

Renal circulation and microcirculation during intra-abdominal hypertension in a porcine model

F. SUI¹, Y. ZHENG¹, W.-X. LI¹, J.-L. ZHOU²

¹Department of Surgical Intensive Care Unit, ²Department of Orthopedic Surgery; Beijing Chaoyang Hospital Affiliated to Capital Medical University, Beijing, China

Abstract. – OBJECTIVE: The purpose of the study is to further investigate the effects of increased intra-abdominal pressure (IAP) on renal hemodynamics and renal perfusion, and to evaluate the renal cortical and sublingual microcirculation by sidestream dark field (SDF) imaging, both with a porcine model.

MATERIALS AND METHODS: IAP was increased stepwise to 10, 15, 20, 25 mmHg, during which hemodynamic parameters, urinary output, renal contrast-enhanced ultrasound (CEUS), sublingual and renal SDF videos were all recorded from 12 pigs.

RESULTS: Wash in time (WT) and time to peak (TTP) prolonged significantly ($p < 0.05$), while peak intensity (PI) wash in slope (WS) and AUC (area under curve) in CEUS declined significantly ($p < 0.05$) compared with baseline when IAP elevated to 25 mm Hg. With an increase of abdominal pressure, sublingual microvascular flow index (MFI) drop significantly, especially upon IAP was over 20 mmHg. But other parameters such as the total vascular density (TVD), De Backer Score, proportion of perfused vessels (PPV), perfused vessel density (PVD), and heterogeneity index (HI) of tongue were not significantly changed. With increasing IAP, renal vascular resistance increased and MFI decreased about 30%. RFG, instead of RFG showed a moderate correlation with AUC ($R = 0.47$, $p < 0.05$) and MFI ($R = 0.49$, $p < 0.05$).

CONCLUSIONS: CEUS is a safe, real-time dynamic, noninvasive and simple technique to evaluate renal microvascular perfusion in intra-abdominal hypertension. Descending slope, PI and AUC can be used for the diagnosis of the renal microvascular damage in a porcine model of IAP-induced renal impairment. Also, SDF on the surface of the kidney is a useful tool to evaluate the microcirculation of kidney but sublingual SDF imaging was barely useful.

Key Words:

Intra-abdominal pressure, Intra-abdominal hypertension, Sidestream dark field imaging, Sublingual microcirculation, Contrast-enhanced ultrasound, Pig.

Introduction

Abdominal compartment syndrome (ACS) and intra-abdominal hypertension (IAH) are increasingly recognized as potential complications in intensive care unit (ICU) patients. ACS/IAH affects blood flow to various organs and plays a significant role in the prognosis of the patients. It was not until 2006 that the World Society on Abdominal Compartment Syndrome (WSACS) established consensus definitions for intra-abdominal hypertension (IAH) and for abdominal compartment syndrome (ACS), which is revised in 2013^{1,2}. Thus, “the intra-abdominal pressure (IAP) is defined as the steady-state pressure concealed within the abdominal cavity”, while “ACS is a sustained IAP > 20 mmHg [with or without abdominal perfusion pressure (APP) < 60 mmHg] that is associated with a new organ dysfunction/failure”. ACS and IAH affect the overall body systems, most notably the cardiac, respiratory, renal, and neurologic systems. However for now, the pathophysiologic consequences of IAH are not uniformly understood.

It is well known that IAH can affect cardiac output and blood supply of abdominal organs, yet the relationship between systemic hemodynamics, renal circulation and renal microcirculation remains a matter of debate. The increasing abdominal pressure severely decreases abdomi-

nal perfusion pressure, which is calculated as the difference between the mean arterial pressure (MAP) and IAP and determines the blood flow into abdominal organs and tissues. Thereby abdominal pressure can be used in predicting organ failure and outcomes (3,4), and has been suggested as a surrogate marker to assess hemodynamic changes related to IAH. However, it should be noted that the correlation between APP and renal perfusion or microcirculatory perfusion has never been reported during the process of IAP elevation. Although the characteristics of renal arterial blood flow in such setting have been well described, on the change of renal microcirculation has been rarely studied. Most of the studies used invasive transonic devices or transit-time flow probe to investigate the kidney blood flow, but as presented from an animal model, the artery is vulnerable and the limitation that such procedure may change the “normal state”, the kidney blood flow cannot “truly” reflect the physiological abnormalities. Nowadays, ultrasound, especially contrast-enhanced ultrasound (CEUS), is considered to be an accurate, rapid and repeatable examination for detecting renal perfusion change in early stage of diseases^{5,6}. It has been applied as an important tool to enable the study of severity, timing, and change over time of renal perfusion as well as the intra-renal distribution of perfusion abnormalities. However, to the best of our knowledge, there is no relative ultrasonic sonographic data in IAH state reported yet, which promoted us to do this study in hope to add more useful information to bedside physicians.

In addition to systemic hemodynamic alterations, microvascular alterations are frequently observed in critically ill patients⁷. As microcirculation is the place of oxygen exchange, its dysfunction in critical states can contribute to cellular hypoxia even when global oxygen delivery is still preserved. It is confirmed that these alternations are associated with organ dysfunction and impairment⁷. Sidestream dark field (SDF) imaging is available as an optical microscopic technique for visualizing microcirculation on organ surfaces⁸ and has benefited investigators in studying microcirculatory alterations in critically ill patients. Microcirculatory change is associated with a development of organ failure and mortality, especially death due to sepsis^{9,10}. The sublingual area is the vascular bed with most easy access for the visualization of microcirculation is the sublingual area. Recent studies¹¹ have demonstrated that the microcirculatory indices detected from this area may

be used as surrogate measures for splanchnic blood flow condition, as the tongue and related areas share a common embryonic origin with gut, and there is a close correlation between sublingual capnometry and gastric tonometry. Also, some studies¹² have used SDF method to describe renal cortical microcirculation. But, there is no data about the relationship between the microcirculation of kidney and tongue that commonly monitored with SDF in clinics.

In this study, SDF is used to visualize sublingual capillary in the setting of elevated IAP. The relations among indicators including IAP, APP, hemodynamic parameters and microcirculation are investigated with IAH porcine models. We raised the hypothesis that increased IAP causes alterations in the renal cortical microcirculation, and these changes are related to the parameters of CEUS, which can be further utilized in the clinic.

Materials and methods

Ethical Considerations

This study was approved by the Animal Investigation Committee at the Capital Medical University, Beijing, China.

Experimental Preparation

After overnight fasting with free access to water, 12 domestic pigs (mean body weight of 35 ± 5 kg) supplied by Institute of Animal Sciences, Chinese Academy of Agricultural Sciences were pre-medicated with midazolam (1-1.5 mg/kg intramuscularly) one hour before induction of anesthesia. Anesthesia was maintained with a continuous infusion of propofol (6-8 mg·kg⁻¹·h⁻¹) and fentanyl (0.01 mg·kg⁻¹·h⁻¹) given via a peripheral catheter into an ear vein. Movement, motor response to painful stimulus, and heart rate were monitored to assess the depth of anesthesia during the experiment. The piglets were positioned supine after anesthesia. After tracheotomy, trachea cannula (internal diameter, 7.5 mm) was inserted. Piglets were ventilated using a volume-controlled mode (Evita 4, Dräger, Lübeck, Germany) with 8 ml/kg tidal volume, a positive end-expiratory pressure of 5 cmH₂O, a respiratory rate of 15 breaths/min; inspiration: expiration ratio of 1:1.5 and a fraction of inspired oxygen of 40%. The respiratory rate was adjusted to maintain the pressure of end-tidal CO₂ from 35 to 45 mmHg.

A 5F thermodilution PCCO catheter (PV 2014L16, Pulsion Medical Systems AG, Munich, Germany) was placed in the descending aorta via the right femoral artery. Via a mid-neck incision, a two-lumen catheter (Arrow-Flex, Arrow International, Reading, PA, USA) was placed in the right external jugular vein.

An ultrasound-guided catheter was inserted into the bladder by a transcutaneous suprapubic puncture to monitor urinary output (UO). All catheters were fixed carefully. After that, a left mini-lumbotomy was performed, and the left kidney surface was identified retroperitoneally to assess the cortical microcirculation.

To induce IAH, a trocar was inserted then connected to an electronic variable-flow insufflator (UHI-4, Olympus Corporation, Tokyo, Japan) for direct intra-abdominal pressure measurement and induction of IAH by nitrogen pneumoperitoneum. Ringer's solution was administered at a constant rate of 10 ml/kg/h throughout the experiment. Body temperature was maintained between 37°C and 39°C with a heating blanket. On average, each pig received just under 5000 ml during the experiment.

Experimental Protocol

A total of 12 pigs were studied (mean weight, 32 kg; range, 30-35 kg). 30 minutes after induction of anesthesia, control hemodynamic measurements were performed. A timeline of the experimental procedure is provided in Figure 1. After the preparation, 30 minutes were allowed for stabilization. The baseline values were recorded. The average values of hemodynamic parameters were taken by PiCCO monitor after 10 ml of iced normal saline (<8°C) being injected for three times repeatedly. Sublingual microcirculation was visualized by an SDF imaging device (Microscan; Microvision Medical, Amsterdam, The Netherlands). Three videos in 3 different fields were captured. The protocol was then repeated at an IAP of 15, 20 and 25 mm Hg by nitrogen pneumoperitoneum. Each state was maintained for about one and half an hour. The animals were sacrificed at the end of the experiment by overdose pentobarbital. The total duration of each experiment was about 9 hrs.

Measurements and Calculations: Global Hemodynamics

Mean arterial pressure (MAP), heart rate (HR), and central venous pressure (CVP) were measured. All pressures were zeroed at the mid-

chest level. CO was taken by PiCCO monitor after 10 ml of iced normal saline (<8°C) being injected via the port. Three repeated measurements were performed to calculate the average value. All injections of iced normal saline were performed by the same person. The interval between two injections was more than 70 seconds. If one measurement differed from the average by more than 10%, that measurement would be replaced by a second measurement and a new average would be obtained. Systemic vascular resistance (SVR) was calculated as $(\text{MAP}-\text{CVP}) \times 80/\text{CO}$.

Measurements and Calculations: Renal Hemodynamics and Renal Function

The kidneys were scanned using a 10-5 MHz transducer and IU22 ultrasound system (Philips, Amsterdam, The Netherlands). The frequency, gain, focus point and focus scope were adjusted in the area of kidney cortex so that the images of microvascular perfusion were displayed perfect-

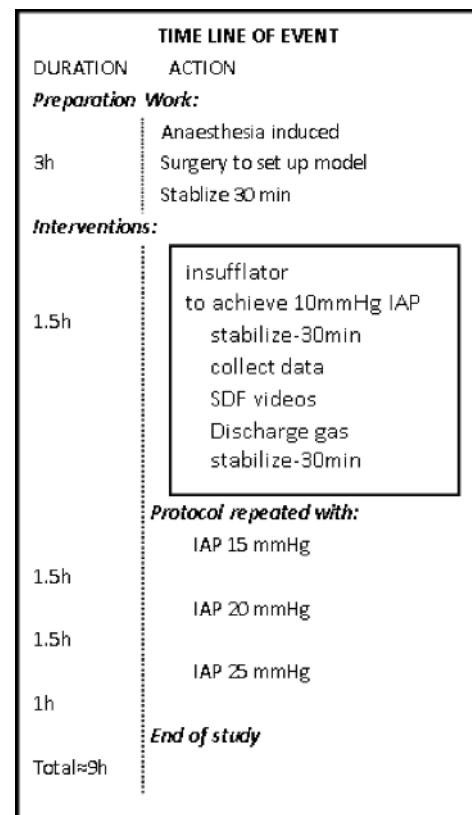


Figure 1. Outline of the experimental protocol showing the order and duration of each step. IAP, intra-abdominal pressure.

Table I. Haemodynamic parameters at various intra-abdominal pressure stages.

Haemodynamic	Parameters baseline	IAP 10 mmHg	15 mmHg	20 mmHg	25 mmHg
MAP (mmHg)	96.42 ± 17.48	97.33 ± 19.25	93.67 ± 16.87	89.83 ± 14.74 ^a	81.08 ± 14.60 ^a
APP (mmHg)	95.42 ± 17.48	87.33 ± 19.25	78.67 ± 16.87 ^a	69.83 ± 14.74 ^a	56.08 ± 14.60 ^a
RFG (mmHg)	94.42 ± 17.48	77.33 ± 19.25	63.67 ± 16.87 ^a	49.83 ± 14.74 ^{ab}	31.08 ± 14.60 ^{abc}
HR (beats/min)	99.00 ± 15.89	106.00 ± 18.04	110.08 ± 13.65	108.33 ± 7.33	117.83 ± 13.04 ^a
CVP (mmHg)	3.50 ± 2.60	5.67 ± 2.95 ^a	9.50 ± 2.72 ^{ab}	12.33 ± 2.81 ^{ab}	15.83 ± 2.82 ^{ab}
GEDV (ml)	421.75 ± 138.09	417.33 ± 158.91	404.42 ± 144.32	407.25 ± 148.99	415.33 ± 155.40
SVR (dyn·s/cm ⁵)	2164.27 ± 347.78	2141.92 ± 352.34	1961.98 ± 323.94	1849.73 ± 270.08	1855.52 ± 363.00
CO (L/min)	3.48 ± 0.65	3.45 ± 0.61	3.48 ± 0.75	3.37 ± 0.57	2.87 ± 0.69 ^a

All the data are presented as mean ± SD. IAP, intra-abdominal pressure; MAP, mean arterial pressure; HR, heart rate; CVP, central venous pressure; GEDV, Global End-Diastolic Volume; SVR, systemic vascular resistance; CO, cardiac output; a, $p < 0.05$ vs. baseline; b, $p < 0.05$ vs. IAP at 10 mmHg; c, $p < 0.05$ vs. IAP at 15 mmHg; d, $p < 0.05$ vs. IAP at 20 mmHg.

ly. The ultrasound transducer was mounted on a fixed stand with a flexible arm. SonoVue (Bracco, Milan, Italy) was prepared according to the manufacturer's instructions. According to the aseptic procedure, 5 mL normal saline was injected into the ampule contained 25 mg of freeze-dried powder. Then, the ampule was shaken in order to form microbubbles suspension. About 1.2 mL (dosage: 0.05 mL/kg) of suspension was used for quick infusion into the central vein. Then, 5 mL of normal saline was immediately used for infusion. While the contrast agent was injected, the images were collected simultaneously. The major section of the kidney was selected and the echo change from the kidney was observed in real-time for 2 min. The dynamic image changes were continuously recorded. The perfusion intensity and equality of the kidney were observed. ROI (region of interest) was drawn over the superficial peripheral cortex of kidney in the same depth with the same size and shape. The software QLAB was applied to analysis time-intensity curve (TIC).

Measurements and Calculations: Sublingual and Renal Cortical Microcirculation

At every IAP level, the optical probe was placed gently on the right kidney surface and the sublingual mucosa after the removal of saliva with a gauze swab. Two videos in 3 different fields were captured, while precautions were taken to avoid pressure and moving artifacts when capturing movies. Every 6 videos were taken at the tongue and the kidney at each time point in total. Microcirculatory videos were analyzed with the aid of specialized software (Au-

tomated Vascular Analysis 3.02; Academic Medical Centre, University of Amsterdam, The Netherlands) every ten seconds by two separate investigators who were blind to the study protocol. The Automated Vascular Analysis software gives the following parameters including the total vascular density (TVD), De Backer Score (DBS), proportion of perfused vessels (PPV), perfused vessel density (PVD), and heterogeneity index (HI) of tongue. Microvascular flow index (MFI) of kidney and tongue was then calculated on the offline movies. For each movie, the frame was divided into four equal quadrants and flow was scored per quadrant as no flow (0), intermittent flow (1), sluggish flow (2), and continuous flow (3). MFI is the sum of each quadrant-score divided by the number of scored quadrants, averaged over the different observers.

Statistical Analysis

Hemodynamic data collected during the study were stored on a laptop computer and later transferred to Microsoft Excel (Microsoft, Redmond, WA, USA) for analysis. Statistical analysis was performed using SPSS 16 software (San Francisco, CA, USA). Normal distribution was confirmed by Kolmogorov-Smirnov test. The changes in each hemodynamic variable after the onset of IAP were compared using the analysis of variance for repeated measures. Friedman's test with Dunn's post hoc test was used for repeated comparisons of not normally distributed data. For correlation analysis, interobserver variability was calculated separately for each parameter through the Bland-Altman analysis for assessing agreement between two opinions using

SPSS software. Bonferroni was used for post hoc analysis. All the data are reported as the mean $\pm$ standard deviation (SD). Differences were considered significant at $p < 0.05$.

Results

Effects of Elevated IAP on Hemodynamics

All 12 animals completed the experiment without complications and fatal arrhythmia. No drugs except anesthetics and Ringer solution were given. The baseline of IAP was 1 ± 1 mmHg, and the duration from the increase of IAP to the end of the experiment was about 9 hour, as indicated in Figure 1.

The hemodynamic parameters at different IAP stages are listed in Table I. In the IAH state, increasing IAP was associated with serial decreases in APP, CO, and RPF ($p < 0.05$) and increases of CVP. But SVR and GEDV did not change significantly during different IAP stages. Similar serial changes were observed with MAP and HR ($p < 0.05$) (Table I).

Effects of IAP on Renal Circulation and Renal Function

After injection of SonoVue, renal artery, cortex, pyramid and renal vein were displayed in sequence. When IAH is above 15mmHg, the display of the renal cortex was slower compared to the control group ($p < 0.05$).

The CEUS data at different IAP stages are listed in Table II. The renal perfusion curve after renal contrast enhancement in all groups was an asymmetrical, single-peak curve, with an obvious ascending slope, peak and descending slope. The ascending slope was steep, while the descending slope was flat. For the baseline condi-

tion, the ascending slope showed quick ascending then descended after reaching the peak. For TIC in pigs with relatively low hypertension, the ascending slope was flatter and milder than that of the control group and descended gradually after reaching the peak. While, for pigs with relatively high IAH, the ascending and descending slope was much flatter and milder, and the peak was lower than that of the other stages.

IAH groups with hypertension higher than 15mmHg showed significantly longer, wash in time (WT) and time to peak (TTP) ($p < 0.05$), but significantly decreased wash in slope (WS) and peak intensity (PI) ($p < 0.05$) (Table II). In IAH group with hypertension of 25 mmHg, significant change of WT, WS and AUC was observed ($p < 0.05$).

UO at the end of every state at around 1.5 hours were collected. The UO value decreased significantly from 76 at baseline to less than 10 ml at IAH state of 25 mmHg ($p < 0.01$).

Effects of IAP on Sublingual Microcirculation

To assess the sublingual microcirculation, 383 videos were captured. Eighty-seven percent of them were confirmed of eligible quality and were included in final analysis. Main reasons for exclusion included movement or partial artifacts (9%), brightness (1%), and excessive saliva (3%). Pressure artifacts were observed in less than 1% analyzed videos. The videos were watched by two investigators and one video with more stable image was selected at each time point. Interobserver bias for TVD was -0.01 n/mm² (SD, 0.84 n/mm²; 95% limits of agreement, 1.66 to -1.68 n/mm²). The bias for PVD, PPV, MFI and De Backer score was 0.22 n/mm² (SD, 0.91 n/mm²; 95% limits of agreement, 2.03

Table II. CEUS data of kidney at various intra-abdominal pressure stages.

CEUS data	IAP baseline	10 mmHg	15 mmHg	20 mmHg	25 mmHg
RT (s)	4.53 $\pm$ 0.54	4.54 $\pm$ 0.46	4.86 $\pm$ 0.74	5.52 $\pm$ 0.81	6.21 $\pm$ 0.77 ^a
TTP (s)	23.91 $\pm$ 4.65	25.42 $\pm$ 4.63	28.35 $\pm$ 4.85	29.59 $\pm$ 4.31 ^a	31.30 $\pm$ 4.74 ^a
WT (s)	21.18 $\pm$ 2.78	21.92 $\pm$ 3.42	24.17 $\pm$ 3.88	25.78 $\pm$ 4.27 ^a	27.03 $\pm$ 5.73 ^{ab}
WS (dB/s)	6.99 $\pm$ 1.13	5.95 $\pm$ 1.02	5.32 $\pm$ 0.97	4.86 $\pm$ 1.14 ^a	4.21 $\pm$ 1.29 ^{ab}
PI (dB)	27.95 $\pm$ 4.26	26.59 $\pm$ 3.54	26.07 $\pm$ 3.96	24.13 $\pm$ 4.53 ^a	22.70 $\pm$ 4.33 ^a
AUC (dB/s)	1632.83 $\pm$ 177.62	1552.25 $\pm$ 158.66	1462.00 $\pm$ 207.61	1363.83 $\pm$ 212.70 ^a	1267.75 $\pm$ 222.49 ^{ab}

RT, rise time; TTP, time to peak; WT, wash in time; WS, wash in slope; PI, peak intensity; AUC, area under the curve; ^a, $p < 0.05$ vs. baseline; ^b, $p < 0.05$ vs. IAP at 10 mmHg; ^c, $p < 0.05$ vs. IAP at 15 mmHg; ^d, $p < 0.05$ vs. IAP at 20 mmHg.

Table III. Parameters of sublingual microcirculation at various intra-abdominal pressure stages.

Variable	IAP baseline	10 mmHg	15 mmHg	20 mmHg	25 mmHg
TVD (n/mm ²)	14.94 ± 3.16	15.21 ± 3.64	14.96 ± 2.97	13.63 ± 3.23	12.13 ± 2.56
DBS	10.78 ± 3.50	11.87 ± 3.50	11.27 ± 3.20	11.34 ± 2.90	10.04 ± 4.11
PPV (%)	83.43 ± 9.24	84.94 ± 8.43	84.98 ± 9.50	84.04 ± 8.52	80.01 ± 9.02
PVD (n/mm ²)	10.32 ± 4.11	10.51 ± 3.84	10.55 ± 4.10	11.18 ± 4.27	9.43 ± 4.05
MFI	2.76 ± 0.10	2.63 ± 0.16	2.55 ± 0.13	2.41 ± 0.16	2.05 ± 0.28 ^{abcd}
HI	0.29 ± 0.10	0.28 ± 0.13	0.27 ± 0.11	0.27 ± 0.2	0.26 ± 0.10

TVD, total vessel density; PVD, PPV, DBS, De Backer score, MFI, and HI TVD, arriving time; A1, slope rate of ascending curve; A2, slope rate of descending curve; RT, rise time; TTP, time to peak; PI, peak intensity; AUC, area under the curve; a, $p < 0.05$ vs. baseline; b, $p < 0.05$ vs. IAP at 10 mmHg; c, $p < 0.05$ vs. IAP at 15 mmHg; d, $p < 0.05$ vs. IAP at 20 mmHg.

to -1.59 n/mm²), 0.87% (SD, 3.06% ; 95% limits of agreement, 6.98% to -5.24%), -0.10 (SD, 0.25 ; 95% limits of agreement, 0.39 to -0.59), and -0.06 (SD, 1.13 ; 95% limits of agreement, 2.21 to -2.33), respectively.

The parameters of sublingual microcirculation at different IAP stages are tabulated in Table III. The TVD of small vessels at the sublingual area was 14.9 vessels per square millimeter at baseline, among which 83.43% were well perfused. The calculated indices suggested relatively good blood flow in capillaries: the De Backer score was 10.78 , and the MFI was 2.76 . The blood flow HI was 0.3 at baseline. No significant changes in the vascular density or flow characteristics except MFI were detected upon further IAH stages (Table III). MFI dropped significantly when abdominal pressure increases above 20 mmHg. Correlation analysis adjusted for repeated measurements revealed weak positive correlations between MFI and APP (Table III).

Effects of IAP on Renal Cortical Microcirculation

288 quadrants (of 72 movies) were scored by two observers in total, among which eight percent were dropped out due to image artifacts. Figure 2 shows some typical screenshots of the microcirculation of the renal cortex. MFI decreased significantly from 2.79 ± 0.1 at baseline to 2.0 ± 0.3 at 25 mmHg. MFI correlated moderately with RFP ($R = 0.64$, $p < 0.05$), and MFI correlated significantly with APP ($R = 0.49$, $p < 0.05$). The correlation of MFI between tongue and renal surface barely reached significance. Moreover, the AUC is positively related with RFG ($R = 0.47$, $p < 0.05$).

Discussion

We reported several new findings as follows: CEUS is a useful tool to describe the blood supply change under IAH state; renal vein is most affected by IAH; SDF is a more accurate in eval-

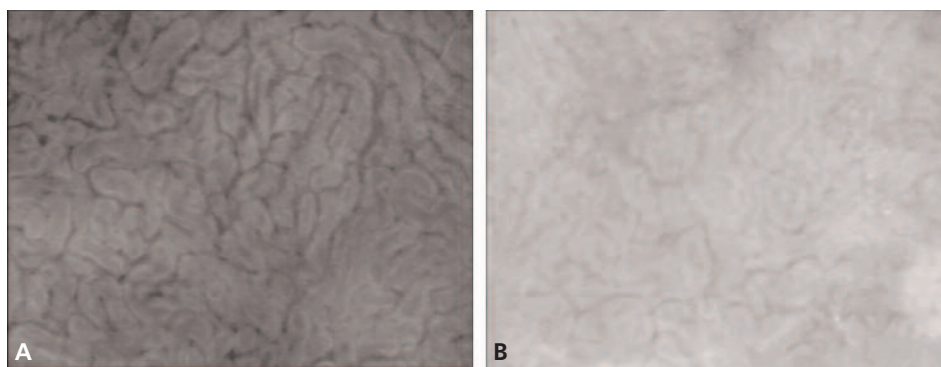


Figure 2. Renal cortical microcirculation at different intra-abdominal pressure stages, (A) Microcirculation at baseline; (B) Microcirculation at an intra-abdominal pressure (IAP) of 25 mmHg.

uating microcirculation of renal cortex than sublingual imaging; APP, as a clinical estimator of renal perfusion, correlated moderately with renal microcirculatory perfusion and renal blood flow.

UO, as a marker of renal function, decreased significantly with raising IAP. The effect of elevated abdominal pressure on end-organ function has been widely studied with both experimental animals and in human subjects. UO is a universally acknowledged indicator to assess renal function not only in acute kidney injury (AKI) but also during elevated IAP^{13,14}. Moreover, UO had good correlations during elevated IAP with insulin clearance¹⁵. The cardiopulmonary system is also frequently affected by IAH. However, the decreasing cardiac output was not paralleled with the deterioration of renal function. Local blood change of kidney remains to be the focus of controversy.

Alterations of renal perfusion are a common sign in critically ill patients, such as septic shock, low cardiac output states and intra-abdominal hypertension. Most previous studies focused on blood flow change in IAH models with ultrasonic flow probes¹⁶, a direct but invasive detecting method and has not been accepted in daily clinical practices. Recent advance in ultrasonographic imaging allows evaluation of the flow of blood through the kidney in a completely noninvasive manner. In the current study, ultrasound was used to evaluate blood flow of abdominal solid organs. To the best of our knowledge, this is the first study on CEUS in IAH state. A noninvasive imaging tool with the potential to be used in clinical to evaluate renal perfusion and blood flow would be of critical diagnostic value. Moreover, CEUS provides us with information such as severity, timing and change over time of renal perfusion as well as the intra-renal distribution of perfusion. It has been proven that CEUS can determine and quantify changes in renal perfusion which play an important role in transplant medicine^{17,18}.

In the present study, the images of renal cortex microvascular beds were rapidly and clearly displayed after the injection of SonoVue in every piglet. Their enhancement and faded images of renal perfusion in different abdominal pressure stages could be easily discriminated with all the necessary signals continuously recorded. The quantitative analysis showed that WT, TTP and AUC increased, and WS and PI decreased significantly in pigs in IAH State III and IV compared with the baseline ($p < 0.05$). It has also been re-

ported by some studies that a close relationship between AUC and GFR was observed⁶. These data strongly demonstrated that CEUS could accurately assess renal cortex microvascular perfusion and hemodynamic characterization of abdominal hypertension in pigs.

It is known that renal artery and veins are compromised in IAH state, and acute renal ischemia and congestion are the key factors in the development of renal injury. Raising pressure increases the hemodynamic impedance of renal microcirculation, reduces renal perfusion and leads to ischemia, causing fewer contrast microbubbles enter the renal parenchyma, and PI decrease significantly. In our study, the renal artery, cortex, pyramid and renal vein were clearly displayed in sequence. In IAH states, the display of the renal cortical was slower compared to the control baseline. The ascending slope of renal perfusion curve was milder in piglets with IAH, while the WT and TTP were longer than that of healthy controls. These changes became more obvious as the abdominal pressure increased. It means that in a porcine model of renal impairment induced by abdominal hyper-pressure, the perfusion velocity or the time that the contrast agent reaches the renal microcapillary bed is prolonged. The decreased WS and PI in the IAH state indicated that less contrast agent entered the renal microvascular bed in unit time. These phenomena might be related to the renal microvascular functional ischemia or some structural pathological changes, and perfusion sluggish in high pressure. As the kidney injury progresses, the quantitative parameters such as the WT, WS, TTP, PI and AUC changed significantly, suggesting that WS, TTP, PI, and AUC would be used in the assess-

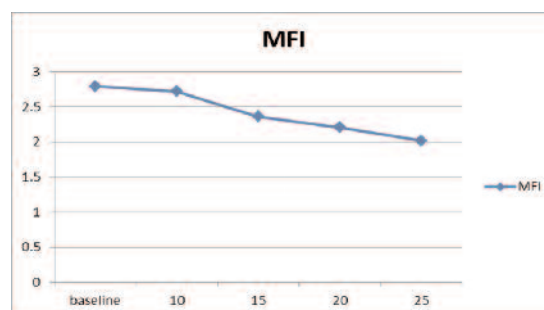


Figure 3. Effect of intra-abdominal pressure (IAP) on microvascular flow index (MFI). Baseline = 1 mmHg. Data are expressed as mean ± SD. * $p < 0.05$ versus baseline.

ment of renal microcirculation perfusion in pathological conditions such as IAH.

The intensity of the contrast-enhancement signal is related to the contrast microbubbles. The microbubbles concentration that permeates into the renal tissue is related to the blood volume of that particular renal tissue¹⁹. AUC, defined as the total area under the curve, is determined by the maximum volume of renal perfusion and the clearance of renal perfusion volume. In IAH state, the average value of AUC was significantly decreased ($p < 0.05$), indicating a decrease in renal cortex microvascular beds which is featured with decreased maximum volume of microvascular perfusion and kidney clearance. This may contribute to hypoperfusion status in IAH state. Thus, the AUC is a sensitive parameter reflecting the renal microvascular perfusion volume.

With a similar porcine model, Bloomfield et al²⁰ showed that an increase in RVP with increasing IAP associated with a significant drop in UO. Doty¹³ showed that isolated renal vein constriction in pigs led to decreased RAF and renal dysfunction. In our study similar results were observed that contrast agents significantly delayed cleaning. As the arterial pressure is difficult to be compressed over 25 mmHg, we could only hypothesize that acutely elevated IAP will compromise renal outflow by generating venous back-pressure, leading to decreased RVF and RAF, which follows the principle “inflow = outflow,” and resulting ultimately in renal mal-perfusion and dysfunction.

Interestingly, the piglet models with IAH, Stages I and II and Stages and IV corresponding to the four representative pressures showed uniform contrast medium dynamics. The changing curves of the renal cortex were characterized by significant increases in TTP and AT, while the PI, AUC and A2 significantly decreased compared with healthy control, which suggests that AUC and A2 could be used for the diagnosis of renal damage in both early and late stage of IAH patients.

Conventional vascular ultrasound (US) techniques such as color-duplex ultrasonography only depict vessels with a diameter more than 30 μ m, making it difficult to accomplish this work using a conventional US. SonoVue, known as second-generation US contrast agent, makes up such limitation. After administration of SonoVue, microbubbles are cleared through breathing and lack of renal excretion explains the absence of kidney toxicity of these agents, which can

therefore, be used in nephropathic patients²¹. SonoVue consists of an aqueous dispersion of lipid stabilized perfluorobutane-filled gas microbubbles with a median volume diameter of 3 μ m. The microbubbles are stabilized with a shell of a phospholipid monolayer; some evidence showed that SonoVue microbubbles could be used for the characterization of the small blood vasculature over prolonged periods of observation. All the perfusion images detected in the kidney cortex tissue were clearly observed in our present study. All the data were easily recorded and quantified. None of the study participants reported any side effects of CEUS. Our renal perfusion curve of all subjects turned out to be with a single peak, which is in consensus with the results by Kay et al²². This evidence demonstrated that the CEUS by SonoVue microbubbles is a safe and simple technique to detect the severity of kidney microvascular perfusion deficits²³.

More attention has been paid to microcirculatory monitoring, from macrohemodynamics to microhemodynamics, in pathologic conditions. Nowadays, bedside SDF imaging and its predecessor, orthogonal polarization spectral imaging, has recently been used to study different microcirculatory in humans and animals sublingual region, brain, small and large intestine, liver surface. SDF is always used to observe the sublingual capillary network because it is easy to access and evaluate. The IAH condition and sublingual microcirculation have been discussed, but general circulation under such state remains to be studied. Verdant et al²⁴ demonstrated with a porcine model of severe sepsis that the severity and time course of microcirculatory changes are similar in the sublingual and gut regions, which suggested that the sublingual assessment of microcirculation may be relevant in this clinical setting. We also tried to visualize renal cortical microcirculation with elevated IAP via SDF. Tough fibrous capsule wrapped the kidneys outside, which was not decapsulated in our study. The results observed in this study were somehow different from previous researches on microcirculation of kidney using SDF¹⁶. We must remove the renal capsule carefully to get a better view because the cortical surface sometimes can't be observed covered by the thick layer. The renal parenchyma is very crisp and easy to bleed. To prevent dehydration, we covered some tissue adjacent to the surface of the kidney at an interval of measurement. But in clinics, we use SDF to observe the sublingual capillary vessel; there is

yet no data about the relationship between the tongue and kidney.

We used the MFI to assess renal cortical microcirculatory perfusion. MFI was validated by Boerma et al²⁵ as a simple and reproducible semi-quantitative flow score for sublingual and stoma microvascular beds in septic patients. We found that MFI decreased by 29% with elevated IAP (up to 25 mm Hg) accompanied with decreasing UO, decreasing renal flows and decreasing perfusion pressures. Our results are in agreement with previous results¹⁶ which also showed decreased renal parenchymal microperfusion with elevated IAP. McDougall et al²⁶ found that cortical and medullar perfusion, measured both by contrast magnetic resonance imaging and radio-labeled microspheres, to be decreased in parallel by about 30% at an IAP of 15 mm Hg during 3 hours, without signs of shunting or redistribution. Associated loss of urine concentrating capacity was reported to reflect medullar injury. Because functioning glomeruli and peritubular capillaries are located in the renal cortex, decreased cortical perfusion may be responsible for decreased UO and decreased GFR, irrespective of medullar shunting. In conditions severe enough stimulated with a porcine model, we also found the blood redistributed to the medullar through CEUS.

We want to underline that the OPS technique can only investigate tissues covered by a thin epithelial layer, and movement artifacts, mostly but not exclusively induced by breathing, do not allow reliable quantitative measurements of microvascular blood flow. Up to now, only semiquantitative analysis can be realized, and a cumbersome offline analysis limits its application in reanimation-guided therapy. The novel method cannot be replace a renal biopsy, which is commonly recognized as the gold standard. Some study had confirmed that during the early stage of IAH there was no change of renal tissue except edema. Studies with a larger group of subjects with renal biopsy will still be needed to explore the close relationship between the sensitive parameters and the kidney pathological changes. We failed to find a specific parameter of CEUS to evaluate renal status to guide our clinical practice.

Conclusions

CEUS allows us to observe renal microvascular perfusion status in a real-time and dynamic manner. WS, PI and AUC could be used for the

diagnosis of the renal microvascular damage in the IAH state, which can be detected with renal SDF imaging. CEUS with SonoVue microbubbles is an accurate, minimally invasive and simple technique to detect the severity of kidney microvascular perfusion deficits. We expect that this method can be applied in evaluating the effect of IAH that alters blood flow to the kidney or other organs.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81341056).

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- 1) BALOGH Z, DE WAELE JJ, KIRKPATRICK A, CHEATHAM M, D'AMOURS S, MALBRAIN M, WORLD SOCIETY OF THE ABDOMINAL COMPARTMENT S. Intra-abdominal pressure measurement and abdominal compartment syndrome: the opinion of the World Society of the Abdominal Compartment Syndrome. *Crit Care Med* 2007; 35: 677-678.
- 2) KIRKPATRICK AW, ROBERTS DJ, DE WAELE J, JAESCHKE R, MALBRAIN ML, DE KEULENAER B, DUCHESNE J, BJORCK M, LEPPANIEMI A, EJIKE JC, SUGRUE M, CHEATHAM M, IVATURY R, BALL CG, REINTAM BLASER A, REGLI A, BALOGH ZJ, D'AMOURS S, DEBERGH D, KAPLAN M, KIMBALL E, OLVERA C, PEDIATRIC GUIDELINES SUB-COMMITTEE FOR THE WORLD SOCIETY OF THE ABDOMINAL COMPARTMENT S. Intra-abdominal hypertension and the abdominal compartment syndrome: updated consensus definitions and clinical practice guidelines from the World Society of the Abdominal Compartment Syndrome. *Intens Care Med* 2013; 39: 1190-1206.
- 3) MALBRAIN ML, VIAENE D, KORTGEN A, DE LAET I, DITS H, VAN REGENMORTEL N, SCHOONHEYDT K, BAUER M. Relationship between intra-abdominal pressure and indocyanine green plasma disappearance rate: hepatic perfusion may be impaired in critically ill patients with intra-abdominal hypertension. *Ann Intensive Care* 2012; 2 Suppl 1: S19.
- 4) CHEATHAM ML, WHITE MW, SAGRAVES SG, JOHNSON JL, BLOCK EF. Abdominal perfusion pressure: a superior parameter in the assessment of intra-abdominal hypertension. *J Trauma* 2000; 49: 621-626.
- 5) STENBERG B, CHANDLER C, WYRLEY-BIRCH H, ELLIOTT ST. Post-operative 3-dimensional contrast-enhanced ultrasound (CEUS) versus Tc99m-DTPA in the detection of post-surgical perfusion defects in kidney transplants - preliminary findings. *Ultraschall in der Medizin* 2014; 35: 273-278.

- 6) MA F, CANG Y, ZHAO B, LIU Y, WANG C, LIU B, WU T, SONG Y, PENG A. Contrast-enhanced ultrasound with SonoVue could accurately assess the renal microvascular perfusion in diabetic kidney damage. *Nephrol Dial Transplant* 2012; 27: 2891-2898.
- 7) TRZECIAK S, DELLINGER RP, PARRILLO JE, GUGLIEMI M, BAJAJ J, ABATE NL, ARNOLD RC, COLILLA S, ZANOTTI S, HOLLENBERG SM, MICROCIRCULATORY ALTERATIONS IN R, SHOCK I. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Ann Emerg Med* 2007; 49: 88-98.
- 8) DE BACKER D, ORTIZ JA, SALGADO D. Coupling microcirculation to systemic hemodynamics. *Curr Opin Crit Care* 2010; 16: 250-254.
- 9) BOERMA EC, KOOPMANS M, KONIJN A, KAIFEROVA K, BAKKER AJ, VAN ROON EN, BUTER H, BRUINS N, EGBERS PH, GERRITSSEN RT, KOETSIER PM, KINGMA WP, KUIPER MA, INCE C. Effects of nitroglycerin on sublingual microcirculatory blood flow in patients with severe sepsis/septic shock after a strict resuscitation protocol: a double-blind randomized placebo controlled trial. *Crit Care Med* 2010; 38: 93-100.
- 10) JUNG C, FERRARI M, RODIGER C, FRITZENWANGER M, GOEBEL B, LAUTEN A, PFEIFER R, FIGULLA HR. Evaluation of the sublingual microcirculation in cardiogenic shock. *Clin Hemorheol Micro* 2009; 42: 141-148.
- 11) BEZEMER R, BARTELS SA, BAKKER J, INCE C. Clinical review: Clinical imaging of the sublingual microcirculation in the critically ill—where do we stand? *Crit Care* 2012; 16: 224
- 12) BURBAN M, HAMEL JF, TABKA M, DE LA BOURDONNAYE MR, DUVEAU A, MERCAT A, CALES P, ASFAR P, LEROLLE N. Renal macro- and microcirculation autoregulatory capacity during early sepsis and norepinephrine infusion in rats. *Crit Care* 2013; 17: R139.
- 13) DOTY JM, SAGGI BH, SUGERMAN HJ, BLOCHER CR, PIN R, FAKHRY I, GEHR TW, SICA DA. Effect of increased renal venous pressure on renal function. *J Trauma* 1999; 47: 1000-1003.
- 14) KELLUM JA, LAMEIRE N, GROUP KAGW. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care* 2013; 17: 204.
- 15) LINDSTROM P, KALLSKOG O, WADSTROM J, PERSSON AE. Blood flow distribution during elevated intraperitoneal pressure in the rat. *Acta Physiol Scand* 2003;177: 149-156.
- 16) WAUTERS J, CLAUS P, BROSENS N, McLAUGHLIN M, MALBRAIN M, WILMER A. Pathophysiology of renal hemodynamics and renal cortical microcirculation in a porcine model of elevated intra-abdominal pressure. *J Trauma* 2009; 66: 713-719.
- 17) GRZELAK P, KURNATOWSKA I, NOWICKI M, MARCHWICKA-WASIAK M, PODGORSKI M, DURCZYNSKI A, STRZELCZYK J, STEFANCZYK L. Perfusion disturbances of kidney graft parenchyma evaluated with contrast-enhanced ultrasonography in the immediate period following kidney transplantation. *Nephron Clin Pract* 2013; 124: 173-178.
- 18) PENG ZY, CRITCHLEY LA, JOYNT GM, GRUBER PC, JENKINS CR, HO AM. Effects of norepinephrine during intra-abdominal hypertension on renal blood flow in bacteremic dogs. *Crit Care Med* 2008; 36: 834-841.
- 19) MAHONEY M, SORACE A, WARRAM J, SAMUEL S, HOYT K. Volumetric contrast-enhanced ultrasound imaging of renal perfusion. *J Ultrasound Med* 2014; 33: 1427-1437.
- 20) BLOOMFIELD GL, BLOCHER CR, FAKHRY IF, SICA DA, SUGERMAN HJ. Elevated intra-abdominal pressure increases plasma renin activity and aldosterone levels. *J Trauma* 1997; 42: 997-1004.
- 21) SCHNEIDER AG, GOODWIN MD, SCHELLEMAN A, BAILEY M, JOHNSON L, BELLOMO R. Contrast-enhanced ultrasound to evaluate changes in renal cortical perfusion around cardiac surgery: a pilot study. *Crit Care* 2013; 17: R138.
- 22) KAY DH, MAZONAKIS M, GEDDES C, BAXTER G. Ultrasonic microbubble contrast agents and the transplant kidney. *Clin Radiol* 2009; 64: 1081-1087.
- 23) KOGAN P, JOHNSON KA, FEINGOLD S, GARRETT N, GURACAR I, ARENDSHORST WJ, DAYTON PA. Validation of dynamic contrast-enhanced ultrasound in rodent kidneys as an absolute quantitative method for measuring blood perfusion. *Ultrasound Med Biol* 2011; 37: 900-908.
- 24) VERDANT CL, DE BACKER D, BRUHN A, CLAUSI CM, SU F, WANG Z, RODRIGUEZ H, PRIES AR, VINCENT JL. Evaluation of sublingual and gut mucosal microcirculation in sepsis: a quantitative analysis. *Crit Care Med* 2009; 37: 2875-2881.
- 25) SALLISALMI M, OKSALA N, PETTILA V, TENHUNEN J. Evaluation of sublingual microcirculatory blood flow in the critically ill. *Acta Anaesth Scand* 2012; 56: 298-306.
- 26) McDougall EM, BENNETT HF, MONK TG, SIEGEL CL, LI D, McFARLAND EG, CLAYMAN RV, SHARP T, RAYALA HJ, MILLER SB, HAACKE EM. Functional MR imaging of the porcine kidney: physiologic changes of prolonged pneumoperitoneum. *JSLs* 1997; 1: 29-35.