BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023981
Article Type:	Protocol
Date Submitted by the Author:	09-May-2018
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Keywords:	sepsis, acute kidney injury, contrast-enhanced ultrasound, prediction

A prospective observational study protocol for examining the ability of contrast-enhanced ultrasound to predict sepsis-associated acute kidney injury

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ABSTRACT

Introduction: Sepsis-associated acute kidney injury (SA-AKI) increases morbidity and mortality among critically ill patients. This study aims to evaluate renal microcirculation perfusion after sepsis using contrast-enhanced ultrasonography (CEUS), and to explore whether CEUS is useful for predicting the risk of developing SA-AKI.

Methods and analysis: This prospective observational study will enrol patients who are diagnosed with sepsis and divide them into AKI and non-AKI groups based on the Acute Kidney Injury Network criteria. Conventional ultrasonography and CEUS scans will be performed on days 0, 1, 3, and 7 after admission to the emergency intensive care unit. The CEUS scan data will be used to create time-intensity curves that characterize renal microcirculation, including the blood volume and velocity parameters. Ultrasound results, demographic information, and routine haematology and biochemistry results will be compared between the two groups. We will also assess the patients' survival outcomes in the intensive care unit, at 1 month, at 3 months, at 1 year, and at 2 years after discharge.

Ethics and dissemination: The study protocol was approved by the ethics committee of Sir Run Run Shaw Hospital (Zhejiang University Medical College). The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Trial registration: ISRCTN 14728986.

Strengths and limitations of this study:

1. This study will be the first to evaluate contrast-enhanced ultrasonography for predicting the risk of sepsis-associated acute kidney injury.

2. This uncomplicated and non-nephrotoxic procedure can be performed at the patient's bedside, which is especially useful in the emergency setting.

3. Subjective differences, patient heterogeneity, and inter-operator variability may limit the generalizability of the findings.

INTRODUCTION

Sepsis is a syndrome than involves an over-activated systemic inflammatory response to infection. This condition can lead to multiple organ dysfunction, with the kidneys being a commonly involved organ.[1] In the field of critical care, sepsis remains a major cause of death, despite the widespread use of antibiotics and ongoing research to improve resuscitation methods.

Acute kidney injury (AKI) is characterized by the loss of kidney function,[2] and has many aetiologies that include sepsis-associated AKI (SA-AKI), ischemia reperfusion injury, extensive skin burns, severe pancreatitis, diabetic ketoacidosis, and drug poisoning.[3] Sepsis is one of the most common causes of AKI, accounting for 50% of all cases,[4] and SA-AKI is thought to involve microvascular dysfunction during sepsis that leads to both local injury and multiple organ dysfunction. Current epidemiological data indicate that AKI and chronic kidney disease are closely-related, rather than independent conditions, and that they share various risk factors. Moreover, both conditions are risk factors for cardiovascular disease,[5] with SA-AKI causing increased morbidity and mortality among critically ill patients. Thus, early prediction and recognition of SA-AKI are important steps in the early and effective initiation of proper therapy, which can prevent further complications and multiple organ dysfunction. Research regarding the pathogenesis of SA-AKI has mainly focused on inflammation and global renal blood flow, although recent attention has been paid to renal microcirculation alternations. Therefore, early detection of renal

microcirculation dysfunction during and after sepsis may facilitate subsequent treatment and prognostic prediction.

Contrast-enhanced ultrasonography (CEUS) is an imaging technique that provides real-time observations and accurate identification of blood flow to an organ of interest. Unlike conventional ultrasonography, which cannot visualize small blood vessels and low-velocity blood flow, CEUS visualizes vessels that feed a lesion or organ as well as their perfusion status. Thus, CEUS has become widely used in the emergency department for evaluating cases of abdominal blunt trauma,[6] and is useful for evaluating microcirculation perfusion in pancreatic, cardiac, hepatic, and renal transplant applications. However, the use of CEUS in sepsis cases is limited and it remains unclear whether it can provide useful information for predicting SA-AKI risk.[7, 8] Therefore, the present study aims to use CEUS at an emergency intensive care unit (EICU) to evaluate renal microcirculation perfusion after sepsis, as well as its ability to predict SA-AKI risk.

METHODS AND ANALYSIS

Study design

This prospective observational study will enrol patients with sepsis who are treated at an interdisciplinary EICU (Sir Run Run Shaw Hospital, Zhejiang University) between August 11, 2017 and September 30, 2019. All patients will be educated regarding the study's protocol and purposes, and will only be enrolled after receiving written informed consent from the patient or their family members.

Cohort descriptions

Patients with sepsis, severe sepsis, and septic shock will be screened for eligibility if they are >18 years old. The exclusion criteria are 1) an EICU stay of <24 h or the presence of a do-not-resuscitate order, 2) the presence of chronic kidney disease and long-term haemodialysis, 3) critically ill patients who have started renal replacement therapy because of AKI before their EICU admission, 4) a history of kidney transplantation, and 5) AKI caused by obstruction. Sepsis is defined as an acute change of ≥2 points in the total Sequential Organ Failure Assessment score as a result of anti-inflammatory function. Septic shock is defined as sepsis with persistent hypotension requiring vasopressors to maintain a mean arterial pressure of ⩾65 mmHg and serum lactate levels of >2 mmol/L (18 mg/dL) despite adequate resuscitation volume.[9]

We plan to recruit 200 patients, who will be divided into AKI and non-AKI groups based on their serum creatinine (SCr) levels. We will consider AKI to be present when blood urea nitrogen levels are >80 mg/dL or creatinine levels are >3 mg/dL.[10, 11] Conventional ultrasonography and CEUS will be used to monitor renal perfusion after the EICU admission.

Patient and Public Involvement

No patients were involved in developing plans for design or implementation of the study, nor were they involved in setting the research question or the outcome measures. No patients were requested to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community.

Study protocol

Demographic information and chronic disease history will be collected as baseline characteristics during the hospitalization. The Acute Physiology And Chronic Health Evaluation II score will be used to assess the severity of the disease within 24 h after EICU admission. Each patient will be followed for at least 1 week using routine haematology and biochemistry testing for complete blood count, haemoglobin, arterial blood gas, arteriovenous oxygen difference, pro-brain natriuretic peptide, plasma protein, C-reactive protein, SCr, and estimated glomerular filtration rate. Work sheets will also be maintained to record the patients' vital signs (temperature, heart rate, respiration, blood pressure, mean arterial pressure, central venous pressure, SpO_2), neurological signs (Glasgow coma score, responsiveness, Patient at Risk Score, motor response), medication, intake, and output. The dosage and frequency will be recorded for vasoactive drugs in cases of shock. Conventional ultrasonography and CEUS scans will be performed on days 0, 1, 3, and 7 after EICU admission, although the CEUS examinations will be discontinued if the patient recovers and is transferred to the floor ward or is discharged. All imaging will be performed by highly experienced EICU physicians with >10 years of experience performing bedside ultrasonography. Survival outcomes in the EICU, at 1 month, at 3 months, at 1 year, and at 2 years after discharge will be determined using outpatient visits or telephone interviews.

Conventional ultrasonography

Conventional two-dimensional ultrasonography scans will be performed using a 3.5–5 MHz convex ultrasound probe (Mindray M9, China) to evaluate the size, morphology, and macrovascular supply of the kidneys, which will be consistently imaged in the longitudinal plane. Colour Doppler flow imaging will be used to evaluate the peak systolic velocity, resistance index, and end diastolic velocities of the segmental artery, interlobar artery, and arcuate artery. Cardiac and lung functions will be assessed using echocardiography and lung ultrasonography, with data including the left ventricular ejection fraction, E-point to septal separation, B lines of the lung, and the respiratory variation of the inferior vena cava diameter.

CEUS

The CEUS will be performed immediately after the long-axis view of the kidney has been obtained using conventional ultrasonography. SonoVue® is a commercial ultrasound contrast agent (Bracco, Milan, Italy) that will be administered intravascularly with a bolus of 1–2.4 mL based on the patient's weight, height, and age. The bolus will then be followed by a 10-mL injection of physiological saline via a peripheral antecubital vein. The timer and imaging recorder will be activated simultaneously with the contrast agent injection, and the procedure will last for a total of 4–6 min. A lower mechanical index will be applied, although the mechanical parameters will be standardized for all patients. We will manually define three circular regions of interest (diameters of 1.5–2.0 cm) at the renal cortex and medulla, which are at the same approximate location, to analyse renal microcirculation perfusion. Care will be taken to avoid nearby vessels (Figure 1).

The two phase terms will be the cortical phases (from 10–15 s after injection until 30–45 s) and the medullary phases (approximately 30–45 s after injection until the microbubble echoes disappear completely).[12] The renal cortex may become enhanced earlier than the renal medulla, and a second contrast agent injection will be performed at an interval of approximately 15 min if necessary. In that scenario, the microbubbles should be destroyed during the interval using the highest acoustic power setting. Images and video clips from the CEUS will be digitally stored for subsequent quantitative analysis.

The time-intensity curves will be created using software to analyse blood volume and velocity based on the following parameters: baseline intensity (intensity before the contrast agent arrives in the microcirculation), arrival time (the time when the contrast agent appears), time-to-peak (the time to the maximum contrast intensity), peak intensity (the maximum contrast intensity value), ascending slope, descending time/2, descending slope, and area under the curve (Figure 2).[13]

Statistical analysis

All statistical analyses will be performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL) and MedCalc® software (Mariakerke, Belgium). Differences will be considered statistically significant at two-tailed *P*-values of <0.05. Categorical data will be compared using the chi-square test. Continuous data will be reported as mean ± standard deviation and compared using Student's t test.[14] Variables that are significant in the univariate analyses will be included in multivariate logistic regression analysis.[15] A receiver operating characteristic curve will also be created

to examine the ability of CEUS to predict AKI based on the sensitivity, specificity, accuracy, positive predictive, and negative predictive values.

Follow up study

Non-invasive measurements of cardiovascular functions and peripheral tissue oxygenation may be useful for critically ill patients. Transcutaneous oxygen pressure ($TcPO_2$) is a non-invasive and convenient way to detect tissue ischemia or inadequate perfusion, and may help evaluate microcirculation perfusion and predict fluid responsiveness during the follow-up of patients with SA-AKI.[16, 17] During sepsis, the skin is the simplest and most convenient tissue for evaluating microcirculation perfusion and treatment effectiveness, as it is prone to vasoconstriction during shock and is the last tissue to recover perfusion after fluid resuscitation. Furthermore, skin oxygenation can be monitored using $TcPO_2$, which might better reflect PaO_2 than SaO_2.[18] Moreover, $TcPO_2$ is closely related to PaO_2 during anaesthesia [19] and can accurately reflect local ischemic and global shock.[16] Therefore, we want to explore whether the combination of CEUS and $TcPO_2$ could be useful for evaluating the microcirculation during SA-AKI, which can facilitate early and effective treatment in the patients with circulatory failure.

DISCUSSION

AKI is common among critically ill patients and is an important cause of ICU admission with significant morbidity and mortality. The incidence of AKI in the ICU is approximately 55–60% and the mortality rate is approximately 27%.[20] Although AKI is manageable, progression to chronic renal failure can lead to tremendous

psychological and economic burdens on the patient. Thus, many studies have explored biomarkers for predicting AKI development among critically ill patients, although those studies have revealed limited predictive values.[21-23]

To the best of our knowledge, SA-AKI is a common condition that lacks an effective therapy. Continuous renal replacement therapy (CRRT) is an useful method for managing AKI,[24] although improvements in CRRT have failed to reduce the high mortality rate among patients with AKI.[25] This lack of efficacy can be related to the patient's status and the timing of CRRT,[26] with recent studies indicating that early CRRT can improve the patient's prognosis. However, there is no specific definition of early CRRT initiation, with some studies defining the timing of CRRT initiation as during the "high risk" stage or the "early injury period".[27] Therefore, it is important to identify a method that can predict SA-AKI risk and facilitate CRRT as early as possible, which could improve outcomes among patients with severe sepsis.[28]

The underlying pathophysiology of SA-AKI is complex and multifactorial, although recent studies have indicated that it may be related to microvascular dysfunction that leads to the release of microparticles, inflammatory mediators, and cytokines. For example, patients at high risk of developing SA-AKI have increased levels of microparticles,[29] vascular endothelial growth factor,[30] and endothelial progenitor cells.[31] These biomarkers can predict the occurrence of AKI and are useful during the monitoring of treatment response. However, these pathways and biomarkers have only been evaluated in the pre-clinical stage, and additional research is needed to verify their clinical utility.

Patients with SA-AKI exhibit altered blood flow to the macrovascular and microvasculature beds, with the most obvious pathophysiological change being microvascular dysfunction.[32] The main feature of microvascular dysfunction leads to the decreased nutrient delivery capillaries,which may play an important role in the development of hypoxia and multiple organ dysfunction during SA-AKI.[33, 34] Thus, CEUS may be useful for providing continuous real-time monitoring of the capillary microcirculation, and this ability is enhanced with the use of ultrasound contrast agents.[35] SonoVue® is a second-generation contrast agent that is composed of sulphur hexafluoride-filled microbubbles (2–10 μm), and is considered an ideal intravascular tracer that facilitates more sensitive, accurate, and non-invasive evaluation of capillary perfusion. Furthermore, sulphur hexafluoride is not nephrotoxic [35] and can be completely eliminated via the lungs within a few minutes after its injection, which is unlike the traditional nephrotoxic contrast agents that are used during computed tomography and magnetic resonance imaging. Finally, the phospholipid shell of the SonoVue® microbubbles is very compliant and is not destroyed at a low mechanical index (<0.16) during CEUS.[35]

A map of the kidney microvasculature can be provided with high temporal and spatial resolution using CEUS,[7] and three-dimensional CEUS can provide detailed information regarding abnormal blood flow based on perfusion volumes or lack thereof. For example, Dong et al. have indicated that CEUS might provide clinically useful tissue perfusion data for the early diagnosis of diabetic nephropathies using quantitative evaluation of renal cortex perfusion.[36] Furthermore, CEUS is widely

used in the dynamic assessment of postoperative complications, such as tissue hypoperfusion, vessel stenosis, dilatation or embolization, active bleeding, and the development of inflammation or infection.[37, 38] Tumour microvasculature and angiogenesis can also be assessed using CEUS,[39] and Fischer et al. have reported that CEUS was superior to traditional ultrasonography for identifying acute rejection after renal transplantation.[40] Wang et al. have also recently evaluated CEUS for monitoring renal microcirculation after kidney transplantation,[41] and suggested that peak systolic velocity, end diastolic velocity, resistance index, and pulsatility index from colour Doppler ultrasound scans could not differentiate between patients with normal and abnormal SCr levels. However, CEUS in that study revealed a significant inter-group difference in the renal parenchymal microcirculation, with the CEUS-based perfusion index of the transplanted kidney being correlated with renal function. Nevertheless, there are limited reports regarding the application of CEUS among patients with SA-AKI, and the present study revealed that CEUS could be combined with the SonoVue® agent, specific regions of interest, and the time-intensity curve to dynamically analyse renal microcirculation perfusion. This information may be useful for predicting the risk of developing AKI after sepsis, which could facilitate early and effective treatment that may improve the patient's prognosis.

The use of CEUS for evaluating renal microcirculation has several important advantages, such as being a relatively uncomplicated procedure that can be performed at the patient's bedside during emergency management. In addition, there is no

exposure to radiation or risk of nephrotoxicity, which can impair renal perfusion and increase the risk of nephrogenic systemic fibrosis. Moreover, CEUS is safe for patients of all ages, including children, critically ill patients, and older individuals. The Food and Drug Administration has approved CEUS for use in paediatric patients, and ongoing clinical studies are evaluating the safety of the SonoVue® agent in this setting.[42] Finally, CEUS overcomes the shortcomings of conventional B-mode sonography, which cannot detect small blood vessels and has poor sensitivity at low velocities and amplitudes.

The present study also has several limitations that will need to be considered. For example, individual-level differences may limit the generalizability of the results, and hypothermia is known to alter blood flow and ultimately the distribution of the contrast agent.[43] However, the present study will be unable to determine whether heart rate, blood pressure, pulmonary artery pressure, or cardiac chamber pressure affect enhancement after the contrast agent injection. In addition, inter-operator variability may affect the findings, which we considered a subjective error. Third, there is a fairly long interval between the CEUS examinations, which may delay the diagnosis in patients who develop SA-AKI.

In conclusion, the present study aims to use CEUS to evaluate renal microcirculation changes in patients with and without AKI, which should provide information regarding whether CEUS can predict the risk of developing SA-AKI.

Contributors: All listed authors have participated in the study, and have reviewed and approved the submitted manuscript. NL, ZZ, and YH designed this study. JD, PZ, SL, LL, BZ, XQ, and QJ drafted and submitted the report. HC, HZ, BL, YZ, and LX revised the report.

Funding: The study was supported by the Zhejiang Provincial Natural Science Foundation of China (LGF18H150005) and Scientific research project of Zhejiang Education Commission (Y201737841).

Competing interests: None declared.

Ethics approval: The study protocol was approved by the ethics committee of Sir Run Run Shaw Hospital (Zhejiang University Medical College).

Provenance and peer review: Not commissioned, externally peer reviewed.

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FIGURE LEGENDS

Figure 1: A color-coded map with circular regions of interest (ROIs) at the renal cortex and medulla.

Figure 2: A color-coded graph comparing the quantitative contrast-enhanced ultrasound parameters between the groups with and without acute kidney injury. GOF: goodness of fit, BI: baseline intensity, AT: arrival time, TTP: time-to-peak, PI: peak intensity, AS: ascending slope, DT/2: descending time/2, DS: descending slope, AUC: area under the curve.

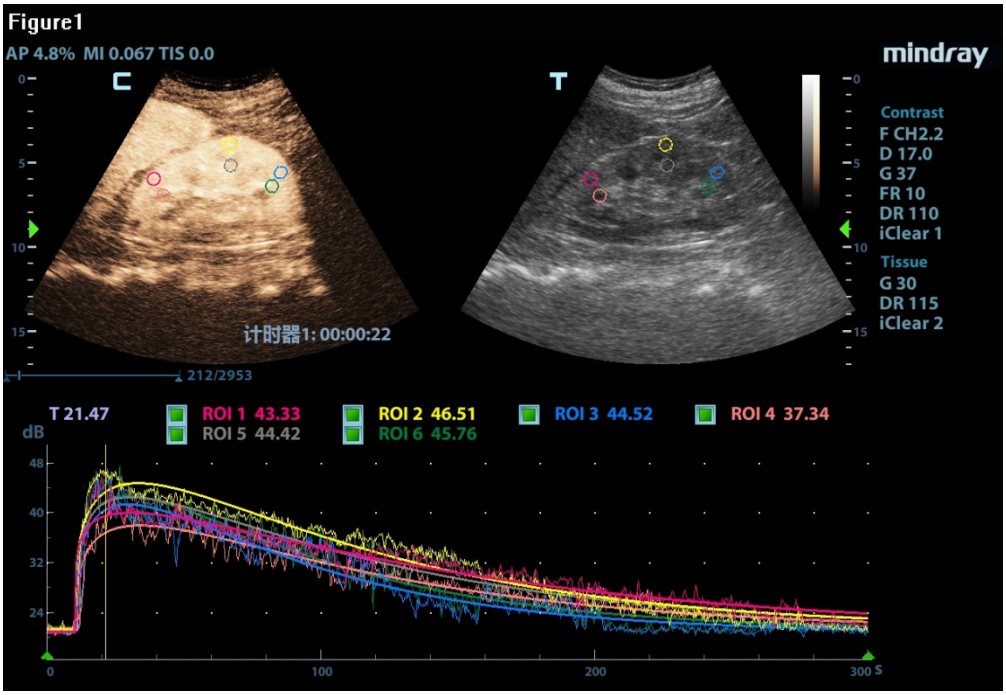

Figure 1: A color-coded map with circular regions of interest (ROIs) at the renal cortex and medulla.

106x73mm (300 x 300 DPI)

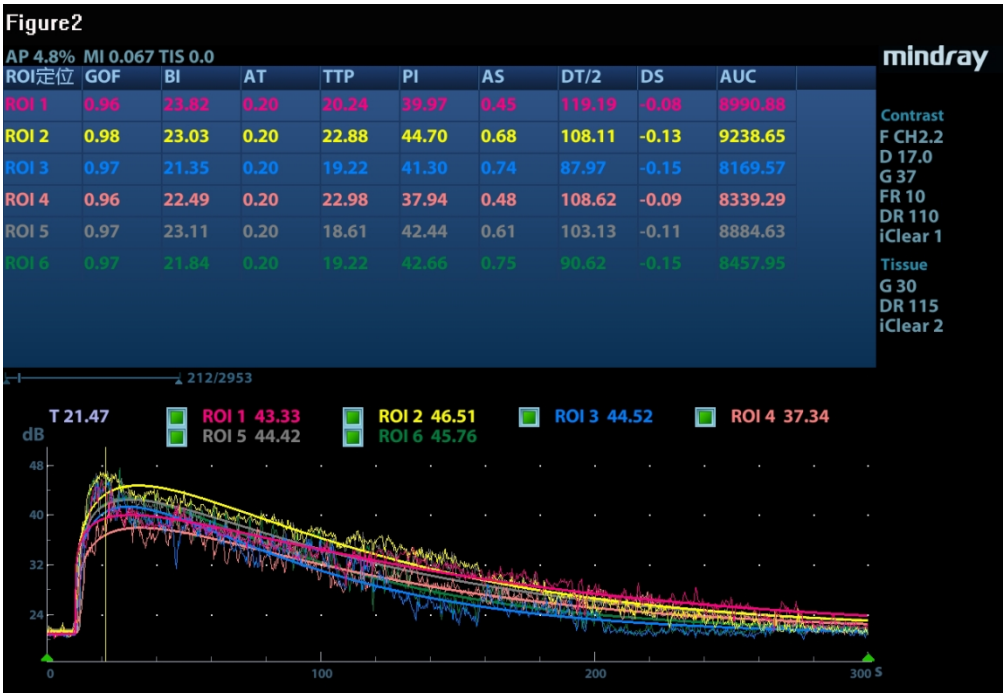

Figure 2: A color-coded graph comparing the quantitative contrast-enhanced ultrasound parameters between the groups with and without acute kidney injury. GOF: goodness of fit, BI: baseline intensity, AT: arrival time, TTP: time-to-peak, PI: peak intensity, AS: ascending slope, DT/2: descending time/2, DS: descending slope, AUC: area under the curve.

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BMJ Open

A prospective observational study protocol for examining the ability of contrast-enhanced ultrasonography to predict sepsis-associated acute kidney injury

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023981.R1
Article Type:	Protocol
Date Submitted by the Author:	19-Oct-2018
Complete List of Authors:	liu, ning; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Zhang, Zhongheng; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Hong, Yucai; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China, emergency medicine Li, Bing; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Cai, Huabo; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Zhao, Hui; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Dai, Junru; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Liu, Lian; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Qian, Xin; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Jin, Qicheng; Department of Ultrasound Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Renal medicine
Keywords:	sepsis, acute kidney injury, prediction, contrast-enhanced ultrasonography

A prospective observational study protocol for examining the ability of contrast-enhanced ultrasonography to predict sepsis-associated acute kidney injury

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ABSTRACT

Introduction: Sepsis-associated acute kidney injury (SA-AKI) increases morbidity and mortality among critically ill patients. This study aims to evaluate renal microcirculation perfusion during sepsis using contrast-enhanced ultrasonography (CEUS), and to explore whether CEUS is useful for predicting the risk of developing SA-AKI and severe SA-AKI occurrence .

Methods and analysis: This prospective observational study will enroll patients who are diagnosed with sepsis-3 definition. Conventional ultrasonography and CEUS scans will be performed on days 0, 1, 3, and 7 after admission to the emergency intensive care unit. In 7 days later, the total of septic patients were stratified into AKI (including stage 1, 2, and 3) and non-AKI groups according to Kidney Disease Improving Global Outcomes (KDIGO) criteria for further statistical analysis. The CEUS scan datas will be used to create time-intensity curves that characterize renal microcirculation, including the blood volume and velocity parameters. Ultrasonography results, demographic information, laboratory and other clinical datas will be compared. We will also assess the patients' survival outcomes in the intensive care unit, at 1 month, at 3 months, at 1 year, and at 2 years after discharge.

Ethics and dissemination: The study protocol was approved by the ethics committee of Sir Run Run Shaw Hospital (Zhejiang University Medical College). The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Trial registration: ISRCTN 14728986.

Strengths and limitations of this study:

1. This study will be the first to evaluate renal perfusion（including cortex and medullary）in patients with sepsis during their first 7 days of care using contrast-enhanced ultrasonography for predicting the risk of sepsis-associated acute kidney injury and its severity.

2. This uncomplicated and non-nephrotoxic procedure with the ability to detect subtle perfusion abnormalities quickly and in real-time has advantages over traditional modes , which is especially useful in the emergency setting.

3. Subjective differences and patient heterogeneity may limit the generalizability of the findings.

INTRODUCTION

Sepsis is a syndrome than involves an over-activated systemic inflammatory response to infection. This condition can lead to multiple organ dysfunction, with the kidneys being a commonly involved organ[1 2]. In the field of critical care, sepsis remains a major cause of mortality , despite the widespread use of antibiotics and ongoing research to improve resuscitation methods.

Acute kidney injury (AKI) is characterized by the loss of kidney function,[3] and has many aetiologies that include SA-AKI, ischemia reperfusion injury, extensive skin burns, severe pancreatitis, diabetic ketoacidosis, and drug poisoning.[4] Sepsis is one of the most common causes of AKI, accounting for 50% of all cases.[5] Many studies revealed that the possible pathophysiology of SA-AKI is profoundly different from AKI of other etiologies and SA-AKI is thought to involve macrovascular and microvascular dysfunction during sepsis that leads to both local injury and multiple organ dysfunction. Current epidemiological data indicate that SA-AKI and chronic kidney disease are closely-related, rather than independent conditions, and that even a mild or short-term of SA-AKI have tended to develop into chronic and end-stage kidney disease in the near future[6-8] ,with causing increased morbidity and mortality among critically ill patients. Thus, early prediction and recognition of SA-AKI are important steps in the early and effective initiation of proper therapy, which can prevent further complications and multiple organ dysfunction. Research regarding the pathogenesis of SA-AKI has mainly focused on inflammation and global renal blood flow, although recent attention has been

paid to renal microcirculation alternations. Therefore, early detection of renal microcirculation dysfunction during and after sepsis may facilitate subsequent treatment and prognostic prediction.

Contrast-enhanced ultrasonography (CEUS) is an imaging technique that provides real-time observations and accurate identification of blood flow to an organ of interest. Unlike conventional ultrasonography, which cannot visualize small blood vessels and low-velocity blood flow, CEUS visualizes vessels that feed a lesion or organ as well as their perfusion status. Thus, CEUS has become widely used in the emergency department for evaluating cases of abdominal blunt trauma,[9] and is useful for evaluating microcirculation perfusion in pancreatic, cardiac, hepatic, and renal transplant applications. However, the use of CEUS in sepsis cases is limited and it remains unclear whether it can provide useful information for predicting SA-AKI risk.[10 11]

Therefore, as a primary objective , the present study aims to use CEUS at an emergency intensive care unit (EICU) to evaluate renal microcirculation perfusion（including cortex and medullary） in the first 7 days of being diagnosed with sepsis. As a second objective, we expected to propose some gold standard parameters which is associated with increasing disorders in renal perfusion in four categories (no AKI, SA- AKI stage 1, stage 2, stage 3) .

METHODS AND ANALYSIS

Study design

This prospective observational study will enroll patients with sepsis who are treated at an interdisciplinary EICU (Sir Run Run Shaw Hospital, Zhejiang University) between August 11, 2017 and September 30, 2019. All conscious patients will be educated regarding the study's protocol and purposes, and will be enrolled after receiving written informed consent from the patient or their family members if patients were ventilated and sedated.

Cohort descriptions and definitions

Patients with sepsis and septic shock will be screened for eligibility if they are >18 years old. The exclusion criteria are 1) an expected stay of <24 h, 2) the presence of end-stage kidney disease or long-term haemodialysis, 3)critically ill patients who have started renal replacement therapy because of SA-AKI before their EICU admission, 4) a history of kidney transplantation, and 5) SA-AKI caused by obstruction.

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. For clinical operationalization, organ dysfunction can be represented by an acute change of ≥2 points in the total Sequential Organ Failure Assessment score. Septic shock is defined as sepsis with persistent hypotension requiring vasopressors to maintain a mean arterial pressure of ⩾65 mmHg and serum lactate levels of >2 mmol/L (18 mg/dL) despite adequate resuscitation volume.[1]

We plan to recruit 200 patients.

In order to identify patients with SA-AKI as early as possible, we could apply the Kidney Disease Improving Global Outcomes (KDIGO) guidelines to our subjects, which

stratifies SA-AKI in three different stages according to changes in creatinine and urine output (Table1)[12] . Baseline creatinine value (μmol/L) was either registered from 6 months previous clinical files or estimated, when data was not available from clinical records, by solving the Modification of Diet in Renal Disease (MDRD) equation assuming a glomerular filtration rate of 75 ml/min/1.73 $m^{(2)}$[13 14]

Table 1 Staging of Sepsis-Associated Acute Kidney Injury (SA-AKI)

Stage	Serum Creatinine	Urine Output
1	1.5–1.9 times baseline OR ≥0.3 mg/dl (≥26.5 mmol/l) increase	<0.5 ml/kg/h for 6–12 hours
2	2.0–2.9 times baseline	<0.5 ml/kg/h for ≥12 hours
3	3.0 times baseline OR Increase in serum creatinine to ≥4.0 mg/dl (≥353.6 mmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2	<0.3 ml/kg/h for ≥24 hours OR Anuria for ≥12 hours

eGFR: estimated glomerular filtration rate

Patient and Public Involvement

No patients were involved in developing plans for design or implementation of the study, nor were they involved in setting the research question or the outcome measures. No patients were requested to advise on interpretation or writing up of results. There are no

plans to disseminate the results of the research to study participants or the relevant patient community.

Study protocol

Demographic information and chronic disease history will be collected as baseline characteristics during the hospitalization. The Acute Physiology And Chronic Health Evaluation II score will be used to assess the severity of the disease within 24 h after EICU admission. Source of infection, use of mechanical ventilation before/after AKI development, and medication for treatment were also collected. Each patient will be followed for at least 7 consecutive days using routine haematology and biochemistry testing for complete blood count, haemoglobin, arterial blood gas, arteriovenous oxygen difference, pro-brain natriuretic peptide, plasma protein, C-reactive protein, SCr, and estimated glomerular filtration rate. The frequency of laboratory tests in patients with stable vital signs is once a day, however, for septic shock patients, laboratory tests may be performed every 8 hours.

Work sheets will also be maintained to record the patients' vital signs (temperature, heart rate, respiration, blood pressure, mean arterial pressure, central venous pressure, SpO_2), neurological signs (Glasgow coma score), medication, intake, and output. The dosage and frequency will be recorded for vasoactive drugs in cases of shock. Conventional ultrasonography and CEUS scans will be performed on days 0, 1, 3, and 7 after EICU admission,for septic shock patients, we performed CEUS before fluid resuscitation, and then we would re-evaluate renal microvascular alterations by the use of CEUS with

resuscitation targeted at normalization of blood pressure. All imaging will be performed by highly experienced EICU physicians with >10 years of experience performing bedside ultrasonography. Survival outcomes in the EICU, at 1 month, at 3 months, at 1 year, and at 2 years after discharge will be determined using outpatient visits or telephone interviews.

Conventional ultrasonography

Conventional two-dimensional ultrasonography scans will be performed using a 3.5–5 MHz convex ultrasound probe (Mindray M9, China) to evaluate the size, morphology, and macrovascular supply of the kidneys, which will be consistently imaged in the longitudinal plane. Colour Doppler flow imaging will be used to evaluate the peak systolic velocity, resistance index, and end diastolic velocities of the segmental artery, interlobar artery, and arcuate artery. Cardiac and lung functions will be assessed using bedside echocardiography and lung ultrasonography[15 16] , with data including the left ventricular ejection fraction, E-point to septal separation , and the respiratory variation of the inferior vena cava diameter.

Performance of CEUS

The CEUS will be performed immediately after the long-axis view of the kidney has been obtained using conventional ultrasonography. SonoVue® is a commercial ultrasound contrast agent (Bracco, Milan, Italy) that will be administered intravascularly with a bolus of 1–2.4 mL based on the patient's weight, height, and age. The bolus will then be followed by a 10-mL injection of physiological saline via a peripheral antecubital vein.

The timer and imaging recorder will be activated simultaneously with the contrast agent injection, and the procedure will last for a total of 4–6 min. A lower mechanical index will be applied, although the mechanical parameters will be standardized for all patients. We will manually define three circular regions of interest (diameters of 1.5–2.0 cm) at the renal cortex and medulla, which are at the same approximate location, to analyse renal microcirculation perfusion. Care will be taken to avoid nearby vessels (Figure 1).

The two phase terms will be the cortical phases (from 10–15 s after injection until 30–45 s) and the medullary phases (approximately 30–45 s after injection until the microbubble echoes disappear completely).[17] The renal cortex may become enhanced earlier than the renal medulla, and a second contrast agent injection will be performed at an interval of approximately 15 min if necessary. In that scenario, the microbubbles should be destroyed during the interval using the highest acoustic power setting. Images and video clips from the CEUS will be digitally stored for subsequent quantitative analysis.

The time-intensity curves will be created using software to analyse blood volume and velocity based on the following parameters: baseline intensity (intensity before the contrast agent arrives in the microcirculation), arrival time (the time when the contrast agent appears), time-to-peak (the time to the maximum contrast intensity), peak intensity (the maximum contrast intensity value), ascending slope, descending time/2, descending slope, and area under the curve (Figure 2).[18]

Statistical analysis

All statistical analyses will be performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL) and MedCalc® software (Mariakerke, Belgium). Differences will be considered statistically significant at two-tailed *P*-values of <0.05. Categorical data will be compared using the chi-square test. Continuous data will be reported as mean ± standard deviation and compared using Student's t test.[19] Variables that are significant in the univariate analyses will be included in multivariate logistic regression analysis.[20] A receiver operating characteristic curve will also be created to examine the ability of CEUS to predict AKI based on the sensitivity, specificity, accuracy, positive predictive, and negative predictive values.

DISCUSSION

SA-AKI is common among critically ill patients and is an important cause of ICU admission with significant morbidity and mortality. The incidence of SA-AKI in the ICU is approximately 55–60% and the mortality rate is approximately 27%.[21] Although SA-AKI is manageable, progression to chronic renal failure can lead to tremendous psychological and economic burdens on the patient. Thus, many studies have explored biomarkers for predicting SA-AKI development among critically ill patients, although those studies have revealed limited predictive values.[22-24]

To the best of our knowledge, SA-AKI is still a common condition that lacks an effective therapy. Logically, a better understanding of the pathophysiology of SA-AKI is critical important, which can help to develop effective measures to improve the prognosis, disease management and long-term follow-up.

The underlying pathophysiology of SA-AKI is complex and multifactorial, growing evidences suggest that patients with SA-AKI exhibit altered blood flow to the macrovascular and microvasculature beds. The global renal ischemia, cellular damage, and acute tubular necrosis [25-27]were once regarded as the three main causes leading to the change of the renal macrocirculation in SA-AKI. However, some new study demonstrated that , in the first 48 hours, pathophysiological alterations of SA-AKI may be functional rather than structural in nature[28], and potentially reversible.[29] Furthermore, histological assessment of postmortem kidneys from patients dying of SA-AKI showed an absence of acute tubular necrosis and tubular cell apoptosis.[30 31] Therefore, it is crucially important to identify the SA-AKI as early as possible within 48 hours.

Recently , some researchs indicated that renal microvascular abnormalities play a central role in SA-AKI[32]. Someone have confirmed that the onset ischemia tissue in the early stage of SA-AKI was the medulla of the kidney ,which may change several hours prior to development of oliguria and increased plasma creatinine. [33 34] Then, changes in cortical renal perfusion were heterogeneous from non-AKI to evolving SA-AKI ,even at different stages of SA-AKI development. In large animal models, including pigs and sheep, SA-AKI was considered to be associated with a hyperdynamic circulation in which the renal cortical tissue was highly perfused and oxygenated.[35 36]However, some reports suggested that the decrease in cortical renal perfusion was associated with severe AKI occurrence.[33]

In agreement with these studies, our study expected to explore changes in the renal cortex and medullary microcirculation with septic patients during their first 7 days of care. In this study, we would separate patients into four categories (no SA-AKI, stage 1, stage 2, stage 3 SA- AKI) after 7 consecutive days of monitoring , and then try to find the correlation between the change of microcirculation in kidney and the occurrence or progression of SA-AKI .

In fact, similar studies have been carried out in which the perfusion of the renal cortex is significantly reduced in patients with severe AKI occurrence (KDIGO stages 2 or 3) in course of sepsis shock by using CEUS.[37] However, they only measured cortical perfusion in patients with septic shock while medullary perfusion was not mentioned. Because renal perfusion patterns are heterogeneous in patients with sepsis, and thus, CEUS may be useful for providing continuous real-time monitoring of the capillary microcirculation, and this ability is enhanced with the use of ultrasound contrast agents.[38] SonoVue® is a second-generation contrast agent that is composed of sulphur hexafluoride-filled microbubbles (2–10 μm), and is considered an ideal intravascular tracer that facilitates more sensitive, accurate, and non-invasive evaluation of capillary perfusion. Furthermore, sulphur hexafluoride is not nephrotoxic [38] and can be completely eliminated via the lungs within a few minutes after its injection, which is unlike the traditional nephrotoxic contrast agents that are used during computed tomography and magnetic resonance imaging. Finally, the phospholipid shell of the

SonoVue® microbubbles is very compliant and is not destroyed at a low mechanical index (<0.16) during CEUS.[38]

A map of the kidney microvasculature can be provided with high temporal and spatial resolution using CEUS,[10] and three-dimensional CEUS can provide detailed information regarding abnormal blood flow based on perfusion volumes or lack thereof. For example, Dong et al. have indicated that CEUS might provide clinically useful tissue perfusion data for the early diagnosis of diabetic nephropathies using quantitative evaluation of renal cortex perfusion.[39] Furthermore, CEUS is widely used in the dynamic assessment of postoperative complications, such as tissue hypoperfusion, vessel stenosis, dilatation or embolization, active bleeding, and the development of inflammation or infection.[40 41] Tumour microvasculature and angiogenesis can also be assessed using CEUS,[42] and Fischer et al. have reported that CEUS was superior to traditional ultrasonography for identifying acute rejection after renal transplantation.[43] Wang et al. have also recently evaluated CEUS for monitoring renal microcirculation after kidney transplantation,[44] and suggested that peak systolic velocity, end diastolic velocity, resistance index, and pulsatility index from colour Doppler ultrasound scans could not differentiate between patients with normal and abnormal SCr levels. However, CEUS in that study revealed a significant inter-group difference in the renal parenchymal microcirculation in terms of the slope rate of the cortical ascending curve , the medullary ascending curve , and the peak intensity. Nevertheless, there are limited reports regarding the application of CEUS among patients with SA-AKI, and our present study revealed

that CEUS could be combined with the SonoVue® agent, specific regions of interest, and the time-intensity curve to dynamically analyse renal microcirculation perfusion. This information may be useful for predicting the risk of developing SA-AKI, which could facilitate early and effective treatment that may improve the patient's prognosis.

The use of CEUS for evaluating renal microcirculation has several important advantages, such as being a relatively uncomplicated procedure that can be performed at the patient's bedside during emergency management. In addition, there is no exposure to radiation or risk of nephrotoxicity, which would impair renal perfusion and increase the risk of nephrogenic systemic fibrosis. Moreover, CEUS is safe for patients of all ages, including children, critically ill patients, and older individuals. The Food and Drug Administration has approved CEUS for use in paediatric patients, and ongoing clinical studies are evaluating the safety of the SonoVue® agent in this setting.[45] Finally, CEUS overcomes the shortcomings of conventional B-mode sonography, which cannot detect small blood vessels and has poor sensitivity at low velocities and amplitudes.

The present study also has several limitations that will need to be considered. For example, individual-level differences may limit the generalizability of the results, and hypothermia is known to alter blood flow and ultimately the distribution of the contrast agent.[46] However, there were not enough evidences to determine whether heart rate, blood pressure, pulmonary artery pressure, or cardiac chamber pressure affect enhancement after the contrast agent injection especially for critically ill patients. In addition, inter-operator variability may affect the findings, which we considered a

subjective error. Third, there is a fairly long interval between the CEUS examinations,and thus some important microcirculatory alteration nodes may not be detected promptly..

In conclusion, the present study aims to use CEUS to evaluate renal microcirculation changes in septic patients and attempt to find out the correlation between the change of microcirculation and the occurrence or progression of SA-AKI.

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FIGURE LEGENDS

Figure 1: A color-coded map with circular regions of interest (ROIs) at the renal cortex and medulla.

Figure 2: A color-coded graph comparing the quantitative contrast-enhanced ultrasound parameters between the groups with and without acute kidney injury. GOF: goodness of fit, BI: baseline intensity, AT: arrival time, TTP: time-to-peak, PI: peak intensity, AS: ascending slope, DT/2: descending time/2, DS: descending slope, AUC: area under the curve.

Footnotes

Contributors: All listed authors have participated in the study, and have reviewed and approved the submitted manuscript. LN, ZZH, and HYC designed this study. DJR, LL, QX, and JQC drafted and submitted the report. CHB, ZH, and LB revised the report.

Funding: The study was supported by the Zhejiang Provincial Natural Science Foundation of China (LGF18H150005) and Scientific research project of Zhejiang Education Commission (Y201737841).

Competing interests: None declared.

Ethics approval: The study protocol was approved by the ethics committee of Sir Run Run Shaw Hospital (Zhejiang University Medical College， registration number: 2016C91401).

Provenance and peer review: Not commissioned, externally peer reviewed.

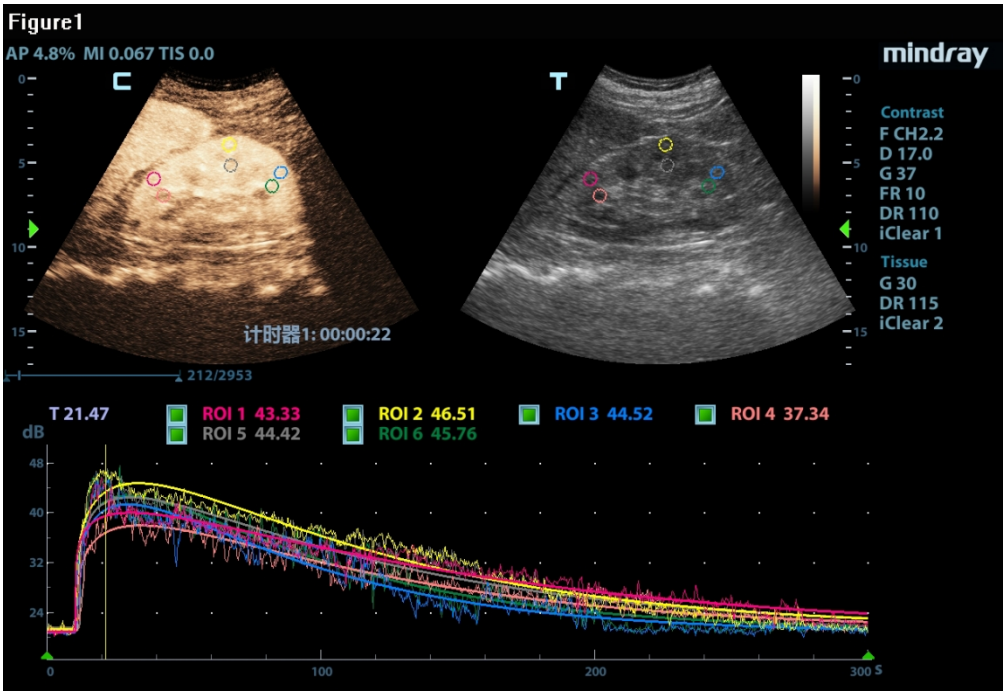

Figure 1: A color-coded map with circular regions of interest (ROIs) at the renal cortex and medulla.

106x73mm (300 x 300 DPI)

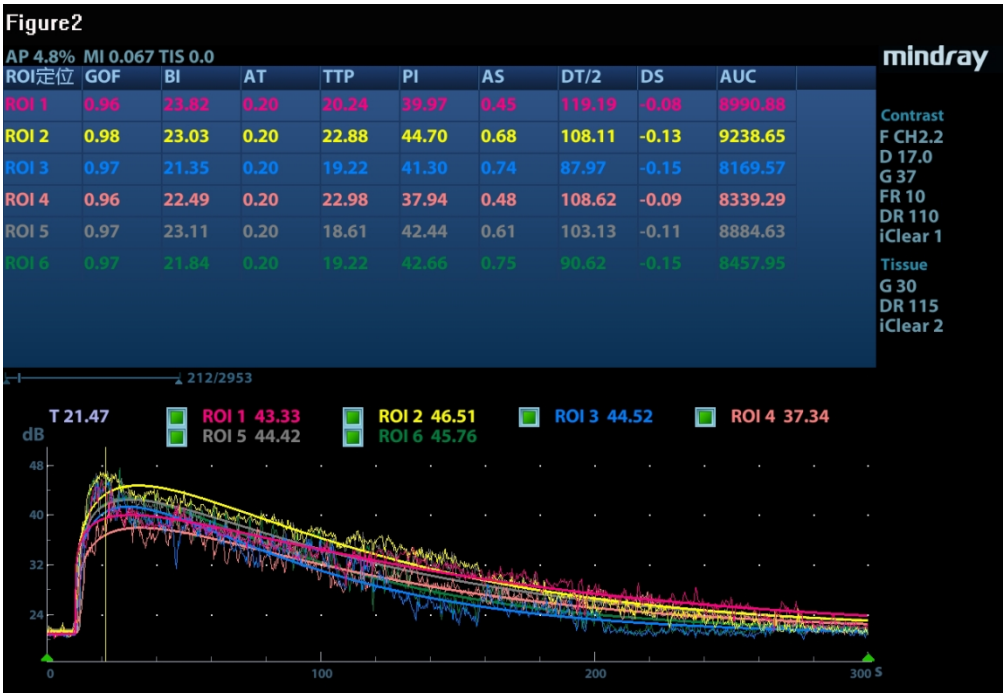

Figure 2: A color-coded graph comparing the quantitative contrast-enhanced ultrasound parameters between the groups with and without acute kidney injury. GOF: goodness of fit, BI: baseline intensity, AT: arrival time, TTP: time-to-peak, PI: peak intensity, AS: ascending slope, DT/2: descending time/2, DS: descending slope, AUC: area under the curve.

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BMJ Open

A protocol for a prospective observational study on the association of variables obtained by contrast-enhanced ultrasonography and sepsis-associated acute kidney injury

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023981.R2
Article Type:	Protocol
Date Submitted by the Author:	05-Mar-2019
Complete List of Authors:	liu, ning; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Zhang, Zhongheng; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Hong, Yucai; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China, emergency medicine Li, Bing; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Cai, Huabo; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Zhao, Hui; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Dai, Junru; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Liu, Lian; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Qian, Xin; Department of Emergency Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China Jin, Qicheng; Department of Ultrasound Medicine, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Renal medicine
Keywords:	sepsis, acute kidney injury, prediction, contrast-enhanced ultrasonography

A protocol for a prospective observational study on the association of variables obtained by contrast-enhanced ultrasonography and sepsis-associated acute kidney injury

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ABSTRACT

Introduction: Sepsis commonly results in acute kidney injury (AKI), whereas about 50% of AKI cases are due to sepsis. Sepsis-associated acute kidney injury (SA-AKI) increases morbidity and mortality especially among critically ill patients. This study aims to monitor renal microcirculation perfusion during sepsis using contrast-enhanced ultrasonography (CEUS), and to explore whether CEUS is useful for predicting the development of SA-AKI.

Methods and analysis: This prospective observational study will enroll patients who were diagnosed with sepsis-3 definition. The total of septic or septic shock patients were stratified into AKI (including stage 1, 2, and 3) and non-AKI groups according to Kidney Disease Improving Global Outcomes (KDIGO) criteria on days 0, 1, 2, and 7 after admission to the emergency intensive care unit, meanwhile, the CEUS technique will be performed to monitor renal microcirculation perfusion. A multivariable model including all CEUS variables were expected to create for predicting the development of AKI during sepsis. Ultrasonography results,

demographic information, therapeutic interventions, survival outcomes, laboratory and other clinical datas will also be collected for further analysis.

Ethics and dissemination: The study protocol was approved on 2 August 2017 by the Ethics Committee of Sir Run Run Shaw Hospital (Zhejiang University Medical College) ,(approval numbe: 2016C91401). The results will be published in a peer-reviewed journal and shared with the worldwide medical community within 2 years after the start of the recruitment.

Trial registration: ISRCTN 14728986; Pre-results

Strengths and limitations of this study:

1. In this study, we expected to monitor changes of the renal microcirculation including cortex and medullary by CEUS among patients with sepsis in their first 7 days and try to certify the possibility of redistribution of intra-renal microcirculatory blood flow.

2. The CEUS is an uncomplicated and non-nephrotoxic procedure with the rare occurrence of serious side effects and anaphylactic reactions , which should be especially considered in the intensive care unit.

3. Limited patients enrolled in the group which will make the available datas inconclusive.

INTRODUCTION

Sepsis is a syndrome that involves an over-activated systemic inflammatory response to infection. This condition can lead to multiple organ dysfunction, with the kidneys being a commonly involved organ.[1 2] Acute kidney injury is characterized by the loss of kidney function with an increase in serum creatinine and/ or a decrease in urinary output.[3] Sepsis is one of the most common causes of AKI, accounting for 50% of all cases, and up to 60% of patients with sepsis have AKI.[4 5] SA-AKI is defined by the simultaneous presence of the recent Sepsis-3 consensus criteria for sepsis[1] and the Kidney Disease Improving Global Outcomes (KDIGO) consensus criteria for AKI.[6]

Current studies indicated that SA-AKI and chronic kidney disease are closely-related rather than independent conditions, and even a mild or short-term of SA-AKI have a tendency to develop into chronic and end-stage kidney disease in the near future.[7 8] Thus, early prediction and recognition of SA-AKI are important steps in the early and effective initiation of proper therapy, which can prevent further complications and multiple organ dysfunction. Former researches regarding the pathogenesis of SA-AKI mainly focused on the renal macrocirculation resulting from global renal ischemia, cellular damage, and acute tubular necrosis.[9-11] However, recent advances have enhanced our knowledge of the pathobiology of SA-AKI. [12-14] In SA-AKI, microvascular dysfunction is characterized by a wide heterogeneity in blood fow distribution across the renal tissues and the same microcirculatory derangements have been detected in the renal capillaries.[15] Therefore, early detection of renal microcirculation dysfunction during and after sepsis may facilitate subsequent treatment and prognostic prediction.

Contrast-enhanced ultrasonography which is a relatively novel modality with microbubble-based contrast agents have provided real-time anatomic and functional information in the study of microvascular fow in patients with SA-AKI without ionizing radiation.[16] Currently, CEUS has become widely used in the emergency department for evaluating cases of abdominal blunt trauma,[17]however, the use of CEUS in SA-AK cases is limited and it remains unclear whether it can provide useful information for predicting the development.

Therefore, as a primary objective, the present study aims to use CEUS at an emergency intensive care unit to monitor renal microcirculation perfusion（including cortex and medullary）in the first 7 days of being diagnosed with sepsis.Then, we expected to create a multivariable model including all CEUS parameters to predict the development of SA- AKI.

METHODS AND ANALYSIS

Study design

This prospective observational study will enroll patients with sepsis and septic shock who are treated at an interdisciplinary EICU (Sir Run Run Shaw Hospital, Zhejiang University) between August 11, 2017 and September 30, 2019. All conscious patients will be educated regarding the study's protocol and purposes. If patients were diagnosed with septic shock or even were ventilated and sedated, informed consent will be written by their family members, instead.

Cohort descriptions and definitions

We plan to recruit 200 patients. Patients with sepsis and septic shock will be screened for eligibility if they are >18 years old. The exclusion criteria are 1) an expected stay of <24 h, 2) the presence of end-stage kidney disease or long-term haemodialysis, 3)critically ill patients who have started renal replacement therapy caused by SA-AKI before EICU admission, 4) a history of kidney transplantation, and 5) Urinary tract obstruction.

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. For clinical operationalization, organ dysfunction can be represented by an acute change of ≥2 points in the total Sequential Organ Failure Assessment score. Septic shock is defined as sepsis with persistent hypotension requiring vasopressors to maintain a mean arterial pressure of ⩾65 mmHg and serum lactate levels of >2 mmol/L (18 mg/dL) despite adequate resuscitation volume(Table 1).[1]

Table 1 Sepsis-3 definitions and quick SOFA (qSOFA) criteria

Sepsis-3 definitions
Sepsis—Life threatening organ dysfunction caused by a dysregulated host response to infection
Septic shock—sepsis with a requirement for vasoactive therapy to maintain mean arterial pressure⩾65mmHg and lactate elevation to >2mmol/L despite adequate volume resuscitation

qSOFA criteria
•Respiratory rate⩾22 breaths per minute • Altered mentation •Systolic blood pressure ≤100 mmHg

qSOFA, quick SOFA; SOFA: Sequential [Sepsis-related] Organ Failure Assessment.

In order to identify patients with SA-AKI as early as possible, we could apply the Kidney Disease Improving Global Outcomes (KDIGO) guidelines to our subjects, which stratifies SA-AKI in three different stages according to changes in creatinine and urine output (Table 2)[6]. Baseline creatinine value (μmol/L) was either registered from 6 months previous clinical files or estimated, when data was not available from clinical records, by solving the Modification of Diet in Renal Disease (MDRD) equation assuming a glomerular filtration rate of 75 ml/min/1.73 $m^{(2)}$. [18 19]

Table 2 Staging of Sepsis-Associated Acute Kidney Injury (SA-AKI)

Stage	Serum Creatinine	Urine Output
1	1.5 – 1.9 times baseline OR ≥0.3 mg/dl (⩾26.5 mmol/l) increase	<0.5 ml/kg/h for 6 – 12 hours
2	2.0 – 2.9 times baseline	<0.5 ml/kg/h for ⩾12 hours
3	3.0 times baseline OR Increase in serum creatinine to ⩾4.0 mg/dl (⩾353.6 mmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2	<0.3 ml/kg/h for ⩾24 hours OR Anuria for ⩾ 12 hours

eGFR: estimated glomerular filtration rate

Patient and Public Involvement

No patients were involved in developing plans for design or implementation of the study, nor were they involved in setting the research question or the outcome measures. No patients were requested to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community.

Data collection

Demographic information and chronic disease history will be collected as baseline characteristics during the hospitalization. The Acute Physiology And Chronic Health Evaluation II score will be used to assess the severity of the disease within 24 h after EICU admission. Source of infection, use of mechanical ventilation before/after AKI development, and therapeutic interventions (the volume and kind of fluid resuscitation , vasopressor therapy, antimicrobial therapy, renal replacement therapy) will be also collected. Each patient will be followed for at least 7 consecutive days using routine haematology and biochemistry testing for complete blood count, haemoglobin, arterial blood gas, arteriovenous oxygen difference, pro-brain natriuretic peptide, plasma protein, C-reactive protein, SCr, and estimated glomerular filtration rate. The frequency of laboratory tests in patients with stable vital signs is once a day, however, for septic shock patients, laboratory tests may be performed every 8 hours.

Work sheets will also be maintained to record the patients' vital signs (temperature, heart rate, respiration, blood pressure, mean arterial pressure, central venous pressure, SpO_2), neurological signs (Glasgow coma score), medication, intake, and output. The dosage and frequency will be recorded for vasoactive drugs in cases of shock. Conventional B-mode ultrasonography and CEUS scans will be performed on days 0, 1, 2, and 7 after EICU admission. For septic shock patients, we performed CEUS before fluid resuscitation, and then we would re-evaluate renal microvascular alterations by the use of CEUS with resuscitation targeted at normalization of

blood pressure(mean arterial pressuree of CE). All ultrasonography examinations were performed by the collaboration of experienced EICU physicians and ultrasonologist. Perfusion time intensity curve (TIC) analysis [20] was performed using the integrated computer workstation (LOGIQ E9, GE). Clinical and biochemical data were retrieved from a clinical database. Data collection was permitted by the ethical review committee of the Sir Run Run Shaw Hospital (Zhejiang University Medical College). Survival outcomes in the EICU, at 1 month, at 3 months, at 1 year, and at 2 years after discharge will be determined using outpatient visits or telephone interviews.

Conventional B-mode ultrasonography

Conventional B-mode ultrasonography scans will be performed using a 3.5–5 MHz convex probe (Mindray M9, China) to evaluate the size, morphology, and macrovascular supply of the kidneys, which will be consistently imaged in the longitudinal plane. Colour Doppler flow imaging will be used to evaluate the peak systolic velocity, resistance index and end diastolic velocities of the segmental artery, interlobar artery and arcuate artery. Cardiac and lung functions will be assessed using bedside echocardiography and lung ultrasonography ,[21 22] with data including the left ventricular ejection fraction, E-point to septal separation, and the respiratory variation of the inferior vena cava diameter.

Performance of CEUS

The CEUS will be performed immediately after the long-axis view of the kidney has been obtained using conventional B-mode ultrasonography. SonoVue® is a commercial ultrasound contrast agent (Bracco, Milan, Italy) that will be administered intravascularly with a bolus of 1–2.4 mL based on the patient's weight, height, and age. The bolus will then be followed by a 10-mL injection of physiological saline via a peripheral antecubital vein. The timer and imaging

recorder will be activated simultaneously with the contrast agent injection, and the procedure will last for a total of 4–6 min. A lower mechanical index will be applied, although the mechanical parameters will be standardized for all patients.We will manually define three circular regions of interest (diameters of 1.5–2.0 cm) at the renal cortex and medulla, which are at the same approximate location, to analyse renal microcirculation perfusion. Care will be taken to avoid nearby vessels (Figure 1). A second contrast agent injection will be performed at an interval of approximately 15 min if necessary. Images and video clips from the CEUS will be digitally stored for subsequent quantitative analysis.

The time-intensity curves will be created using software to analyse blood volume and velocity based on the following parameters: baseline intensity (intensity before the contrast agent arrives in the microcirculation), arrival time (the time when the contrast agent appears), time-to-peak (the time to the maximum contrast intensity), peak intensity (the maximum contrast intensity value), ascending slope, descending time/2, descending slope, and area under the curve (Figure 2).[23]

Statistical analysis

All statistical analyses will be performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL) and MedCalc® software (Mariakerke, Belgium). Differences will be considered statistically significant at two-tailed *P*-values of <0.05. Categorical data will be compared using the chi-square test. Continuous data will be reported as mean ± standard deviation and compared using Student's t test.[24] Variables that are significantly different between AKI and non-AKI groups in the univariate analyses will be included in multivariate logistic regression analysis.[25] All CEUS parameters that are statistically significant in univariate analysis will be included in a multivariable regression model to establish a combined score for the predictionof SA-AKI. [26]

A receiver operating characteristic curve will also be created to examine the diagnostic performance of CEUS (a multivariable model including all CEUS parameters) to predict AKI. Other diagnostic statistics such as the sensitivity, specificity, accuracy, positive predictive, and negative predictive values will be reported for the model.

DISCUSSION

SA-AKI is common among critically ill patients and is an important cause of ICU admission with significant morbidity and mortality. The incidence of SA-AKI in the ICU is approximately 55–60% and the mortality rate is approximately27%.[27] Volume resuscitation, vasopressor therapy, antimicrobial therapy, and renal replacement therapy remain the mainstays of the management for SA-AKI in the ICU.[28] Although SA-AKI is manageable, progression to chronic renal failure can lead to tremendous psychological and economic burdens on the patient. Thus, a better understanding of the pathophysiology of SA-AKI is essential in order to improve the prognosis, disease management and long-term follow-up.

The underlying pathophysiology of SA-AKI is complex and multifactorial. Evidences suggested that the global renal ischemia, cellular damage, and acute tubular necrosis [9-11]were once regarded as the three main causes leading to the change of the renal macrocirculation in SA-AKI. At present , a growing body of experimental and clinical evidence now shows that, at least in the early phase of SA-AKI, renal blood flow is normal or even increased.[14 29] Some new study demonstrated that , in the first 48 hours, pathophysiological alterations of SA-AKI may be functional rather than structural in nature[30], and potentially reversible.[31]Therefore, it is crucially important to identify the SA-AKI as early as possible within 48 hours.

To the best of our knowledge , the levels of serum creatinine and blood urea nitrogen are the classical index to evaluate kidney function, but with much higher delayed diagnosis and missed

diagnosis rate.[32]Currently, there are still no diagnostic tools for the earlier detection of the SA-AKI development, although some studies have revealed limited predictive values.[33-35] [30][31][36 37]Recently, a major researchs indicated that renal microvascular abnormalities play a central role in SA-AKI.[36-39] Disturbances in microcirculatory oxygen delivery may include both decreased fow and diffusion limitation in the setting of organ edema and inflammation.[40]The microcirculatory derangements such as capillary plugging and micro-thrombi have been detected in the renal capillaries in animal models of sepsis-associated acute kidney injury.[41 42]

Previously, CEUS measurement as a diagnostic tool in monitoring renal microcirculatory perfusion have shown promising results, however, most of them provided information regarding cortical perfusion.[43-46] Most recently, someone have confirmed that the onset ischemia tissue in the early stage of SA-AKI was the medulla of the kidney which may change several hours prior to development of oliguria and increased plasma creatinine. [47 48] Medullary hypoxia due to intrarenal blood fow redistribution may be one of the factors causing AKI in sepsis.[47] Another study, in an experimental model in mice subjected to ischemia-reperfusion injury (IRI) demonstrated that CEUS was able to monitor changes in renal microvascular perfusion in space and time. They reported that the outer medullary perfusion decreased disproportionately to the reduction in the cortical and inner medullary perfusion after ischemia.[49] The outer medulla appears to be particularly sensitive to development of hypoxia, because of its poor blood supply and the large amount of reabsorptive work performed in the proximal tubules and thick ascending limbs of the loop of Henle.[50]

In line with these studies, our study expected to monitor changes in the renal cortical and medullary microcirculation among septic patients on days 0, 1, 2, and 7 after admission to the

emergency intensive care unit. At the same time, we would separate patients into four categories (no SA-AKI, stage 1, stage 2, stage 3 SA- AKI) according to the consensus criteria for sepsis and AKI, and then try to find the correlation between the change of microcirculation in kidney and the development of SA-AKI.

The CEUS for evaluating renal microcirculation has several important advantages, such as being a relatively uncomplicated procedure that can be performed at the patient's bedside during emergency management. Furthermore, a map of the kidney microvasculature can be provided with high temporal and spatial resolution.[51] In addition, there is no exposure to radiation or risk of nephrotoxicity, which would impair renal perfusion and increase the risk of nephrogenic systemic fibrosis. CEUS can be safe for the critically ill patients and even for the paediatric patients.[52]

The present study also has several limitations that will need to be considered. Firstly, the regions of interests of the perfusion map were selected subjectively in the kidney and this might very well represent local micro-heterogeneities.[53] To minimize this parameter, Three ROIs were drawn for each experimental time point and the results averaged in order to minimize heterogeneity of measurement. Secondly, this is a prospective observational study conducted in a 10-bed emergency intensive care unit and only 200 patients were to be enrolled in the study , therefore, these available datas still remains inconclusive and needs to be further validated by multicenter cooperation.

This narrative protocol aims to use CEUS to monitor the renal microcirculation changes in septic patients and attempt to find out the correlation between the changes of microcirculation and the development of SA-AKI.

FIGURE LEGENDS

Figure 1: A color-coded map with circular regions of interest (ROIs) at the renal cortex and medulla.

Figure 2: A color-coded graph comparing the quantitative contrast-enhanced ultrasound parameters between the groups with and without acute kidney injury. GOF: goodness of fit, BI: baseline intensity, AT: arrival time, TTP: time-to-peak, PI: peak intensity, AS: ascending slope, DT/2: descending time/2, DS: descending slope, AUC: area under the curve.

Footnotes

Contributors: All listed authors have participated in the study, and have reviewed and approved the submitted manuscript. LN, ZZH, and HYC designed this study. DJR, LL, QX, and JQC drafted and submitted the report. CHB, ZH, and LB revised the report.

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Competing interests: None declared.

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Provenance and peer review: Not commissioned, externally peer reviewed.

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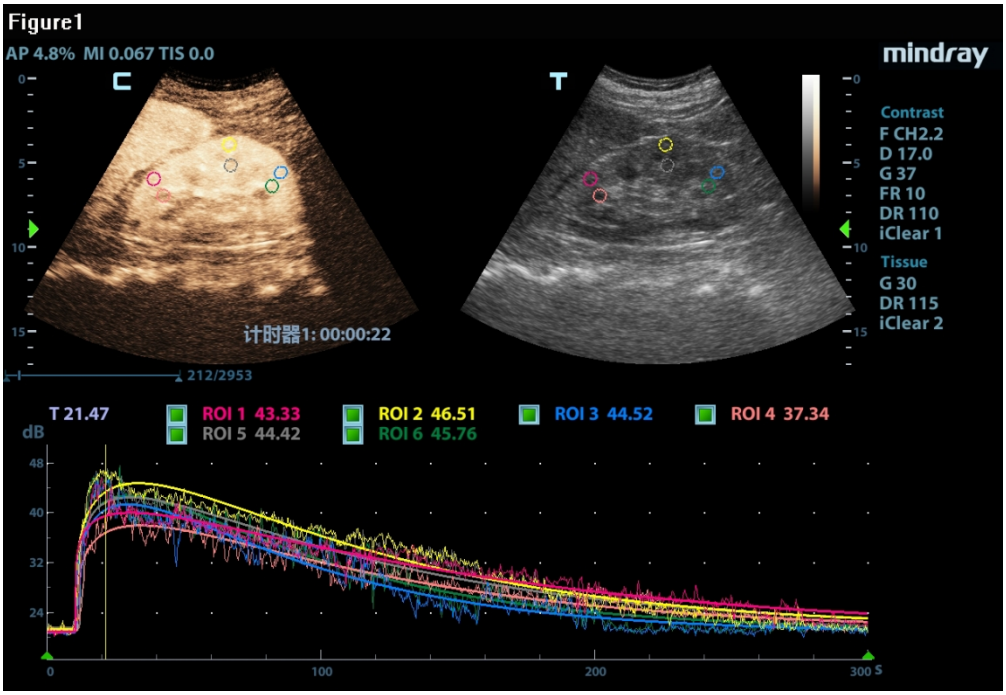

Figure 1: A color-coded map with circular regions of interest (ROIs) at the renal cortex and medulla.

106x73mm (300 x 300 DPI)

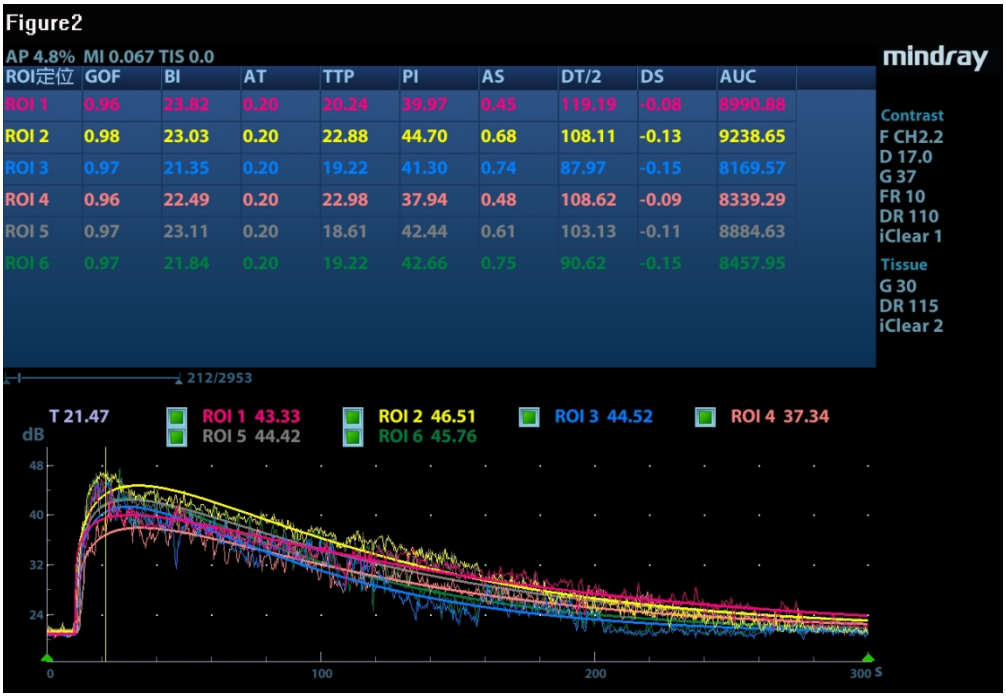

Figure 2: A color-coded graph comparing the quantitative contrast-enhanced ultrasound parameters between the groups with and without acute kidney injury. GOF: goodness of fit, BI: baseline intensity, AT: arrival time, TTP: time-to-peak, PI: peak intensity, AS: ascending slope, DT/2: descending time/2, DS: descending slope, AUC: area under the curve.

106x73mm (300 x 300 DPI)