS:

Bracco (Milano, Italy) provided the contrast agent (Sonovue®) free of charge. Bracco Research (Geneva, Switzerland) provided the VueJect® pump and the VueBox® software free of charge. Both these companies were allowed to read the draft manuscript before submission but had no influence on its content or decision for submission.

5.1. INTRODUCTION

Renal blood flow (RBF) and glomerular filtration rate (GFR) are normally auto-regulated when systemic mean arterial pressure (MAP) is maintained between 60 and 100 mmHg [139]. This autoregulation is thought [140,141] to be mediated by a fast myogenic response of the afferent arteriole to blood pressure changes [142] with a superimposed slower tubuloglomerular feedback mechanism [143]. In the setting of acute kidney injury (AKI), however, as demonstrated in several animal models [144-147] this autoregulation may be lost and further episodes of hypotension may be associated with marked decreases in RBF and GFR. Similarly, in patients with chronic hypertension, the autoregulatory curve is shifted to the right [148], and a higher MAP is required to maintain RBF and GFR.

Based on this knowledge, intensive care physicians often aim at maintaining a higher MAP in patients deemed at risk of AKI and in those with chronic hypertension [149]. An arbitrary target of 70 or 80 mmHg is usually chosen and therapy adapted to reach this goal. Vasoconstrictors such as noradrenaline often need to be started or their infusion rate increased.

However, such increase in MAP target means higher exposure to a drug with recognized dose-dependent side effects [150]. To date, there is no data confirming that such practice improves renal microcirculation. In addition, microcirculatory changes in response to an increase in MAP might depend on individuals' circumstances and a standardised MAP target might not suit every patient [151]. Hence, a technique that allows microvascular renal perfusion quantification at the patient-level might help clinicians to determine the optimal MAP in critically ill patients.

Contrast-Enhanced Ultrasonography (CEUS) is a novel imaging technique which uses low mechanical index ultrasonography and microbubble-based contrast agents. CEUS has been shown to be able to detect changes in microvascular RBF [71,100,101,105]. CEUS is fast, safe and safe, has good inter-observer agreement [107] and can be performed at the bedside without requiring patient transport. It is therefore an ideal technique to be used in the intensive care unit [107].

We have designed a pilot observational study using CEUS to determine changes in renal cortical microvascular blood flow associated with a noradrenaline-induced increases in MAP from 60-65 mmHg to 80-85 mmHg in critically ill patients and related these changes to clinical outcomes.

5.2. METHODS

The study was approved by the Austin Health Research Ethics Committee (H2012/04592).

5.2.1. Participant recruitment and selection

Twelve patients who had an arterial line in situ, required a noradrenaline infusion >5 μg/min and expected to require noradrenaline for >24 hours at the time of inclusion, were approached and consented within 48 hours of ICU admission (consent could be obtained from the patient or its next of kin). Exclusion criteria were: Sonovue® or any ultrasound contrast agent (UCA)

intolerance, intra-cranial hypertension, aortic dissection or aneurysm, decompensated heart failure, severe left ventricular dysfunction (left ventricular ejection fraction <30%), ischemic heart disease, ventricular arrhythmias, end-stage renal disease (pre-morbid plasma creatinine concentration >300 mmol/l or chronic hemodialysis), on-going renal replacement therapy, inability to obtain informed consent, treating physician concern that a MAP of 60 mmHg might be too low or a MAP of 80 mmHg might be too high, and enrolment in a conflicting research study.

5.2.2. Study procedure

After obtaining written consent, renal CEUS scans were performed (procedure detailed below). The first scan was performed at baseline with the commonly applied MAP level of 60-65 mmHg. The noradrenaline infusion was then titrated up to reach a MAP of 80-85 mmHg. After a 30 minutes equilibration period, the CEUS scan was repeated. The rate of the noradrenaline infusion was then titrated back to the original MAP target as per treating physician recommendations.

5.2.3. Safety parameters

All studies were performed within the intensive care unit. Patients received full monitoring according to their clinical stability at the time of the examination as evaluated by the treating physician. At a minimum, invasive blood pressure, blood oxygen saturation (via pulse oxymetry), and continuous three leads electrocardiograms were available in all patients throughout the experiment. In addition, urinary output was monitored on an hourly basis and blood samples regularly drawn for routine blood tests as per clinical practice.

5.2.4. CEUS procedure

For the purpose of this study, we used Sonovue® (Bracco, Milano, Italy) as an ultrasound contrast agent (UCA). The UCA was infused into a peripheral or central vein (according to

availability) through an intravenous cannula using a dedicated syringe pump. Low mechanical index ultrasound of the kidney was performed with a Philips IU22® ultrasound machine and a C5-1® 5 MHz probe. A long axis view of the kidney was obtained by placing the transducer probe over the lower back of the subject. Once adequate images of the kidney were obtained, UCA infusion was started at 1 ml/min. Image depth, focus, gain and frame rate were optimized at the beginning of each experiment and held constant during the study. After a two minutes period required to obtain a steady state, five consecutive destruction/refilling sequences (with 15s refilling time) were obtained[66]. Destruction was obtained by applying a flash of increased ultrasound intensity (5 pulses with high mechanical index (> 1.0).

5.2.5. Sequences analyses

Ultrasound data sets were exported in a digital imaging and communication in medicine (DICOM) format and analysed offline using a dedicated software package, VueBox® (Bracco Research, Geneva, Switzerland). An example of offline analysis is presented in Figure 5.1. For each sequence, one region of interest (ROI) was drawn. In order to minimize the influence of local perfusion heterogeneities, this ROI was drawn so that it enclosed the largest area of visible renal cortex on the surface of the kidney closest to the ultrasound probe. Cortex that was only intermittently visible because of breathing or other artefacts was not included in the ROI. The software generates a perfusion index (PI), which is thought to be proportional to perfusion within a region of interest. Such PI is calculated by dividing the relative blood volume (RBV) by the mean transit time (mTT) and is expressed in arbitrary units (a.u.). These parameters have been described in detail elsewhere [66,97]. In brief, the RBV is a measure of pixel luminance. RBV is proportional to contrast agent concentration within a region of interest (increases with higher level of perfusion) and is expressed in arbitrary units (a.u.). The mTT is a measure of the time to replenishment after flash destruction of the contrast agent (a shorter time indicates higher level of perfusion). MTT is expressed in seconds.

Suboptimal sequences with inadequate insonification or excessive movement artefact were excluded. For each patient and study time, the median value for interpretable measurements was considered for analysis. Given the expected inter-observation variability and based on previous research [105], a change of more than 25% between two measurements was considered to be significant.

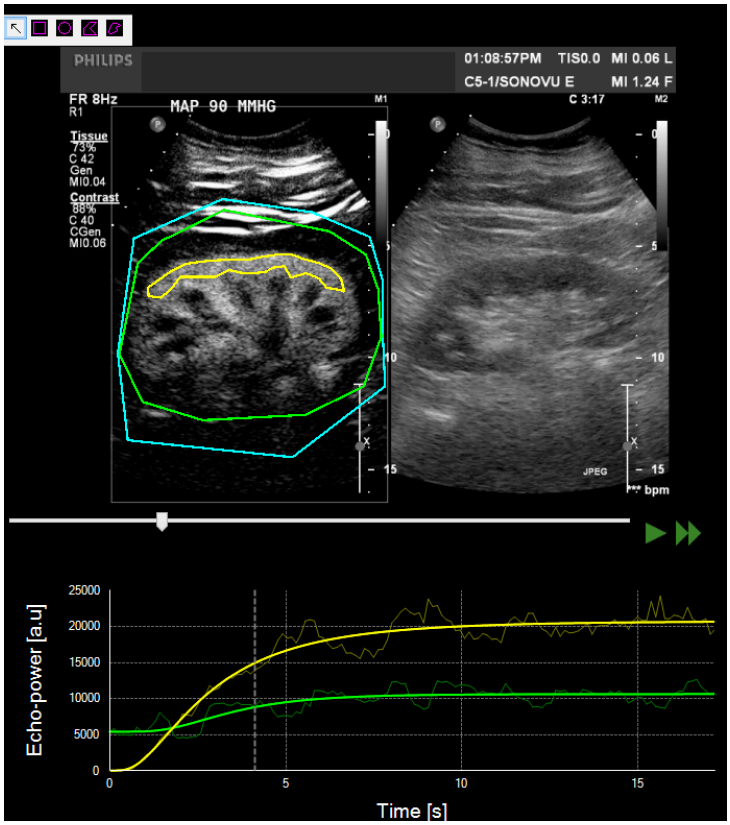


Figure 5.1: Sequence analysis with Vuebox®: A region of interest was drawn (yellow line) in the largest possible area of renal cortex close to the ultrasound probe. The software generates a time intensity curve (in yellow in lower panel). This curve is used to generate CEUS derived parameters. The green curve corresponds to the overall zone (kidney and surrounding tissues) and is not relevant for analysis. T0 corresponds to the destruction of all microbubbles in the scan plan by an ultrasound flash (increase of ultrasound intensity Cf. text for details). Top right panel shows standard "B mode" ultrasound image.

5.2.6. Consistency of CEUS measurements

As perfusion indices are calculated based on two measured parameters (RBV and mTT), we sought to report the agreement between these two measurements. For the purpose of this analysis, we considered that changes in RBV and changes in mTT were consistent when, in a given patient, both parameters increased or decreased by >25% of their baseline value in a direction indicating similar change in blood flow (for instance a >25% *increase* in RBV and a >25% *decrease* in mTT, both indicating an increase in perfusion), or when one parameter increased or decreased by >25% of its baseline value and the other one was unchanged (<25% change). We considered that changes were *not consistent* when both parameters increased or decreased by >25% of their baseline value in a direction indicating *an opposite* change in blood flow. The value of 25% corresponds to the mean coefficient of variation for mTT.

5.2.7. Responders vs non responders

Patients were classified into responders if their PI increased by >25% after noradrenaline-induced increase in MAP compared with baseline. They were classified into "non responders" if PI increased by <25% or decreased after noradrenaline-induced increase in MAP.

5.2.8. Statistical analysis

Analyses were performed using SPSS® version 21 (IBM, Armonk, NY, USA). All outcomes were assessed for normality and as RBV, mTT and PI were all well approximated by log-normal distributions, each was log-transformed prior to analysis.

Precision of measurements was estimated by calculating the coefficient of variation -defined as standard deviation divided by mean value and multiplied by 100 for all sequences obtained at one study time. We report the mean average coefficient of variation for the three CEUS derived parameters.

Normally distributed data are reported as means (standard deviation [SD]) and compared using paired t-test. Non-normally distributed data are presented as median (interquartile range) and were compared using Wilcoxon matched-pairs signed rank test. A two-sided p-value of 0.05 was considered to be statistically significant.

5.3. RESULTS

5.3.1. Patients description and outcomes

Patient's demographics and outcomes are detailed in table 1. Septic shock was the main diagnosis for ten of the twelve patients; six were mechanically ventilated at the time of the study.

Ten patients developed AKI during their hospital stay according to the risk injury failure loss, end-stage kidney disease (RIFLE) classification[152] but none required renal replacement therapy and all had recovered their renal function at the time of hospital discharge or death. Two patients died during their hospital stay.

Table 1: Patients characteristics

	Age	Sex	Main Diagnosis	CKD	DM	HT	Ap III	MV	ICU LOS	AKI	RIFLE	RRT	Renal Recovery	Death
1	51	M	Septic shock	No	No	No	65	Yes	6	1	F	No	Yes	No
2	64	F	Septic shock	No	Yes	No	47	No	5	1	R	No	Yes	No
3	30	M	Septic shock	No	No	No	18	Yes	4	1	I	No	Yes	No
4	71	F	Septic shock	No	No	No	57	No	2	1	I	No	Yes	No
5	84	M	Septic shock	No	No	Yes	73	Yes	10	1	R	No	Yes	Yes
6	42	F	Status epilepticus	No	No	No	24	Yes	12	1	R	No	Yes	No
7	68	M	Septic shock	No	Yes	Yes	60	No	3	0	-	No	Yes	No
8	63	M	Septic shock	No	No	No	61	Yes	4	1	I	No	Yes	No
9	32	F	Septic shock	No	No	No	56	No	2	1	R	No	Yes	No
10	71	M	Septic shock	No	No	Yes	44	No	2	1	F	No	Yes	Yes
11	62	M	Septic shock	No	No	No	80	No	7	1	I	No	Yes	No
12	65	M	Cardiogenic shock	No	No	No	75	Yes	4	0	-	No	Yes	No

M= male F= female NA= Noradrenaline RIFLE= Risk, Injury, Failure, Loss, End-Stage Kidney Failure ACE Inh: Angiotensin converting Enzyme inhibitors ICU= Intensive Care Unit LOS= Length of stay RRT= Renal replacement therapy

5.3.2. Tolerance / Feasibility

Overall 24 CEUS scans were performed using a total of 48 vials of Sonovue® (10 ml per scan). No adverse effect was noted with UCA administration. At least one interpretable sequence was obtained for each patient at the two study times. Each CEUS examination took approximately 15 minutes to complete.

To increase MAP from 60-65 mmHg to 80-85 Hg, the median noradrenaline infusion rate was increased from 10 µg/min (interquartile range [IQR] 5.5–12.5) to 14 µg/min (IQR 10.5-18.5). No adverse event was associated with the increase in MAP and noradrenaline dose.

5.3.3 Precision

The mean coefficients of variation were 12.2% for RBV, 25.2% for mTT and 25.2% for PI.

5.3.4. CEUS derived parameters

Perfusion indices (PI)

Overall (Figure 5.2), there was no difference in perfusion indices (PI) between measurements obtained at baseline (median PI 3056 (2438-6771) a.u.) and those obtained after a noradrenaline-induced increase in MAP (4101 [3067-5981] a.u.); p=0.38.

However, at individual level (Figure 3), large variations were observed. Indeed, a >25% increase was observed in four patients (>75% in three) and a >25% decrease was observed in one. Smaller changes were observed in the seven remaining patients (-19 to + 16%).

Among the three patients with chronic hypertension, noradrenaline-induced MAP increase was associated with a 10% and 93% increase in two but a 50% decrease in one.

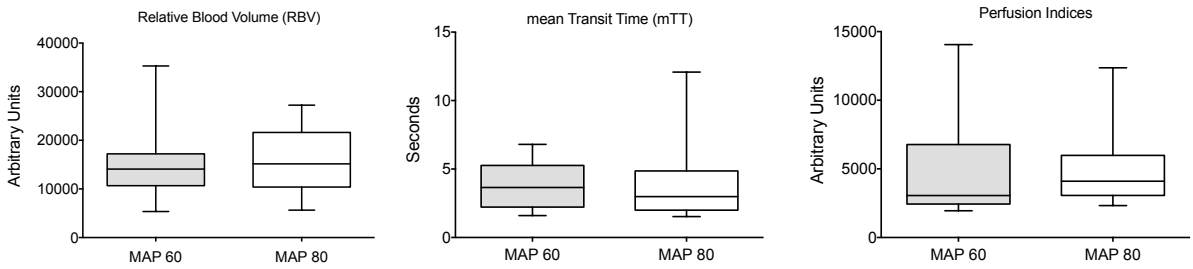
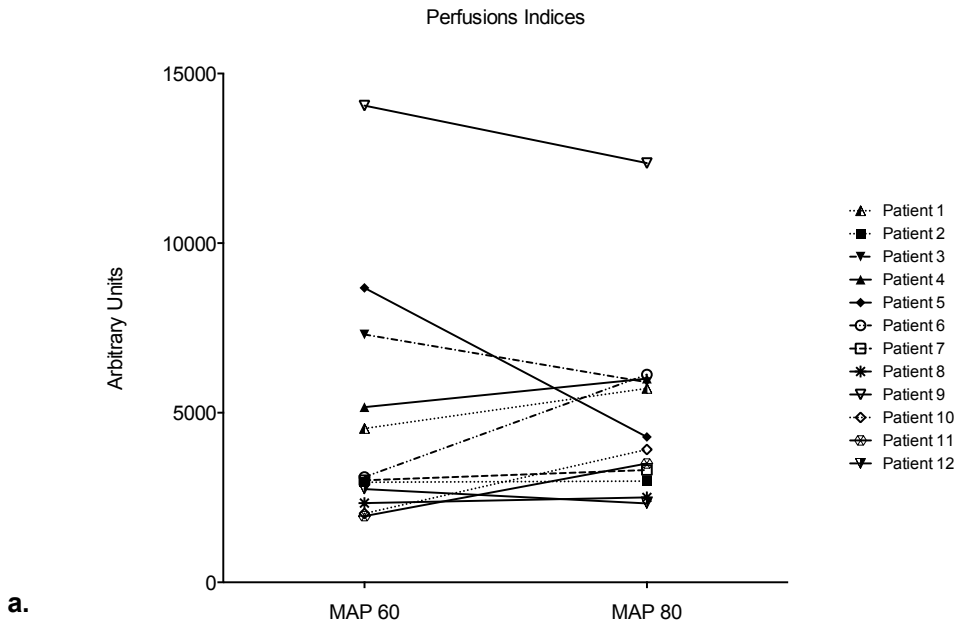
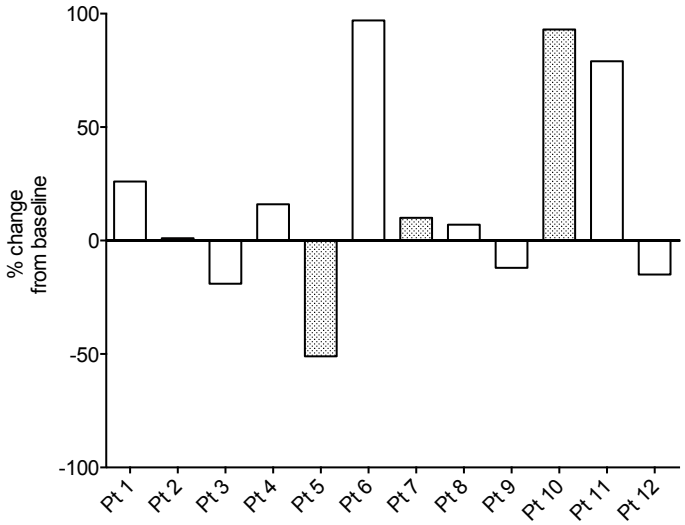


Figure 5.2: Overall results: MAP: mean arterial pressure



a.



b.

Figure 5.3: Perfusion indices patient per patient: 3a: raw data (arbitrary units) , 3b: percentage change from baseline. Greyed bars are for patients with chronic hypertension (patients 5, 7 and 10).

Consistency of changes in parameters

Patient-level changes from baseline for RBV and mTT are presented in Figure 4. Such changes were considered to be consistent with each other (suggesting similar changes in perfusion) in

nine (75%) patients. They were non consistent (suggesting opposite changes in perfusion) in three patients (two, eight and twelve in Table 1).

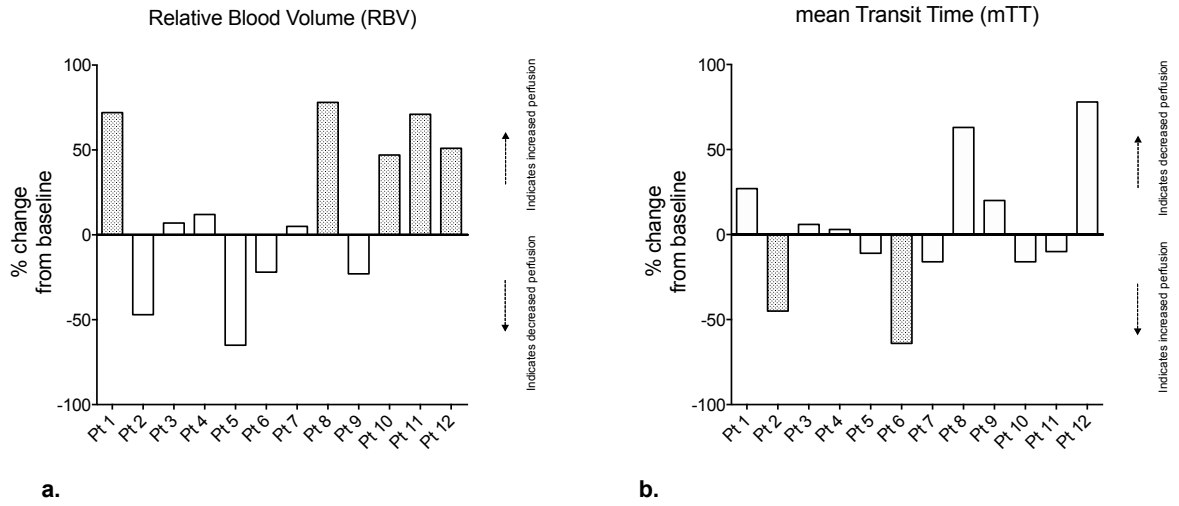


Figure 5.4: Percentage changes from baseline for RBV and mTT parameters
Agreement between the two parameters obtained to determine perfusion indices.
An increase in mTT is suggestive of a decrease in organ perfusion while a increase in RBV is suggestive of an increase in organ perfusion. Changes in RBV and changes in mTT were considered to be consistent when, in a given patient, both parameters increased or decreased by >25% of their baseline value in a direction indicating similar change in blood flow or when one parameter increased or decreased by >25% of its baseline value and the other one was unchanged (<25% change).
Grey bars are for patients with chronic hypertension (patients 5, 7 and 10).

Relationship between CEUS parameters and clinical outcomes

Four patients (one, six, ten and eleven in Table 1) were classified as responders based on a >25% change in PI after noradrenaline induced increase in MAP. These patients had an average APACHE III score of 56.3 and one had hypertension. All four patients developed AKI (RIFLE-F in two, RIFLE-I in one and RIFLE-R in one) and one subsequently died.

Conversely, within the eight patients who were classified as non-responders, the average APACHE III score was 55.9, two had DM and one HT. Six (75%) developed AKI (RIFLE-R in three and RIFLE-I in three).

5.4. DISCUSSION

5.4.1. Key findings

We attempted to quantify renal cortical microvascular perfusion in a non-invasive manner in critically ill patients on vasopressors using CEUS. We found that renal CEUS was feasible and administration of UCA well tolerated even in hemodynamically unstable patients. We found that a noradrenaline-induced increase in MAP was not associated with an overall change in renal perfusion indices as measured by CEUS. In contrast, the intervention was associated with highly heterogeneous responses at a patient-level, with observed increase or decrease by >25% of baseline values in a quarter of the patients.

5.4.2. Relationship to previous studies

Several animal studies [153,154] have demonstrated that noradrenaline may increase RBF in vasodilated / hypotensive states. This effect seems to be mediated by an increase in systemic blood pressure and an associated decrease in renal sympathetic tone through a baroreceptor response [154]. The effect of noradrenaline *per se* on renal vasculature tone was examined in an animal model of septic shock [155]. In this study, although noradrenaline administration was associated with an increase in MAP under all conditions, it was only associated with an increase in RBF (as measured by implanted ultrasonic flowmeters) when renal vascular vasodilatation was present. These findings suggest that noradrenaline infusion, in acute endotoxemia reverses systemic hypotension and may improve RBF independent of perfusion pressure.

However, the human data confirming these experimental findings is extremely limited. There are only a few studies that report RBF measurement in critical illness and its changes in response to noradrenaline administration. In particular, Redfors et al [136] have measured global RBF in critically ill patients, with invasive renal vein blood sampling. In this very detailed

physiological study, an increase from 60 to 75 mmHg of the MAP was associated with an increase in GFR and urine flow but not in RBF.

Other authors have used surrogate measures of RBF and measured renal vascular resistive indices in critically ill patients. These indices, however, have been shown to be poorly correlated with RBF [63]. However, such parameters can be predictive of reversibility of AKI [156] and perform better than urinary indices for diagnosing persistent AKI.

Current recommendations for MAP target [157] in septic shock (Grade 1C) are based on small physiological studies that demonstrated the absence of changes in several physiological parameters [158,159]. A recent large clinical trial [150] randomly allocated 776 patients with septic shock to undergo resuscitation with a MAP target of either 65-70 mmHg or 80-85mmHg. In this trial, there was no difference in 28 or 90-days mortality between the two groups. In the subgroup of patients with chronic hypertension, however, there was a decrease in the need for renal replacement therapy. Our data, suggesting high heterogeneity in renal perfusion in response to a similar change in MAP, could provide an explanation for these findings. Indeed, a pre-determined "one-size fits all" MAP target might not be suitable for a highly heterogeneous group such as critically ill patients. On the contrary, a "tailored" MAP target aiming at restoring tissue perfusion, based on assessment of mental status, skin appearance, urinary output and perhaps CEUS parameters could represent an alternative approach [160].

5.4.3. Strengths and limitations

This study is the first to use CEUS to evaluate renal microvascular perfusion induced by a change in the noradrenaline infusion rate. CEUS is a new technology, which is applicable at the bedside and could improve our understanding of organ perfusion in critical illness. This study provides pathophysiological insight to an important and unresolved question that persists despite large randomized controlled trials. However, this study has several limitations. First, the small sample size precludes advanced statistical analyses and determination of factors

predicting response and the classification of patients into “responders” and “non-responders” remains arbitrary.

Second, no measure of renal vascular resistive indices were performed. This measure would have, if consistent with CEUS data, made our conclusions stronger. However, for technical reasons, such data were not collected.

Changes between measurements could be random variations associated with an overly sensitive technique. Indeed, CEUS measurements can be limited by numerous factors such as organ depth, echogenicity of surrounding tissues, breathing artefacts, US equipment settings, and fluid retention. This is illustrated by the large variability of baseline measurements among patients. However, such parameters are unlikely to have influenced the results because, for each patient, both CEUS scans were performed within a very short (<45 minutes) time window in which ventilation parameters, fluid and medications infusions, patient’s position and US machine settings were all kept constant. Only comparison of measurements obtained in a single patient, as all other factors are kept constant can be interpreted. In addition, CEUS data were obtained by a single experienced operator aware of all these limitations.

In ventilated patients with low tidal volumes, respiration-related renal displacement can be dealt with by selecting of a probe angle limiting this motion and by the use of an advanced image stabilisation algorithm in the VueBox® software. Therefore, a breath-holding manoeuvre was not necessary.

Consistency between parameters used to determine perfusion indices was fairly good however, 25% of measurements suggested changes in perfusion in opposite directions. Further studies would be required to clarify the causes of such disagreements, how to prevent them and how to handle them.

Finally, the clinical relevance of our findings and the applicability of CEUS derived parameters remains to be determined. Indeed, as illustrated by a recent animal study [161] the relationship between renal microcirculation and renal function are complex and still poorly

understood. Our findings suggest the need for further studies aiming at understanding factors that predict changes in CEUS derived parameters and to evaluate whether the presence or absence of change in CEUS derived parameters in response to an increase in MAP are associated with specific clinical outcomes.

5.5. CONCLUSIONS

An increase in MAP as induced by noradrenaline infusion was not associated with overall changes in renal microvascular cortical perfusion as evaluated by CEUS. However, some individual patients seem to have marked responses (either increase or decrease). Further studies are required to establish whether such patients would benefit from tailored MAP targets.

CHAPTER 6

CEUS EVALUATION OF THE RENAL MICROCIRCULATION RESPONSE TO TERLIPRESSIN IN HEPATO-RENAL SYNDROME: A PRELIMINARY REPORT

DECLARATION FOR THESIS CHAPTER 5

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Participated in study design, carried out participant recruitment, performed contrast-enhanced ultrasound studies, participated in date interpretation and drafted the manuscript.	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%)**
Mark D. Goodwin[1]	participated in study design and critically reviewed the manuscript	NA
Anthony Schelleman[1]	participated in study design and critically reviewed the manuscript	NA
Michael Bailey	participated in study design, performed statistical analyses	NA
Lynne Johnson[1]	participated in study design, contributed to contrast-enhanced ultrasound studies	NA
Rinaldo Bellomo	participated to the study design, data interpretation and critically reviewed the manuscript	NA

1= From the radiology department, Austin Health, Heidelberg, Victoria, Australia

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	Date: 15th November 2014
Main Supervisor's Signature	[redacted]	Date: 15th November 2014

The second clinical situation in which CEUS could play a role in clinical practice is the hepato-renal syndrome (HRS). HRS is a rare complication of chronic liver disease in which renal function deteriorates in response liver disease. HRS is thought to be secondary to selective distribution of the blood flow to the mesenteric circulation with associated renal hypoperfusion. It therefore represents a model of purely vascular AKI.

CEUS could be of particular interest in patients with HRS to evaluate response to terlipressin therapy. Indeed, terlipressin has been demonstrated to improve outcomes in patients with HRS. Unfortunately, the drug is only effective in half the patients to which it is administered. In the absence of good predictors for response, current practice is to administer the medication and evaluate for clinical response. Such practice means exposure to a potentially dangerous and relatively expensive drug for patient who will ultimately not benefit from it. Therefore, a method enabling the determination of a patient's responsiveness to terlipressin after the administration a single dose would be of great interest.

Terlipressin is thought to act by redirecting blood flow towards the kidney by preferential vasoconstriction of the mesenteric arteries. Therefore, demonstration of an increase in renal circulation by CEUS might be a predictor of clinical response.

We have designed an exploratory study to explore the potential of CEUS to determine a clinical response to terlipressin in HRS

6. CEUS EVALUATION OF THE RENAL MICROCIRCULATION RESPONSE TO TERLIPRESSIN IN HEPATO-RENAL SYNDROME: A PRELIMINARY REPORT

6.1. INTRODUCTION

Terlipressin improves renal outcomes in type-1 hepato-renal syndrome (HRS)[162,163], a condition associated with a poor prognosis[164]. Terlipressin is thought to redirect blood flow from the splanchnic to the systemic and renal circulations, thereby, improving renal function[165]. However, only half the patients with HRS patients respond to terlipressin [166]. In the absence of robust predictors of response, the drug is usually initiated and its efficacy assessed based on changes in serum creatinine levels over the following few days. This means that some patients are exposed to potentially severe side effects [167] for no benefit and unnecessary cost. Thus, early identification of responders would be desirable.

Contrast-enhanced ultrasonography (CEUS) is a recent imaging modality enabling visualisation and quantification of organ perfusion[70,96]. Renal cortical microcirculation evaluation has been shown with CEUS in healthy volunteers [71,105] and patients undergoing cardiac surgery [107].

We hypothesized that CEUS would detect renal microcirculation changes in patients with type-1 HRS following the administration of single dose of terlipressin and conducted a proof-of-concept study.

6.2. METHODS

The study was approved by the Austin Hospital Human Research Ethics Committee (H2010/04010).

6.2.1. Patients recruitment

Patients admitted to the Austin Health were screened for inclusion criteria between July 2011 and March 2013. Inclusion criteria were established cirrhosis or acute liver failure, type-I HRS, terlipressin prescribed by their medical team in charge and provided written informed consent. Exclusion criteria were treatment limitations, hypersensitivity to Sonovue® components, clinically unstable ischemic heart disease (acute coronary syndrome or myocardial infarction within seven days), acute or chronic (class III/IV) cardiac failure, severe rhythmic disorders (defined as the occurrence of ventricular tachycardia or ventricular fibrillation within seven days), known left-to-right shunt, severe (> 90 mmHg) pulmonary hypertension, pregnancy and lactation.

6.2.2. Study design

Once enrolled, a baseline renal CEUS examination was performed and perfusion indices (PI) calculated (detailed procedure below). At the end of the procedure, 1 mg of terlipressin was administered. After two hours, renal CEUS was repeated under strictly identical conditions (same examiner, patient's position, bed head angulation, ultrasound probe position and angulation and ultrasound settings).

Additional outcomes of interest included: peak and nadir serum creatinine concentration during hospitalisation, need for renal replacement therapy, as well as location, vital status and receipt of liver transplant at day 30 and 90.

6.2.3. CEUS procedure

We used Sonovue® (Bracco, Milano, Italy) as the ultrasound contrast agent (UCA). Low mechanical index renal ultrasound was performed with a Philips IU22® ultrasound machine and a C5-1® probe. A long axis view of the kidney was obtained by placing the transducer probe over the lower back of the subject. Once adequate images of the kidney were obtained, the UCA infusion was started at 1 ml/min using a dedicated syringe pump (VueJect®, Bracco,

Milano, Italy). Image depth, focus, gain and frame rate were optimized at the first examination and held constant throughout the study. During both CEUS examinations, five destruction-replenishment sequences were obtained [66].

6.2.4. Sequences analyses

Ultrasound data sets were analysed offline with dedicated software (VueBox®, Bracco Research, Geneva, Switzerland) (Figure 1). For each sequence, one region of interest (ROI) was drawn (details for ROI selection and sequences exclusions have been described elsewhere[107]). Based on the time-intensity-curve, the software generated a perfusion index (PI), thought to be proportional to perfusion within the ROI. This PI is the ratio of the relative blood volume (RBV) over the mean transit time (mTT) and is expressed in arbitrary units (a.u.). These parameters have been described elsewhere[66,97]. For each patient and each study time, the median value for interpretable measurements was considered for analysis.

6.3. RESULTS

Four patients were included in the study (Table 1). Two patients had severe presentations and required ICU admission. One required a noradrenaline infusion and renal replacement therapy (prior to terlipressin administration). All patients had severe acute kidney injury.

Three patients (one, two and four in table 1) clinically responded to terlipressin as their serum creatinine decreased to less than 150 µmol/l. All patients were alive at to hospital discharge and at day 90. Two patient underwent orthotopic liver transplantation.

	Patient 1	Patient 2	Patient 3	Patient 4
Patients characteristics				
Age and sex	51 yo male	48 yo male	60 yo male	45 yo male
CLD etiology	Alcohol ic liver disease	Chronic HCV infection	Alcohol ic liver disease	Chronic HCV infection
Body weight (kg)	96	105	88.5	83
Hospital and ICU stay				
Hospital LOS	24	36	9	14
ICU admission (LOS)	Yes (7 days)	Yes (6 days)	No	No
APACHE III score	89	68	-	-
Documented infection	N	N	N	*Y (E. asbunriae SBP)*
Noradrenaline (duration)	Yes (84 hrs)	No	No	No
Dose max	17 µg/min			
Terlipressin				
Total number of doses	8	4	25	15
Initiated on hospital day	16	2	2	3
Acute kidney Injury				
RIFLE class	F	F	F	F
Peak creatinine (µmol/l)	389	183	653	262
Baseline creatinine (µmol/l)	84	41	120	91
Day of peak creatinine	D14	D1	D1	D1
Nadir Creatinine (µmol/l)	146 (3 days off RRT)	44	238	85
Clinical response to terlipressin?	Yes	Yes	No	Yes
Liver function tests				
Baseline INR	2.1	1.4	1.6	1.2
Peak INR during hospitalisation				
Baseline Bilirubin (mmol/l)	102	29	34	15
Peak bilirubin during hospitalisation (mmol/l)	225	101	50	124
Outcomes				
RRT (duration)	Yes (46 hrs)	No	No	No
Vital status day 30	Alive	Alive	Alive	Alive
Location at day 30	Home	Ward	Ward	Ward
Vital status at day 90	Alive	Alive	Alive	Alive
Location at day 90	Home	Ward	Home	Home
Discharge destination	Home	Rehabilitation	Home	Home
Liver transplantation within 90 days	Yes	Yes	No	No

Table 1: Patients characteristics and outcomes

CLD: chronic liver disease, HCV: hepatitis C Virus, LOS: length of stay, ICU: intensive care unit, SBP: Spontaneous bacterial peritonitis, RIFLE: risk, Injury, failure, loss and end-stage-renal failure, RRT: renal replacement therapy

6.3.1 CEUS results

All CEUS studies were well tolerated and no adverse event was noted. CEUS-derived parameters are presented in Figure 1 and still CEUS images obtained before and after terlipressin administration are presented in Figure 6.2.

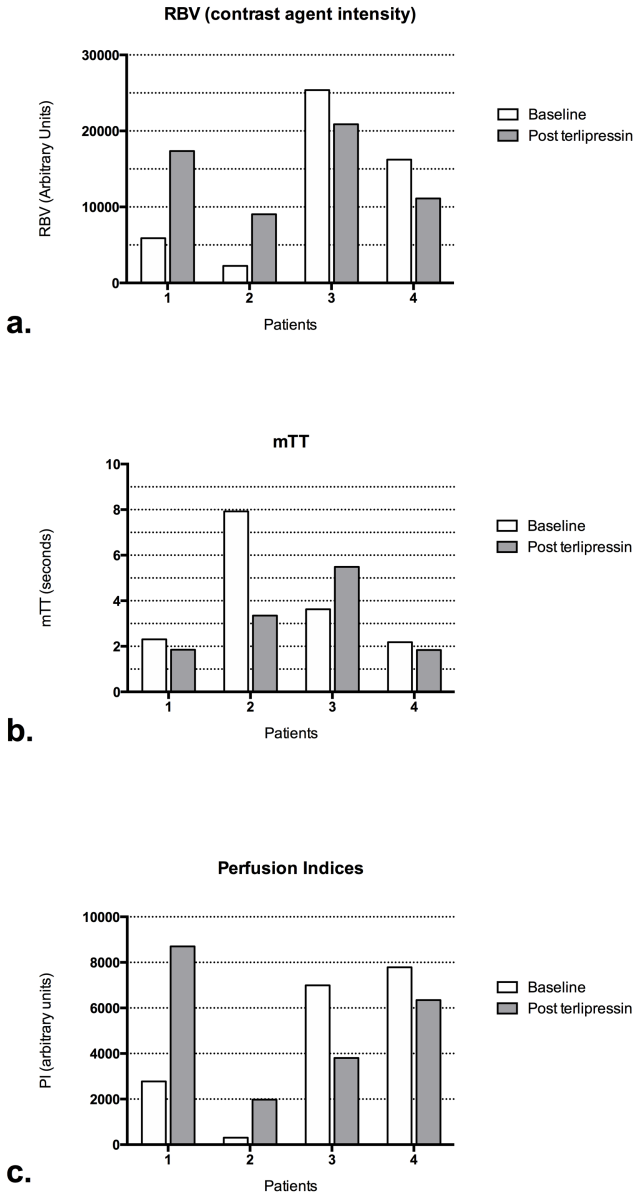


Figure 6.1: CEUS derived parameters
Panel a: RBV: indicates contrast agent intensity - increases with higher perfusion
Panel b: mTT (mean Transit Time): indicates time taken for contrast agent to travel through tissue - increases with lower perfusion
Panel c: Perfusion index: ratio of RBV/mTT

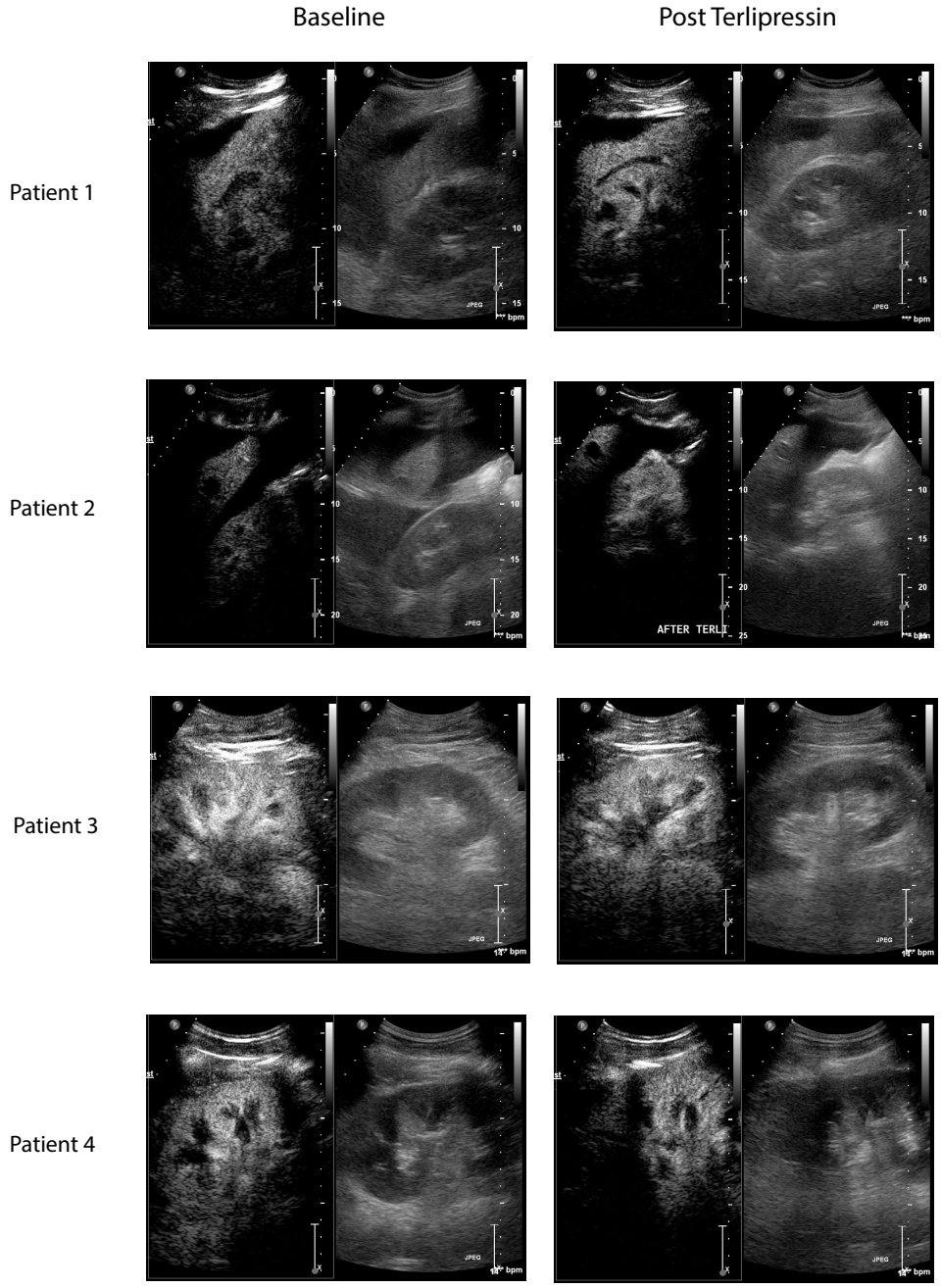


Figure 6.2: Screenshot of CEUS images: Left side of the panel: images obtained at baseline; right side of the panel images obtained two hours after intravenous administration of 1mg terlipressin. Each image is divided into two (left side: contrast enhanced image and right side: conventional "B mode" ultrasound image for orientation).

Perfusion indices

As shown in Figure 6.1, terlipressin administration was associated with a 213% and 548% increase in perfusion indices in patients 1 and 2 (respectively from 2777 to 8703 a.u. and from 304 to 1974 a.u.). It was associated with a 46% and 19% decrease in patients 3 and 4 (respectively from 6991 to 3804 a.u. and from 7786 to 6344 a.u.)

Raw parameters

Consistent with the overall changes in PI (Figure 6.1), terlipressin administration was associated with an increase in the RBV parameter (consistent with an *increase* in microcirculation perfusion) in patients 1 and 2 (respectively from 5882 arbitrary units (a.u.) to 17362 (+195%) and from 2253 to 9034 a.u.(+301%)). Terlipressin administration was associated with a decrease in RBV patients 3 and 4 (respectively from 25382 to 20887 a.u. (-18%) and from 16218 to 11113 a.u. (-31%)).

Terlipressin administration was also associated with a 58% decrease in the mTT parameter (consistent with *increased* perfusion) in patient 2; a 51% (suggestive of *decreased* perfusion) increase in patient 3 and a non-significant (<25%) change was observed in patients 1 and 4.

6.4. DISCUSSION

To the best of our knowledge, this proof-of-concept study is the first to evaluate renal cortical microvascular changes in response to terlipressin in patients with type-1 HRS. We found that CEUS was feasible and well tolerated. We also found that renal cortical perfusion indices as measured by CEUS did not increase in response to terlipressin administration in all patients with type-1 HRS. Indeed, in two patients, with very low perfusion indices at baseline, there was a several fold increase in these values. Both were clinical responders to terlipressin. Conversely, two patients, with higher perfusion indices on baseline, exhibited a modest *decrease* in their perfusion indices. Among theme, only one was a clinical responder to the therapy.

To date, the ability to predict a patient's response to terlipressin is very limited. Nazar et al [168] reported that only baseline serum bilirubin concentration (with a specificity of 61%) and an increase in mean arterial pressure *at day three of treatment* were independent predictors of response. A secondary analysis of a randomized controlled trial[169] found that baseline creatinine was the only independent predictor of response. Yet, none of these parameters are useful in clinical practice. Hence, a strategy to help determine response to terlipressin after a single dose could limit exposure to risk and cost. CEUS could be part of such a strategy. Our study, however, is limited by its small number of patients. Indeed, well documented type-1 HRS syndrome are uncommon and most series involving such patients with this condition are of limited size. CEUS derived parameters are also limited by the high heterogeneity of baseline measurements among patients due to differences in tissue depth and interstitial composition preventing direct patient comparison. This study represents a pilot and feasibility study and justifies further investigations.

Nonetheless, CEUS is a safe, fast[107], non-invasive technique which could enable bedside renal cortical microcirculation quantification in patients with HRS and be also used as a research tool. Such imaging modality could enable to better characterisation of HRS pathophysiology, an entity which remains poorly understood. Indeed, CEUS might improve current diagnostic criteria for HRS type 1, by introducing a direct evaluation of renal perfusion enabling improved differentiation from other causes of AKI. Further studies involving larger number of patients are required to confirm or refute our preliminary findings.

6.5. CONCLUSIONS

Renal cortical microcirculation evaluation with CEUS is feasible in patients with type-1 HRS. CEUS derived perfusion indices can demonstrate dramatic changes after terlipressin therapy

and perfusion responses appear variable. Larger studies are necessary to establish the sensitivity and specificity of CEUS to predict terlipressin responsiveness.

7. CONCLUSIONS

Contrast-enhanced ultrasound is a recent and promising technology. Its development and validation is ongoing. Our studies have enabled to improve technical measurements and determine a reliable protocol to obtain CEUS measurements. We have established the feasibility and safety of CEUS in critical illness. We have proposed two clinical situations in which CEUS could improve management of acutely ill patients.

Further, studies involving larger patients samples are required to establish the relationship between changes in CEUS derived parameters and clinical outcomes. Such studies could involve patients in the two clinical situations tested in our pilots studies, circulatory shock and hepato-renal syndrome.

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