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Observational Study Protocol for Repeated Clinical Examination and Critical Care Ultrasonography within the Simple Intensive Care Studies

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Dear Dr. Myers,

Thank you very much for inviting us to submit a manuscript. Please find enclosed our manuscript, “The Simple Intensive Care Studies-II: a prospective, observational study using repeated clinical examination and critical care ultrasonography measurements to determine the hemodynamic status of critically ill patients.” by Renske Wiersema et al., which we would like to submit for consideration as in the Journal of Visualized Experiments.

All authors have approved the manuscript and agree with its submission to the Journal of Visualized Experiments. We confirm that this manuscript is not under consideration nor has been published by another journal.

I look forward to hearing from you at your earliest convenience.

Thank you for your consideration.

Sincerely,
Renske Wiersema

TITLE:

Observational Study Protocol for Repeated Clinical Examination and Critical Care Ultrasonography within the Simple Intensive Care Studies

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KEYWORDS:

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SUMMARY:

Structured protocols are necessary to provide answers on research questions in critically ill patients. The Simple Intensive Care Studies (SICS) provides an infrastructure for repeated

measurements in critically ill patients including clinical examination, biochemical analysis and ultrasonography. SICS projects have specific focus but the structure is flexible to other investigations.

ABSTRACT:

Longitudinal evaluations of critically ill patients by combinations of clinical examination, biochemical analysis and critical care ultrasonography (CCUS) may detect adverse events of interventions such as fluid overload at an early stage. The Simple Intensive Care Studies (SICS) is a research line that focuses on the prognostic and diagnostic value of combinations of clinical variables.

The SICS-I specifically focuses on the use of clinical variables obtained within 24 hours of acute admission for prediction of cardiac output (CO) and mortality. Its sequel, SICS-II, focuses on repeated evaluations during ICU admission. The first clinical examination by trained researchers is performed within 3 hours after admission consisting of physical examination and educated guessing. The second clinical examination is performed within 24 hours after admission and includes physical examination and educated guessing, biochemical analysis and CCUS assessments of heart, lungs, inferior vena cava (IVC) and kidney. This evaluation is repeated at days 3 and 5 after admission. CCUS images are validated by an independent expert, and all data is registered in an online secured database. Follow-up at 90-days includes registration of complications and survival status according to patient's medical charts and the municipal person registry. The primary focus of SICS-II is the association between venous congestion and organ dysfunction.

The purpose of publishing this protocol is to provide details on the structure and methods of this on-going prospective observational cohort study allowing answering multiple research questions. The design of the data collection of combined clinical examination and CCUS assessments in critically ill patients are explicated. The SICS-II is open for other centers to participate and is open for other research questions that can be answered with our data.

INTRODUCTION:

Patients admitted to the Intensive Care Unit (ICU) are the most critically ill with high rates of co- and multi-morbidities, independent of their admission diagnosis. Therefore, the ICU is the setting to investigate co- and multi-morbidity, their negative impact on patient outcomes, and how critical illness may lead to complications that contribute to additional multi-morbidities. To gain insight in this heterogeneous patient group detailed examination of each individual patient is of utmost interest.

The Simple Intensive Care Studies (SICS) research line is designed with the goal of evaluating the prognostic and diagnostic value of a comprehensive selection of clinical, hemodynamic and biochemical variables in ICU patients collected by a dedicated team of student-researchers coordinated by medical experts. One of the primary objectives of the SICS-I is to investigate the combination of clinical examination findings best associated with shock defined by cardiac output (CO) measured by critical care ultrasonography (CCUS)[1]. The SICS-II uses the structure of the SICS-

I but adds repeated clinical examination, biochemical analysis and CCUS. The primary focus of SICS-II is to quantify venous congestion and identify variables that may contribute to its development. Repeated measurements provide dynamic information on the course of a patient's illness. Studies show that fluid overload is present in critically ill patients and fluid overload is associated with new morbidities. We thus focus on venous congestion in these patients. Moreover, several studies have suggested the possible negative effects of excessive fluid administration[2–6]. Fluid overload can be perceived as venous congestion or venous fluid overload, which may be observed by an increased central venous pressure (CVP) or peripheral edema. Elevated pressure in the central venous system may contribute to reduced organ perfusion followed by organ failure.

Previous studies that suggested negative effects associated with excessive fluid administration used single surrogate measurements of venous congestion such as CVP, IVC collapsibility, fluid balance and/or peripheral edema[7–10]. To the best of our knowledge, the SICS-II is the first study to perform repeated CCUS of multiple organs combined with findings from clinical examination to assess the hemodynamic status of ICU patients. The focus on this multi-organ ultrasonography technique is important as organ failure or diminished organ function always influences the entire hemodynamic system. We expect that data from repeated examinations in SICS-II will help to unravel the pathophysiology and consequences of venous congestion. Consequently, this may help to improve earlier identification of critically ill patients at risk of venous congestion and guide the optimization of fluid administration. Additionally, the association between venous congestion and short- and long-term organ failure can be explored. Finally, the successful implementation of the SICS-II protocol would make evident that carrying out a large prospective study with a dedicated team of student-researchers is feasible and can yield quality data to investigate clinical problems.

Here, the procedure to perform comprehensive clinical examination of ICU patients with the goal of measuring venous congestion is demonstrated. A concise protocol of SICS-II was published on clinicaltrials.gov[11]. After the first initial clinical examination, a maximum of three additional clinical examinations, biochemical analyses and CCUS are conducted. Physical examination comprises of variables which reflect peripheral perfusion/microcirculation such as capillary refill time (CRT) or mottling as well as variables of the macrocirculation such as blood pressure, heart rate and urine output. Also, standard care laboratory values are registered (*e.g.,* lactate, pH). Subsequently, CCUS of the heart, lungs, IVC and kidney is performed to obtain information on perfusion. Further methods will be elaborated within our statistical analysis plan, as was done in the SICS-I[12].

Based on 138 patients included between 14-05-2018 and 15-08-2018, repeated measurements of a broad array of clinical variables within this structure seem feasible. We also show that independent validation is feasible. The SICS-II exemplifies a valuable methodology for enabling researchers to accurately register changes in variables of interest and can thus act as a guide to conducting research that reflects the progression in patients' condition as seen in daily practice. The SICS-II study is carried out daily by a team of 2-3 student-researchers at all times, with a senior supervisor available on call. These student-researchers are trained in performing the

physical examination and CCUS. They execute all the steps of the following protocol and are responsible for patient inclusion both during working hours and in the weekends. In addition, a larger ICU student team of around 30 students participate in evening and night shifts, to conduct the initial clinical examination (within 3 hours of admittance) of new patients. **Figure 1** presents a schematic summary of the study protocol, and **Figure 2 and 3** display the Case Report Forms (CRF) used to register data upon collection.

PROTOCOL:

This study is conducted according to the principles of the Declaration of Helsinki (64th version, Brazil 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO), the guidelines of Good Clinical Practice, and the local institutional review board (Medisch Ethische Toetsingscommissie; M18.228393).

1. Patient Admission to ICU and Screening

NOTE: For screening, a digital list with minimum patient data is updated throughout the day, and inclusions and exclusions are registered. The screening list is stored in the secured hospital electronic system with exclusive access for researchers. To protect patient privacy, all physical copies of the lists are destroyed at the end of the day. Inclusion criteria are: acute and unplanned admission; and patients above the age of 18.

1.1. Screen the patient management system for all new admissions and check whether patients meet the inclusion criteria.

1.2. Immediately exclude readmissions, elective admissions, patients younger than 18 years of age, and those who will not be able to give informed consent.

NOTE: We also exclude patients with a non-trauma neurological admission reason as we have established multiple patient groups in SICS-I, in which this group was hemodynamically stable and ICU admission concerned mainly neurological treatment[1].

1.3. Add the possible inclusions to a continuously updated patient list. Use this list to plan new and repeated measurements daily based on the time of admission/inclusion.

2. Clinical Examination 1

NOTE: The first clinical examination is conducted in all patients who fulfill the inclusion criteria within 3 hours after admission. This examination is performed by the student-researchers if the patient is admitted during the day shift. For patients admitted during the evening or night shifts, this first clinical examination is conducted by a member of the ICU student team and data are processed and finalized the next day by the student-researchers. For a full description of the protocol of the first clinical examination, see clinicaltrials.gov[13]. At the bedside, if possible,

patients are asked if they consent to the clinical examination at that moment. Written informed consent is obtained later on: see Step 1.2 for instructions and Step 7**.**

2.1. Physical examination

2.1.1. Start by ensuring the required safety/isolation rules for the patient: disinfect hands and wrists following standard hospital procedures with 70% alcohol and use non-sterile gloves and a plastic apron or additional precautions such as an isolation gown during patient contact.

2.1.2. Introduce yourself and ask the patient for permission to conduct the examination if they are not sedated, conscious and adequate. Explain to the patient what is being done.

NOTE: Formal informed consent is requested at a later stage during ICU admission or after discharge to the ward, either from the patient themselves or from the next of kin if the patient is unable. This is described in further detail in Step 7.

2.1.3. Register the hemodynamic variables heart rate, respiratory rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and central venous pressure (CVP) from the bedside monitor.

2.1.4. Register the oxygen saturation (SpO_2) and whether the patient receives non-invasive respiratory support or is mechanically ventilated. If so, register the positive end expiratory pressure (PEEP) and the fraction of inspired O_2 (FiO_2).

2.1.5. Determine reperfusion of the knee and sternum by pressing 10 seconds on the skin and letting go, then counting the number of seconds until full reperfusion.

2.1.6. Determine the subjective skin temperature by palpating the extremities with the hands and estimate if they are cold or warm.

2.1.7. Record the bladder temperature from the monitor, which shows the temperature measured by a sensor attached to an indwelling urinary catheter.

2.1.8. Determine the skin temperature on the dorsum of the foot by placing an additional temperature sensor on the middle of the dorsum and connecting it to the monitor. Reconnect the bladder temperature sensor to the monitor after this measurement.

2.1.9. Score the degree of mottling if observable by using the Ait-Oufella knee scale[14].

2.1.10. Register whether the patient receives sedation and, if yes, which drug, at what pump speed, and in which dosage.

2.1.11. Determine and register the patient's Glasgow Coma Scale (GCS)[15].

2.1.12. Estimate the patient's survival in hospital, 6 months survival and ability to return to their original residence based on an educated guess and the results from this clinical examination[16,17]. Ask the nurse and physician for their estimations as well and register all estimations on the CRF.

3. Clinical Examination 2

NOTE: The second clinical examination is conducted within 24 hours after admission and includes CCUS measurements. This examination is always conducted by student-researchers trained in CCUS, and not by members of the ICU student team. In addition, in patients who meet the inclusion criteria and got clinical examination 1 but are shown later to suffer exclusively from a neurological condition (*e.g.,* non-traumatic subarachnoid hemorrhage), repeated measurements including CCUS are not performed, and these are ultimately excluded.

3.1. Obtain informed consent.

NOTE: Under University Medical Centre Groningen (UMCG) regulations for observational measurements, per 1st of January 2016, the ultrasound images collected during clinical examination can be used without expressed consent. However, it is policy of the SICS-II study to seek informed consent from patients at the earliest possible moment while simultaneously upholding the principles of minimizing "stress" to the patient, increase shared decision making, and giving the patient enough time to consider participation. Since most patients are unable to provide consent early in their ICU stay, "delayed" consent is usually obtained. If, conversely, they are able to provide or refuse consent before or after the examinations, these will either not be carried out or all already obtained data is deleted, respectively.

3.1.1. Before starting the examination, determine if the patient is alert/conscious and able to engage with the student-researchers by determining their GCS score. Provide capable patients with an explanation regarding the examination to be conducted, and leave a standardized, written letter which they must sign.

3.1.2. Should the patient not be able to be consulted for consent (due to impaired consciousness, limited mental capacity, *etc.*), monitor their GCS score daily and consider obtaining family consent if family members are reachable (as described in Step 7.1).

3.2. Perform the physical examination following the steps described for Clinical Examination 1.

3.3. Perform CCUS of the heart and the lungs.

NOTE: This protocol is valid when using an ultrasound machine in the **Table of Materials**, the cardiac transducer for the parasternal long axis view (PLAX), and the cardiac phased array transducer for the apical four and five chamber views (AP4CH, AP5CH). For other ultrasound systems, users should consult the operating manuals of their specific device.

3.3.1. Turn on the machine. Register the patient's anonymous study ID, start a new exam, and wait for the automatic 2D imaging mode to be displayed on the screen.

3.3.2. If the patient is clothed, unbutton their gown to expose the chest. Place new electrocardiogram (ECG) stickers and connect them to the ultrasound machine if necessary.

3.3.3. Connect the ECG cable from the machine to the patient's bedside monitor. Wait for it to stabilize and register the heart rate measured by ECG in the CRF.

3.3.4. When possible, position the patient slightly rotated on their left side. This improves the quality of both cardiac and kidney imaging.

NOTE: Technical Considerations: Before starting the examination, click the **Configure** button, and set the image settings to five heart cycles, a depth of 10-15 cm, an image width of 65°, and a frequency of 1.7/3.4 MHz. Check if the correct probe is selected by clicking the **Probe** button.

3.3.5. Put a sufficient amount of ultrasound gel on the ultrasound transducer and place the transducer on the lateral left of the sternum, between the 3rd and 5th intercostal spaces, to obtain the PLAX view in the 2D mode. Adjust the depth as needed to record images for the left ventricular outflow tract measurements and save the image.

NOTE: The maximal width of the left ventricle should be visible with a maximal opening of the mitral valve. No muscle of the valve should be visible. Before placing the transducer on the patient's chest, warn them that the gel is cold and can feel uncomfortable, and that they will feel some pressure (especially around the sternum when recording images for the left ventricular outflow tract (LVOT). Be aware in patients that have costal fractures some places should be avoided as this may be uncomfortable for the patient).

3.3.6. In the 2D mode, adjust the depth to 15-20 cm, and place the transducer over the apex of the heart, caudal to the left areola. The AP4CH view is obtained, with all four chambers clearly visualized. Save the image.

3.3.7. Roll the trackball so that the cursor is on the boundary between the tricuspid valve and the right ventricle wall to obtain the Tricuspid Annular Plane Systolic Excursion (TAPSE). Press the **M-Mode** button to obtain the correct image and save it when defined sinus waves are seen. Save the image.

3.3.8. Place the cursor over the tricuspid valve with the trackball. Decrease the width of the image to increase the number of frames per second, necessary for the RV S' quality. Press the **TVI** button first, and then the **PW** button, to obtain the correct image for the RV S' and save it.

3.3.9. From the AP4CH view, tilt the transducer upwards (*i.e.,* flatten it) to obtain the AP5CH view and get the aortic root on screen. Save the image.

3.3.10. Place the cursor right above the aortic valve and press the **PW** button to obtain the LVOT pulse wave Doppler. Place the cursor at exactly the same place where the LVOT diameter was measured. Save the image with the highest quality (sharp Doppler wave boundaries, hollow on the inside and well distinguishable from retrograde or mitral flow). These will later be used to calculate the velocity time integral (VTI), and subsequently the CO.

NOTE: Always try to obtain at least three flow waves for every measurement. In case of an irregular rhythm, at least five waves should be saved.

3.3.11. Proceed to the lung ultrasound using the same phased array cardiac transducer and changing the settings to a frequency of 3.7 MHz, depth to 15 cm, and record the images only during 2 heart cycles. Place the transducer in 6 different locations, with the light of the transducer at 12 o'clock, according to the BLUE protocol[18]. Make sure to always obtain the image in the same sequence, to avoid confusion when viewing images later.

3.3.12. Obtain a superior anterior mid-clavicular view of the lungs by placing the transducer on the intercostal space of the 2nd and 3rd rib on both sides. Save the images for each side.

3.3.13. Obtain an inferior anterior mid-clavicular view of the lungs by placing the transducer 2 to 3 ribs lower. Save the images for each side.

3.3.14. Obtain a mid-axial view of the lungs by placing the transducer under the patient's arm pits. Save the images for each side.

3.3.15. Once cardiac and pulmonary imaging is complete, wipe excess gel off the patient's chest.

3.4. Perform CCUS of the IVC and the kidney.

3.4.1. Click the **Probe** button and use the trackball to change the active probe to the convex/curvilinear array (abdominal) transducer for the IVC and kidney examination. The light of the transducer, which may be used for orientation, should be at 12 o'clock for both measurements.

3.4.2. Using the 2D mode and with settings set to a depth of 10-20 cm, and a frequency of 2.5/5.0 MHz, place the transducer just below the xiphoid process and displace it approximately 2 cm to patient's right. The IVC should become visible. Save the image.

3.4.3. Place the cursor just above the superior wall of the IVC and outside the lumen using the trackball and press the **M-Mode** button. Save the image.

3.4.4. For the renal ultrasound, start with the 2D mode and adjust the settings to a depth of 10-15 cm, and a frequency of 2.2/4.4 MHz. Place the transducer dorsal and caudal of the rib cage. Get the chosen kidney central in the image and save it.

NOTE: Make sure to place the transducer as dorsal as possible to filter out liver tissue and bowel loops. For reliable measurements of the kidney length, the borders of the kidney should be clearly visible, and the distance between the central sinus complex (the more echogenic center of the kidney) and the cortex should be similar throughout the image.

3.4.5. Press the **Color** button to get a color Doppler image of the kidney and determine flow in the renal vasculature. Place the cursor over any artery at the corticomedullary junction in the center of the kidney where Doppler flow is clearly visible using the trackball.

3.4.6. Adjust the cursor angle and press the **PW** button. Adjust the signal amplitude and the contrast in **Active Mode** if necessary. Save the image.

3.4.7. Determine if there is also sufficient venous signal (*i.e.,* flow visible in the negative half of the y axis), which is required for later measurements. If not, repeat Step 3.4.5 and place the cursor over a vein at the corticomedullary junction in the middle where venous flow is visible. Save the image.

3.4.8. Once all imaging is complete, disconnect all cables, wipe excess gel off the patient and the transducer, redress or cover the patient and clean the transducer with ultrasound-approved disinfecting wipes.

4. Clinical Examinations 3 and 4

NOTE: The third and fourth clinical examinations are conducted on days 3 and 5 after admission if the patient is still in the ICU (*i.e.,* no death or transfer to the ward took place).

4.1. Physical examination

4.1.1. Conduct the physical examination following the steps described for Clinical Examination 1.

4.2. CCUS of the heart and the lungs

4.2.1. Conduct the ultrasonographic examination of the heart and lungs according to Step 3.3. Obtain LVOT only once since it is a static measurement, and therefore does not have to be recorded in Clinical Examination 3 and 4.

4.3. CCUS of the IVC and the kidney

4.3.1. Conduct the ultrasonographic examination of the IVC and kidney according to Step 3.4. Obtain kidney length only once since it is a static measurement, and therefore does not have to be recorded in Clinical Examination 3 and 4.

5. Measurements and Analysis of the Ultrasound Examinations

NOTE: The images saved during the clinical examination are used after each examination to measure the desired variables. The measured values are registered on the CRF and transcribed to an online clinical patient data management system. Images in which measurements are performed and visible should also be saved, in addition to the original images which will later be used for validation.

5.1. LVOT measurement

5.1.1. Click the **Probe** button to select the cardiac transducer in order to start the measurements.

5.1.2. Using the image saved in Step 3.3.5, pause the image when the valves are fully open.

5.1.3. Click the **Measure** button, and then select the **Cardiac-Dimension-LVOT** options on the right-side menu, to start the measurement.

5.1.4. Once the cursor appears, choose two points at the base of the aortic valve, one on each side of the lumen, from inner to inner edge, during end-diastole. Save the image.

NOTE: The LVOT measurement must be done and saved before the CO measurement, so that this can be automatically determined by the machine.

5.2. CO measurement

5.2.1. Using the image saved in Step 3.3.10, trace the left ventricular output. Adjust the horizontal sweep to 100 cm/s.

5.2.2. Select three well-shaped, hollow waves with clear borders which align with the ECG. Click the **Measure** button and use the trackball to select the **Cardiac-Aortic-LVOT Trace** options.

5.2.3. Trace the waveform line, starting and ending at the baseline, and the ultrasound machine automatically calculates the CO. Repeat this for three waves and save this image.

NOTE: In case of an irregular rhythm, record the average CO value obtained for five waves.

5.3. TAPSE

5.3.1. Using the M-mode image saved in Step 3.3.7, click the **Measure** button, and use the trackball to select the **Cardiac-Dimension-TAPSE** options on the right-side menu.

5.3.2. Place the cursor first on the lowest point of a well-defined sinus wave and then on the highest point. The difference between the two (the TAPSE) should appear on the top left corner of the screen. Do this in three sinus waves and take the average of the three TAPSE measurements. Save the image.

5.4. Right Ventricular Systolic excursion (RV S')

5.4.1. Using the image saved in Step 3.3.8, click the **Caliper** button and place the cursor on the highest peak of a well-defined curve. Do this in three curves and take the average. Save the image.

5.5. Kerley B-line artifact assessment

NOTE: The horizontal A-lines which represent a normal lung surface can be used for reference in the detection of B-lines. These arise from the pleura and are hyperechoic compared to A-lines.

5.5.1. Adjust the contrast of the image and/or the gain. B-lines are not always immediately visible in the saved images.

5.5.2. Determine and register the number of Kerley B-lines for each of the six images obtained. Since the number of B-lines is not saved in the machine, it must be immediately registered on the CRF (between 0 and 5).

5.6. IVC diameter and collapsibility

5.6.1. Click the **Probe** button to select the abdominal transducer in order to start the measurements.

5.6.2. Using the 2D image saved in Step 3.4.2, click the **Caliper** button and measure the distance between the two walls of the IVC at 2 cm from the where it enters the right atrium. This is the IVC diameter, save this image.

5.6.3. Using the M-mode image saved in Step 3.4.3, click the **Caliper** button and measure the IVC expiratory and inspiratory diameters. Save this image.

Note: The expiratory and inspiratory diameters of the IVC are the maximum and minimum diameters seen in the M-mode image, respectively.

5.7. Kidney length and blood flow

5.7.1. Using the 2D image saved in Step 3.4.4, click the **Caliper** button and draw the longest line spanning from the caudal to the cranial ends of the renal cortex. This is the kidney length in cm, register this finding in the CRF. Save this image.

5.7.2. Using the Doppler image saved in Step 3.4.6, analyze the venous flow line seen below the baseline as continuous, monophasic or biphasic. Register the findings in the CRF.

5.7.3. Using the image saved in Step 3.4.6, click the **Measure** button and use the trackball to select the **Abdominal-Renal-PS/ED/RI** options on the right-side menu.

5.7.4. Place the cursor on the peak and on the lowest point of the pulsatile flow wave in the positive half of the y axis.

NOTE: The G6 machine can calculate the Doppler renal resistive index (RRI) automatically if a continuous pulsatile flow wave is stored, using the formula: RRI = (peak systolic velocity - end diastolic velocity)/peak systolic velocity. Save the image with the measurement from the ultrasound machine on the screen.

5.7.5. Using the image saved in Step 3.4.6 or 3.4.7, click the **Caliper** button and place the cursor, first, over the peak maximum flow velocity, and then over the maximal flow velocity at nadir (*i.e.,* end diastolic)[19]. Save the image after measurement.

NOTE: The venous impedance index (VII) is calculated from: VII = (peak systolic velocity - end diastolic velocity) / peak systolic velocity[20,21]. The VII is not registered in case of monophasic flow, as then only one peak is visible and no diastolic and systolic phases are distinguishable.

6. Data Registration, Storage, and Validation of Ultrasound Images

NOTE: As **Figure 1** shows, data registration is done after each clinical examination. Below, the procedure of entering the data obtained from the measurements, clinical examination, and biochemical information (**Table 2**) retrieved from the electronic health record into the study subject's anonymized online file is described.

6.1. Access the online secured patient management system and open the dossier of the recently included patient. Register the blood gas analysis values, general serum variables, serum renal variables, and 24-hour urine analysis. The list of all the variables that are obtained and instructions to do so are presented in **Table 2**.

6.2. Validate the cardiac CCUS images.

Note: This validation is done by independent experts of a Cardiovascular Imaging Core Laboratory in accordance with EACVI guidelines[22]. The quality of the images obtained by the student-researchers is assessed and the measurements performed are repeated to ensure the required quality of dimensional measurements and tracings of velocity profiles.

6.2.1. Perform the LVOT measurements at end-diastole, as seen on the ECG signal, just beneath the aortic valve.

6.2.2. Trace the PW signal taken from the LVOT in an AP5CH view to obtain the left ventricular stroke volume and left ventricular CO.

6.2.3. Validate all images and measurements of the IVC and kidneys. For this, employ by an independent experienced abdominal radiologist. Should there be issues with obtaining the

desired images during clinical examination, the independent abdominal radiologist can be called to perform the CCUS, in which case no further validation takes place.

7. Patient Follow-Up

7.1. Registering informed consent

7.1.1. If patient or family consent is obtained during any of the clinical examinations or after the clinical examinations protocol is finished but the patient is still admitted to the hospital, register this in the patient data management system and upload the hand-signed consent form.

7.1.2. If consent was denied, register this in the patient data management system along with the reason for not obtaining consent, and notify the study coordinator, who will delete all patient data.

7.2. Mortality data

7.2.1. For patients who die during admission, register mortality directly from the electronic patient record and the associated cause of death.

7.2.2. For patients without in-hospital mortality, obtain mortality data from the municipal registry in the Netherlands, which is updated every 90 days.

REPRESENTATIVE RESULTS:

The purpose of these representative results is to illustrate the feasibility of the protocol.

Patients

In total, 663 patients were admitted to the ICU between 14-05-2018 and 15-08-2018. Of these, 208 patients were eligible for inclusion (reasons for exclusion are displayed in **Figure 4**). A number of 49 patients were excluded as there was no possibility to perform the CCUS due to ongoing resuscitation efforts. Seven patients refused to participate (no informed consent) and in four patients CCUS was impossible, *e.g.,* due to prone positioning for mechanical ventilation or vacuum assisted closure of large wounds, resulting in 138 included patients with data for analysis.

CCUS validation and image quality

Extensive validation of cardiac imaging is planned for September 2018. Renal ultrasonography validation has been initiated. So far, images of 21 patients (15%) were validated. In 18 patients (86%) images appeared of sufficient quality. All reasons for disapproval of images were listed and returned for feedback to the researcher who performed the ultra-sonography. The name of the researcher who performed the ultrasonography is recorded to be able to asses inter- and intra-observer variability using the Intraclass Correlation Coefficient (ICC). Exact statistical methods will be described in our statistical analysis plan, as was done in the SICS-I[12].

Example case: Patient X, 52-year-old female

Patient X was admitted after she was found with impaired consciousness and low blood pressure. All obtained measurements are shown in **Table 1**. All variables were obtained within the required time set without missing data, illustrating the possible feasibility of this protocol. Within 3 hours after admission the first clinical examination was performed. During this examination the patient was sedated, intubated and needed vasopressor treatment. The second clinical examination was performed ten hours later and showed stable vitals after 700 mL of fluid infusion. Vasopressors were reduced. CCUS and biochemical analysis showed normal cardiac, IVC and renal function (**Figure 5, Figure 6 and Figure 7**). At T3, two days later, the vasopressors were stopped but cumulative positive fluid balance had risen to 6 liters, accompanied by an increased CO, wider IVC and diminished renal perfusion and function reflected by increased serum creatinine. At T4, 5 days after admission, fluid balance and serum creatinine had risen even further, where the patient developed stage 3 AKI. The patient died 2 days later due to multi organ failure with unclear origin, at 7 days after admission.

FIGURE AND TABLE LEGENDS:

Figure 1. **SICS-II study overview.**
Timeline of the SICS-II study from patient admission to Intensive Care to the final step of data registration.

Figure 2. **Case Report Form (CRF) for clinical examination 1.**
CRF to be filled by the ICU team students or student-researchers when conducting the first clinical examination.

Figure 3. Case Report Form (CRF) for clinical examinations 2, 3 and 4.
CRF to be filled by the ICU team students or student-researchers when conducting the second, third and fourth clinical examinations.

Figure 4. SICS-II patient inclusion and exclusion chart.
Flowchart describing the criteria for patient inclusion and exclusion in the SICS-II study until 15-08-2018.

Figure 5. **Apical five chamber (AP5CH) view showing change in cardiac function.**
(**A**) Image of the heart on an AP5CH view during CCUS conducted during clinical examination 2 (T=2); (**B**) Image of the heart VTI pulse wave signal on T=2, showing a CO of 5.6 L/min; (**C**) Image of the heart on an AP5CH view during CCUS conducted during clinical examination 3 (T=3); (**D**) Image of the heart VTI pulse wave signal on T=3, showing a CO of 8.3 L/min.

Figure 6. M-mode image of the inferior vena cava (IVC) for diameter measurements.
Image showing, on top, the IVC in real-time, and, below, the M-mode image representing the IVC diameter changes, from which the collapsibility can be calculated.

Figure 7. The various elements of renal ultrasonography

(**A**) Image of the right kidney during CCUS; (**B**) Image showing, on top, the Doppler flow in the renal arteries, and, below, the flow wave from which the renal resistive index is calculated; (**C**) Image showing, on top, the Doppler flow in the renal veins, and, below, the flow wave from which the venous impedance index is calculated; (**D**) Image illustrating the measurement of renal length.

Table 1. **A random SICS-II patient.**
Patient X, 52-year-old female admitted to the ICU after being found with impaired consciousness. Abbreviations: bpm = beats per minute, CRT = capillary refill time, LVOT = left ventricular outflow tract, TAPSE = tricuspid annular plane systolic excursion, RV S' = right ventricular systolic excursion, IVC = inferior vena cava, RRI = Renal resistive index, VII = venous impedance index, N.A. = not applicable.

Table 2. **List of biochemical variables obtained.**
All patient biochemical variables collected during the study are listed here.

DISCUSSION:
All examinations need to be performed according to the protocol. Physical examination only has value if performed according to pre-specified definitions[23]. Laboratory values should be collected according protocol to obtain all values. Clear, interpretable CCUS images are key to answer the research question of this study, as described in Step 3.3. If poor quality images are obtained, the measurements and analyses described in Step 5 cannot be performed, and the purpose of repeated measurements expires. Three important measures are taken to minimize the risk of obtaining low quality images. First, student-researchers who perform CCUS in our study are trained by an experienced cardiologist-intensivist. Literature shows that a short training program is well suited to obtain basic competence in CCUS[24]. Second, the student researchers are supervised by a senior student-researcher during their first 20 exams so they may receive hands on feedback. Last, all acquired cardiac and kidney images will be reassessed and validated by an independent expert of a Cardiac Imaging Core Laboratory and an experienced abdominal radiologist, respectively to ensure that data is reliable.

To ensure image quality, researchers also need to pay attention to other aspects. Re-applying ultrasound gel or repositioning the probe so that it makes better contact with the skin of the patient is sometimes required to ensure optimal image quality. It is also important to take enough time to acquire the most optimal image and if there are doubts a senior researcher, *i.e.,* a supervising cardiologist-intensivist or core laboratory technician, should be consulted before the clinical examination is completed. Continuous evaluation and validation of all ultrasonographic images is ensured by enforcing the protocolized steps displayed in **Figure 1**. In addition, student-researchers and experts frequently exchange feedback, making it easy to quickly implement protocol changes to further increase the quality of images and measurements. This frequent verification makes systematic errors easy to detect so that the CCUS training for future student-researchers can be adapted accordingly. Furthermore, monthly meetings open to all team members allows thorough evaluation and (if necessary) modifications of the protocol.

Round the clock availability for patient screening and inclusion is another key element for the successful implementation of this study. This can only be achieved by having a dedicated team of student-researchers, a large team of students to provide support, and good coordination with the ICU caregivers. This coordination takes place by regular low stake contact between caregivers and researchers about possible improvements to optimize collaboration with standard care.

A limitation of this protocol is that successfully conducting CCUS is dependent on the accessibility of the pre-specified positions where the probe is placed. During the SICS-I, it was already shown that cardiac CCUS cannot be performed when patients require drains, gauzes or wound dressings which obstruct the theoretically optimal echocardiographic window[1]. Additionally, the possibility to obtain a proper subcostal window *via* transthoracic echocardiography, which is required for the IVC measurements, has previously been shown to be potentially limited in a general ICU population[25]. The 24/7 availability required by this protocol to carry out the different examinations at different time points is also a potential limitation, as some centers may lack the capacity to do so. Even in a large academic hospital such as the UMCG, ensuring this has led to delays in the start of the study. Another limitation intrinsic to ultrasonographic measurements is the inter-observer variability of the measurements. For patient inclusion to be guaranteed 24/7, it is impossible for one researcher to conduct all clinical examinations in all included patients. This study aims to have the same researcher carry out all ultrasound measurement in one same patient to minimize variability at the individual level, but for the entire cohort, inter-observer variability remains an issue.

Ultrasonographic imaging of multiple organs can be a fast, safe, and effective structure for visualizing organ perfusion and function. It is a convenient tool that all medical professionals should be able to use, and for which few measurements based on a simple, standardized protocol should generally provide reliable measurements.

Furthermore, most observational studies evaluating the use of ultrasonography, and particularly of echocardiography, are retrospective in nature or include only a small number of patients.[26] This protocol allows a structural 24/7 screening of an unselected cohort of critically ill patients, of which subpopulations of interest can be defined, thus allowing for the simultaneous investigation of multiple research questions.

Moreover, despite it being known that clinical variables in critical care are highly dynamic and reciprocally influence each other, most studies have only investigated the additive value of singular ultrasound measurements of specific organs[27,28]. This is the first protocol to focus on repeated measures, whole body ultrasound and venous congestion. We expect that the SICS-II will provide a more accurate reflection of patients' hemodynamic status during ICU admission.

The current structure used in SICS can be applied to a large number of settings, and the addition of other elements is currently being studied. Its strength lies in the combination of a basic research line and an adaptive line in which new variables can easily be added to the CRFs so that new research questions can be investigated. An example of this adaptability is the addition of

extensive ventricular wall assessment by deformation imaging, *i.e.,* strain on short term, to the regular protocol in a specific subset of patients.

Moreover, patient inclusion is currently exclusively taking place in the ICU and part of the patients' care trajectory is now missed. ICU patients are often first admitted to the emergency department (ED), and stay in the regular hospital ward after ICU discharge. Therefore, the SICS aims to include patients at an earlier stage by including patients upon ED arrival and register interventions and hemodynamic function from initial hospital admission onwards. Furthermore, plans to conduct CCUS after ICU-discharge to regular wards are also ongoing so that all patients can be measured at each predefined study time. Another important aspect is the expandability of the protocol to other centers: its simplicity allows easy adaptation by centers which can start inclusion themselves.

Lastly, the development and successful implementation of a structured CCUS protocol may also have clinical ramifications. Despite being used for research purposes only, it could be implemented for clinical CCUS by medical doctors after the proposed short training period. It would then be interesting to assess if facilitating CCUS training to (inexperienced) physicians would decrease additional diagnostic testing.

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DISCLOSURES:

Authors have nothing to disclose.

REFERENCES:

1. Hiemstra, B. *et al.* Clinical examination, critical care ultrasonography and outcomes in the critically ill: cohort profile of the Simple Intensive Care Studies-I. *BMJ open*. **7** (9), e017170, 10.1136/bmjopen-2017-017170 (2017).
2. Lee, J. *et al.* Association between fluid balance and survival in critically ill patients. *Journal of Internal Medicine*. **277** (4), 468–477, 10.1111/joim.12274 (2015).
3. Perner, A. *et al.* Fluid management in acute kidney injury. *Intensive Care Medicine*. **43** (6), 807–815, 10.1007/s00134-017-4817-x (2017).
4. Balakumar, V. *et al.* Both Positive and Negative Fluid Balance May Be Associated With Reduced Long-Term Survival in the Critically Ill. *Critical care medicine*. **45** (8), e749–e757, 10.1097/CCM.0000000000002372 (2017).
5. Hjortrup, P.B. *et al.* Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial. *Intensive Care Medicine*. **42** (11), 1695–1705, 10.1007/s00134-016-4500-7 (2016).

6. Prowle, J.R., Kirwan, C.J., Bellomo, R. Fluid management for the prevention and attenuation of acute kidney injury. *Nature reviews.Nephrology*. **10** (1), 37–47, 10.1038/nrneph.2013.232 [doi] (2014).
7. Gambardella, I. *et al.* Congestive kidney failure in cardiac surgery: the relationship between central venous pressure and acute kidney injury. *Interactive CardioVascular and Thoracic Surgery*. **23** (5), 800–805, 10.1093/icvts/ivw229 (2016).
8. Chen, K.P. *et al.* Peripheral Edema, Central Venous Pressure, and Risk of AKI in Critical Illness. *Clinical journal of the American Society of Nephrology : CJASN*. **11** (4), 602–8, 10.2215/CJN.08080715 (2016).
9. Song, J. *et al.* Value of the combination of renal resistance index and central venous pressure in the early prediction of sepsis-induced acute kidney injury. *Journal of critical care*. **45**, 204–208, 10.1016/j.jcrc.2018.03.016 (2018).
10. Zhang, L., Chen, Z., Diao, Y., Yang, Y., Fu, P. Associations of fluid overload with mortality and kidney recovery in patients with acute kidney injury: A systematic review and meta-analysis. *Journal of Critical Care*. **30** (4), 860.e7-860.e13, https://doi.org/10.1016/j.jcrc.2015.03.025 (2015).
11. Simple Intensive Care Studies II - Full Text View - ClinicalTrials.gov. at <https://clinicaltrials.gov/ct2/show/NCT03577405?term=simple+intensive+care+studies &rank=1>.
12. Wetterslev, J. Statistical analysis plan Simple Intensive Care Studies-I DETAILED STATISTICAL ANALYSIS PLAN (SAP) 1. Administrative information 1.1. Title, registration, versions and revisions. at <https://clinicaltrials.gov/ProvidedDocs/24/NCT02912624/SAP_000.pdf>.
13. I.C.C. van der Horst Simple Observational Critical Care Studies - Full Text View - ClinicalTrials.gov. at <https://clinicaltrials.gov/ct2/show/NCT03553069?term=SOCCS&rank=1>.
14. Ait-Oufella, H. *et al.* Alteration of skin perfusion in mottling area during septic shock. *Annals of Intensive Care*. **3** (1), 31, 10.1186/2110-5820-3-31 (2013).
15. Teasdale, G., Jennett, B. Assessment of Coma and Impaired Consciousness: A Practical Scale. *The Lancet*. **304** (7872), 81–84, 10.1016/S0140-6736(74)91639-0 (1974).
16. Detsky, M.E. *et al.* Discriminative Accuracy of Physician and Nurse Predictions for Survival and Functional Outcomes 6 Months After an ICU Admission. *JAMA*. **317** (21), 2187, 10.1001/jama.2017.4078 (2017).
17. Lipson, A.R., Miano, S.J., Daly, B.J., Douglas, S.L. The Accuracy of Nurses' Predictions for Clinical Outcomes in the Chronically Critically Ill. *Research & reviews. Journal of nursing and health sciences*. **3** (2), 35–38 (2017).
18. Lichtenstein, D.A. BLUE-protocol and FALLS-protocol: two applications of lung ultrasound in the critically ill. *Chest*. **147** (6), 1659–1670, 10.1378/chest.14-1313 (2015).
19. Tang, W.H.W., Kitai, T. Intrarenal Venous Flow: A Window Into the Congestive Kidney Failure Phenotype of Heart Failure? *JACC: Heart Failure*. **4** (8), 683–686, 10.1016/J.JCHF.2016.05.009 (2016).
20. Jeong, S.H., Jung, D.C., Kim, S.H., Kim, S.H. Renal venous doppler ultrasonography in normal subjects and patients with diabetic nephropathy: Value of venous impedance index measurements. *Journal of Clinical Ultrasound*. **39** (9), 512–518, 10.1002/jcu.20835 (2011).

21. Iida, N. *et al.* Clinical Implications of Intrarenal Hemodynamic Evaluation by Doppler Ultrasonography in Heart Failure. *JACC: Heart Failure*. **4** (8), 674–682, 10.1016/J.JCHF.2016.03.016 (2016).
22. Lang, R.M. *et al.* Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *European Heart Journal – Cardiovascular Imaging*. **16** (3), 233–271, 10.1093/ehjci/jev014 (2015).
23. Hiemstra, B., Eck, R.J., Keus, F., van der Horst, I.C.C. Clinical examination for diagnosing circulatory shock. *Current opinion in critical care*. **23** (4), 293–301, 10.1097/MCC.0000000000000420 (2017).
24. Vignon, P. *et al.* Basic critical care echocardiography: validation of a curriculum dedicated to noncardiologist residents. *Critical care medicine*. **39** (4), 636–42, 10.1097/CCM.0b013e318206c1e4 (2011).
25. Jensen, M.B., Sloth, E., Larsen, K.M., Schmidt, M.B. Transthoracic echocardiography for cardiopulmonary monitoring in intensive care. *European journal of anaesthesiology*. **21** (9), 700–7, at <http://www.ncbi.nlm.nih.gov/pubmed/15595582> (2004).
26. Koster, G., van der Horst, I.C.C. Critical care ultrasonography in circulatory shock. *Current opinion in critical care*. **23** (4), 326–333, 10.1097/MCC.0000000000000428 (2017).
27. Haitsma Mulier, J.L.G. *et al.* Renal resistive index as an early predictor and discriminator of acute kidney injury in critically ill patients; A prospective observational cohort study. *PloS one*. **13** (6), e0197967, 10.1371/journal.pone.0197967 (2018).
28. Micek, S.T. *et al.* Fluid balance and cardiac function in septic shock as predictors of hospital mortality. *Critical care (London, England)*. **17** (5), R246, 10.1186/cc13072 (2013).

Figure 1.

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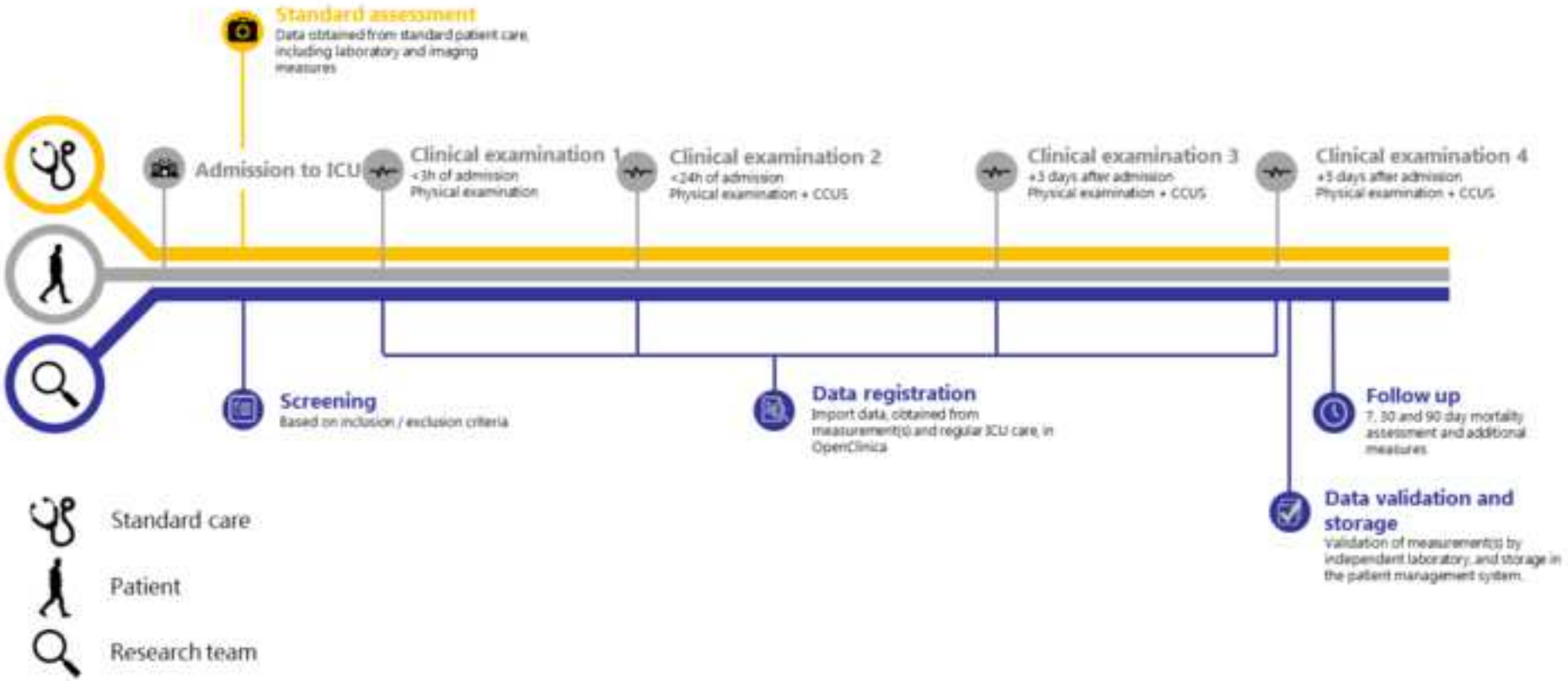


Figure 2. Click here to access/download;Figure;Figure 2.pdf

CRF **ICU bed:** **Study ID:** **Exam no:** **1**

COLLECT

Date		
Time		
Heart rate		bpm
Resp rate		min
SpO2		%
SBP		mmHg
DBP		mmHg
MAP		mmHg
Arm	*Left*	*Right*
CVP		mmHg
Urine previous hour		ml

Non-invasive respiratory support:	*Yes*	*No*
Ventimask/optiflow	*Yes*	*No*
Nasal cannula	*Yes*	*No*
FiO2 / L O2		% / L
PEEP		cm H20
Mechanical ventilation:	*Yes*	*No*
PEEP		cm H20
FiO2		%

Sedative	Dose		Speed	
None				
Midazolam		mg/ml		ml/h
Propofol		mg/ml		ml/h

Inotropics /vasopressors				
None				
Noradrenalin		mg/ml		ml/h
Vasopressin		mg/ml		ml/h
Dobutamin		mg/ml		ml/h
Dopamin		mg/ml		ml/h
Milrinone		mg/ml		ml/h

CHECK

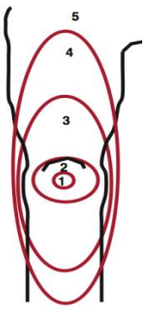
Temperature

Artifical warming / artificial cooling / none

Subjective: *Warm* *Cold*

Moist? *Yes* *No*

Mottling (0-5)



CRT sternum		sec
CRT knee		sec
Bladder		°C
Dorsum foot		°C
E-M-V	- -	
ECG time	:	

Height of arterial line dome compared with height heart

Higher / Lower / Same height

Left ventricular function

Good / Reasonable / Moderate / Poor

Estimations

	Student	**Nurse**	**A(N)IOS**
Return original residence 6 months	*Yes / No*	*Yes / No*	*Yes / No*
6 month survival	*Yes / No*	*Yes / No*	*Yes / No*
In hospital survival	*Yes / No*	*Yes / No*	*Yes / No*

Figure 3. Click here to access/download;Figure;Figure 3..pdf

CRF **Ultrasound:** **Study ID:** **Exam no: 2 3 4**

Date			
Time			
Heart rate		bpm	
Rythm	*Regular*	*Irregular*	*AF*
Resp rate		min	
SpO2		%	
SBP		mmHg	
DBP		mmHg	
MAP		mmHg	
Arm	*Left*	*Right*	
CVP		mmHg	
Fluid balance		mL	
Urine 1h		ml	
Urine ml/x h		ml in	hours

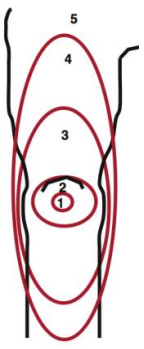
Temperature

Artificial warming / artificial cooling / none

Subjective: *Warm* *Cold*

Moist? *Yes* *No*

Mottling (0-5)



CRT sternum		sec
CRT knee		sec
Bladder		°C
Dorsum foot		°C
E-M-V	- -	

Left ventricular function

Good / Reasonable / Moderate / Poor

Estimations

Non-invasive respiratory support:	*Yes*	*No*
NIV/CPAP	*Yes*	*No*
Ventimask/optiflow/NRM	*Yes*	*No*
Nasal cannula	*Yes*	*No*
PEEP		cm H20
FiO2		%

Invasive respiratory support:	*Yes*	*No*
PEEP		cm H20
FiO2		%

Sedative	**Dose**		**Speed**	
		mg/ml		ml/h
		mg/ml		ml/h
Inotropics				
		mg/ml		ml/h
		mg/ml		ml/h

Student	**Nurse**	**ANIOS/AIOS**
Return original residence 6 months		
Yes / No	*Yes / No*	*Yes / No*
6 month survival		
Yes / No	*Yes / No*	*Yes / No*
In hospital survival		
Yes / No	*Yes / No*	*Yes / No*

Critical care ultrasound measurements

Cardiac

LVOT		cm
Heart rate		bpm
Cardiac Output		L/min
TAPSE		mm
RV S'		cm/s

Number of B -lines on lung ultrasound

	Left	*Right*
Superior		
Inferior		
Lateral		

General

IVC min		cm
IVC max		cm

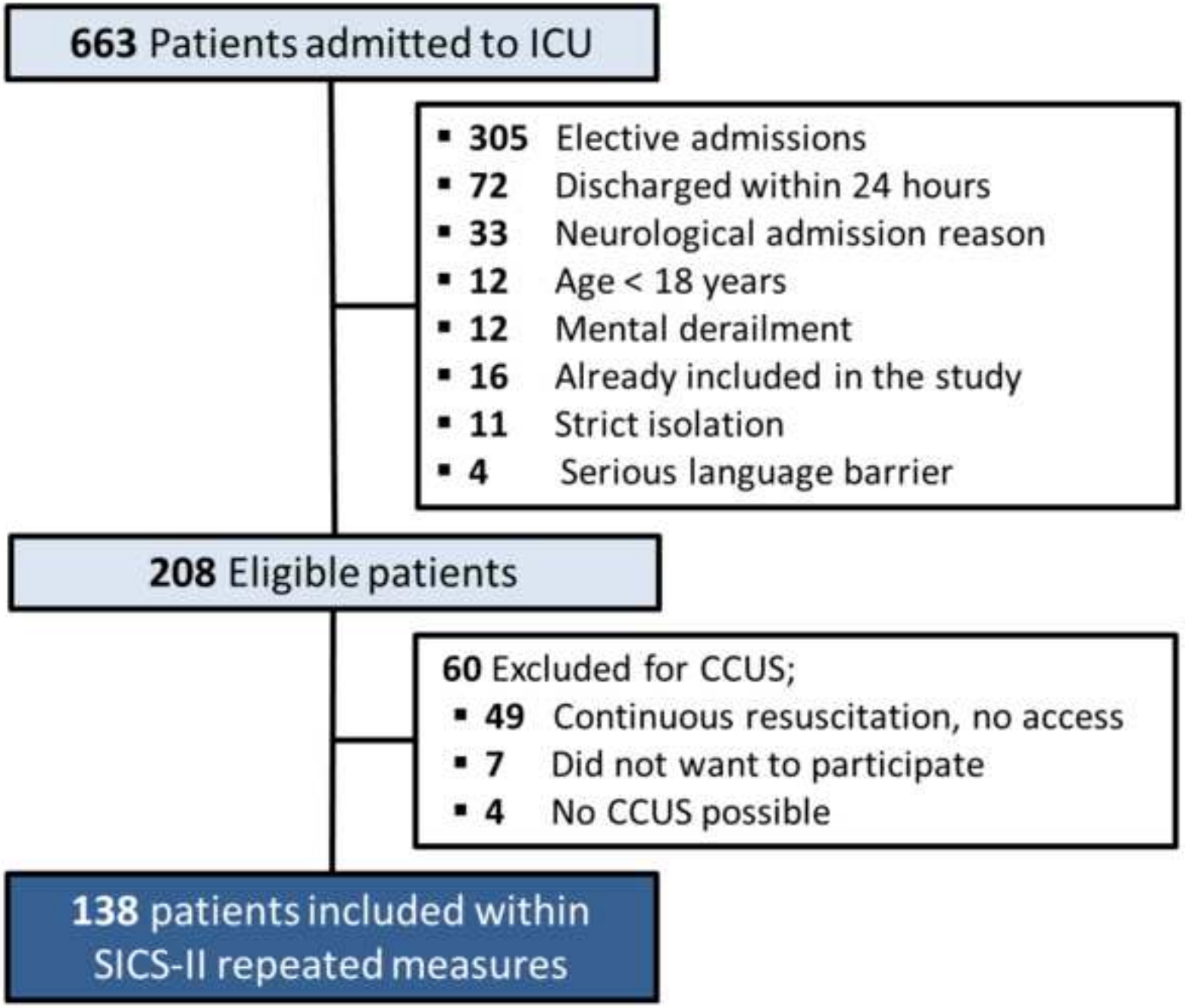
Renal *Left / Right*

IRVF *Continuous / Biphasic / Monophasic*

DRRI	
VII	
Kidney size	cm

CCUS quality? *Poor / Suboptimal / Optimal*

Figure 4.



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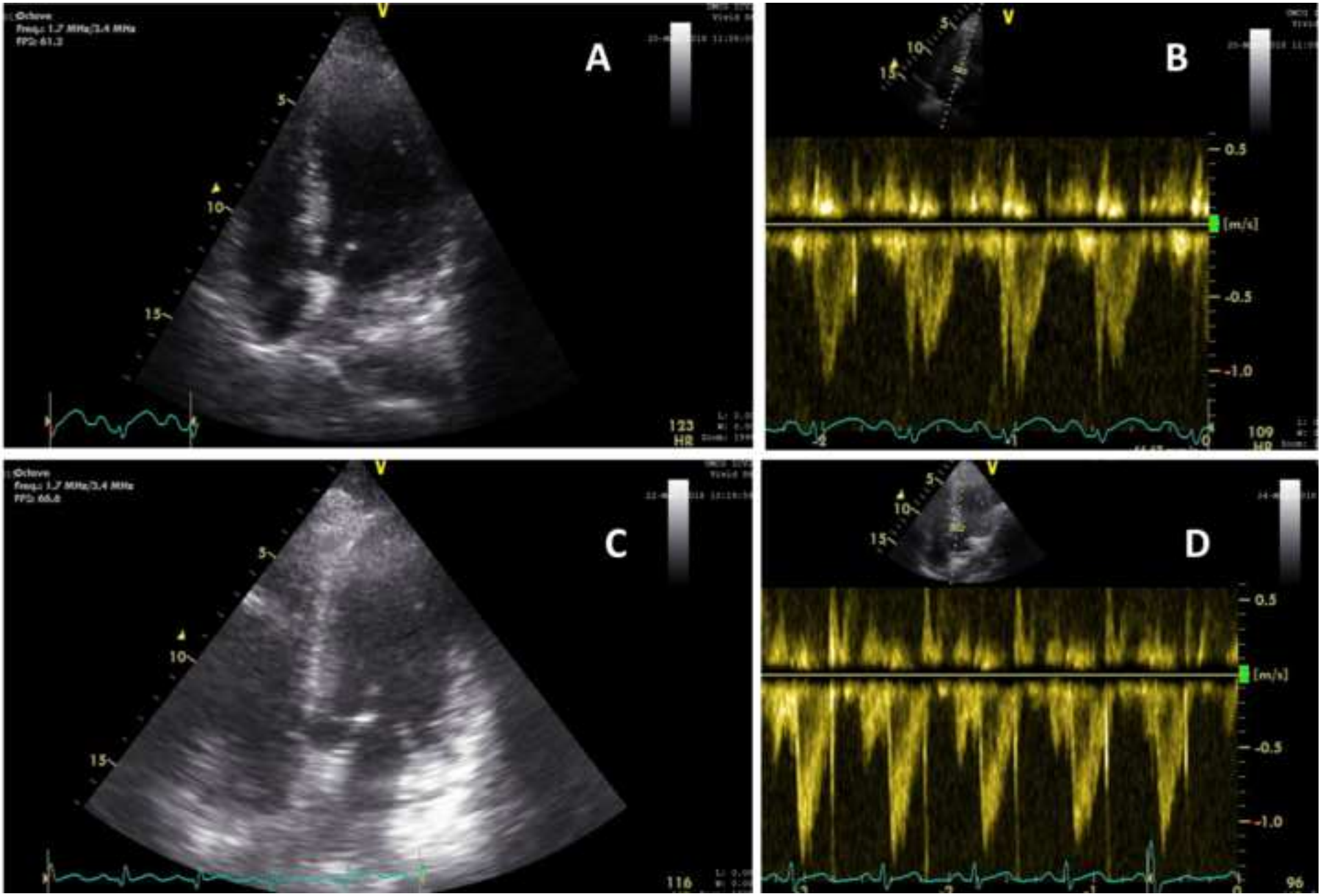

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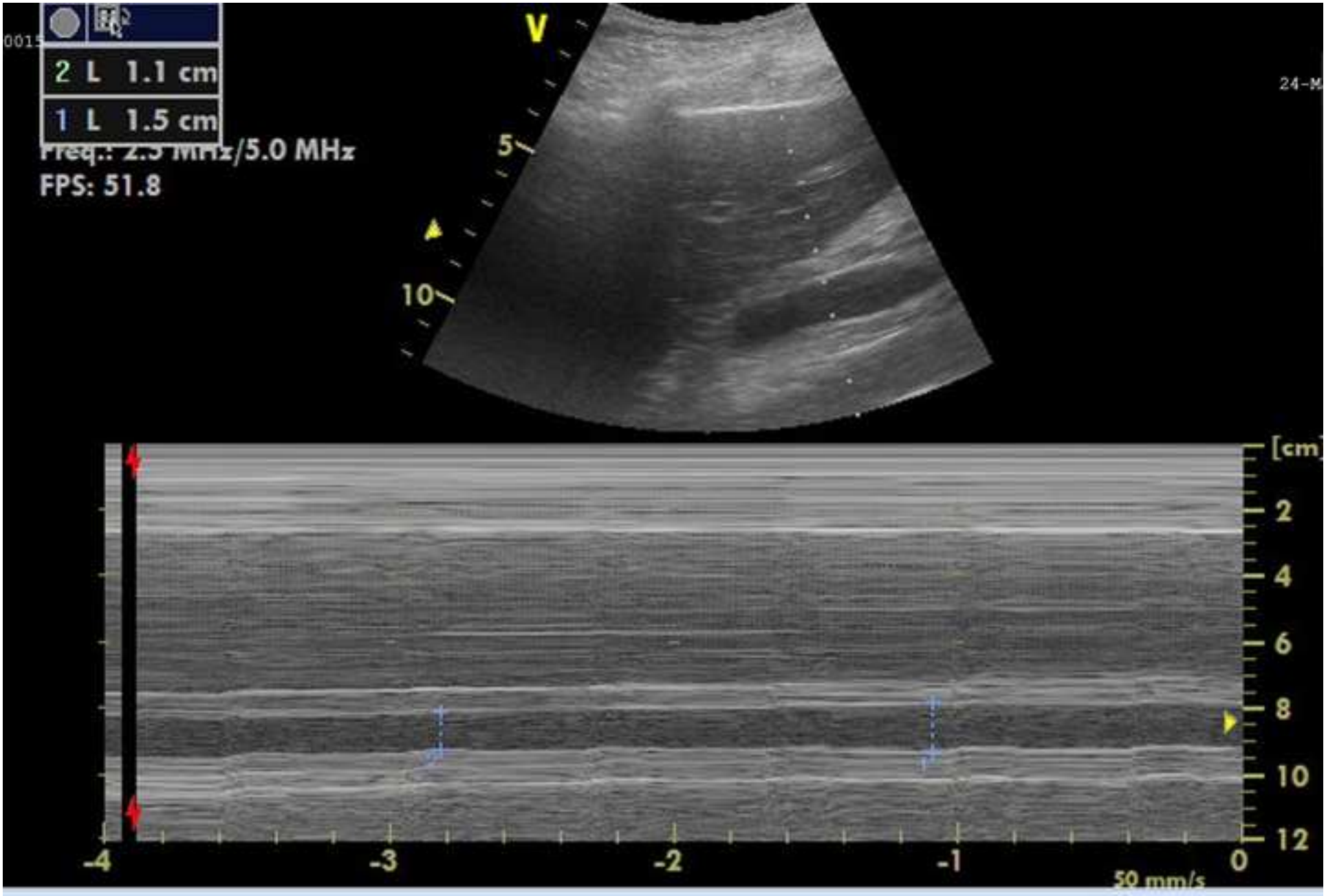

Figure 7.

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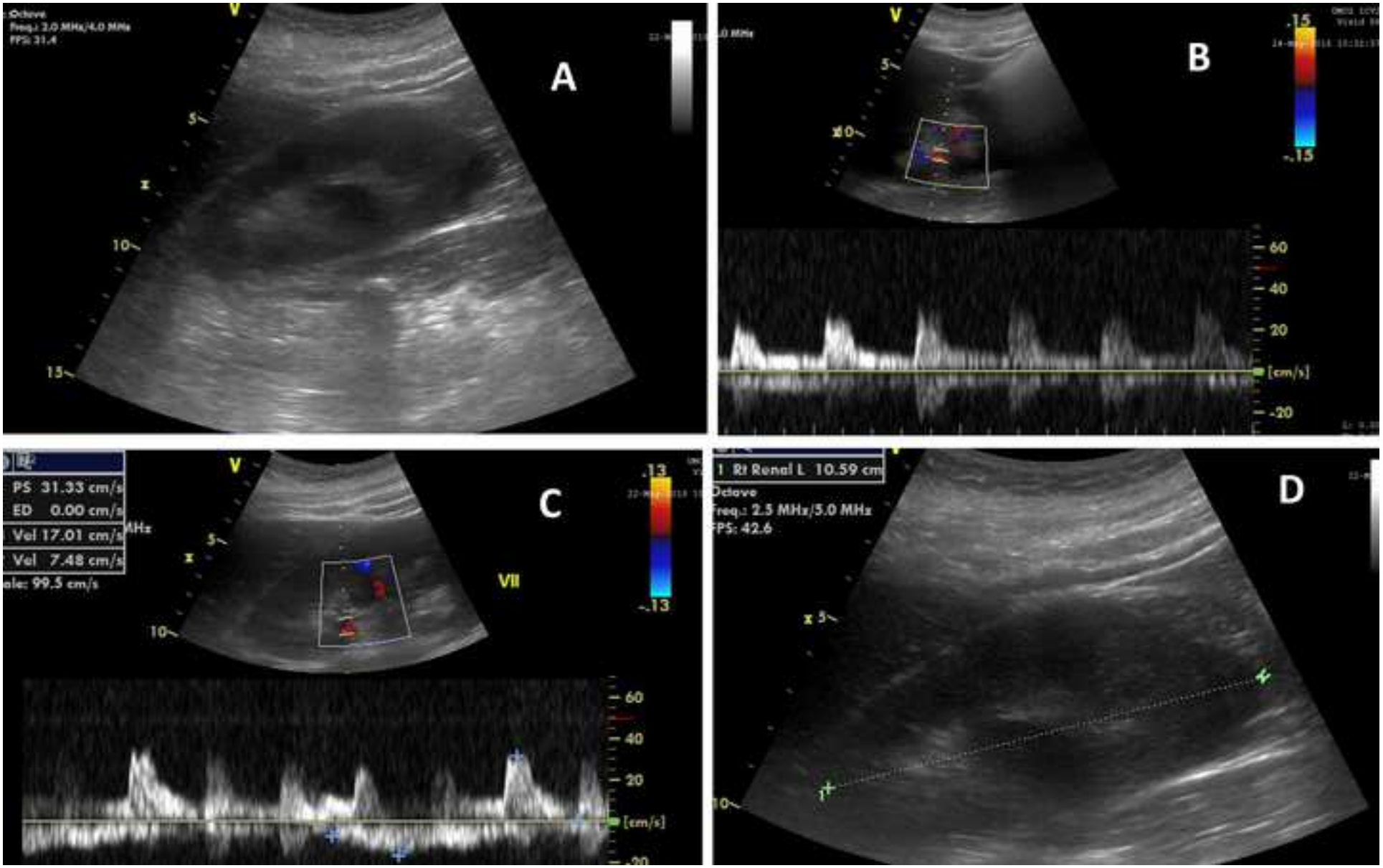

Table 1

Variable	T_1 Day 1, at 00:38	T_2 Day 1, at 10:53	T_3 Day 3, at 10:14	T_4 Day 5, at 10:20
Heart rate (bpm)	110	124	122	98
Respiratory rate (breaths per min)	24	15	26	12
Systolic blood pressure (mmhg)	100	115	130	118
Diastolic blood pressure (mmhg)	61	69	66	65
Mean arterial pressure (mmhg)	73	80	84	81
Cumulative fluid balance (mL)	0	704	7272	12338
Mechanical ventilation	PEEP 5, FiO2 40%	PEEP 5, FiO2 40%	PEEP 5, FiO2 30%	PEEP 5, FiO2 30%
CRT sternum (seconds)	1.5	2	4	3
Central temperature (◦ C)	37.6	37.5	38.0	37.4
Urinary output previous hour (mL)	117	60	0	10
Administered inotropic agents	Noradrenaline 0.1 mg/ml 3.0 ml/h	Noradrenaline 0.1 mg/ml 1.0 ml/h	none	none
Administered sedative agents	Propofol 20 mg/ml 5.0 ml/h	none	none	none
APACHE IV score	92	88	87	90
SOFA score	8	8	5	8
LVOT (cm)	N.A.	2.4	2.4	2.4
Cardiac output (L/min)	N.A.	5.6	8.34	9.89
TAPSE (mm)	N.A.	25	26	21
RV S' (cm/s)	N.A.	14	15	12
IVC inspiratory diameter (cm)	N.A.	1.14	1.24	1.10
IVC expiratory diameter (cm)	N.A.	1.27	1.38	1.50
Kerley B lines (total)	N.A.	6	2	4
Renal length (cm)	N.A.	10.59	N.A.	N.A.
Intrarenal venous flow pattern	N.A.	Continuous	Continuous	Continuous
Doppler Renal RI	N.A.	0.61	0.75	0.70
VII	N.A.	0.33	0.56	0.68

Table 2

Click here to access/download;Table;Table 3. List of biochemical variables obtained.xlsx

Variable	Unit	Source	Obtained at
Lactate	mmol/L	Arterial blood gas analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
Chloride	mmol/L	Arterial blood gas analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
pH		Arterial blood gas analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
PCO2	kPa	Arterial blood gas analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
PaO2	kPa	Arterial blood gas analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
HCO3-	mmol/L	Arterial blood gas analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
Hemoglobin	mmol/L	Arterial blood gas analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
Leukocytes	10^E9/L	Serum analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
Trombocytes	10^E9/L	Serum analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
HS Troponine	ng/L	Serum analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
ASAT	U/L	Serum analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
ALAT	U/L	Serum analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
Total bilirubin	uoml/L	Serum analysis	From standard care, as close as possible too each clinical examination, max 12 hours difference
Creatinine	umol/L	Serum analysis	All measurements from start of ICU admission
Urine volume	ml	Urine collection of 24 hours	All measurements from start of ICU admission
Creatinine	mmol/24h	Urine analysis	All measurements from start of ICU admission

Table of Materials

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Ultrasound machine	GE Healthcare	0144VS6	Ultrasound machine, GE Vivid S6
Ultrasound machine	GE Healthcare	3507VS6	Ultrasound machine, GE Vivid S6
Ultrasound machine	GE Healthcare	0630VS6	Ultrasound machine, GE Vivid S6
Ultrasound gel	Parker	01-08	Aquasonic 100 ultrasound transmission gel
Temperature probe	DeRoyal	81-010400EU	Skin Temperature Sensor

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Title of Article: The Simple Intensive Care Studies-II: a prospective, observational study using repeated clinical examination and critical care ultrasonography measurements to determine the hemodynamic status of critically ill patients.

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Groningen, August 21st , 2018

Dr. DSouza

Senior Review Editor - JoVE

Subject: Revisions of our manuscript “The SICS-II: a prospective, observational study using repeated clinical examination and critical care ultrasonography in critically ill patients”

Dear dr. Dsouza,

We wish to thank you for the opportunity to revise our manuscript “The SICS-II: a prospective, observational study using repeated clinical examination and critical care ultrasonography in critically ill patients” for your journal. We sincerely appreciate the useful and critical comments from the reviewers. Below we have addressed all questions raised in a point-by-point fashion. We additionally made minor amendments to improve readability. All significant changes to the manuscript are highlighted in green, the content for filming is highlighted yellow.

We hope that you will now find our manuscript suitable for publication in your *Journal of Visualized Experiments.*

Yours sincerely,

On behalf of all co-authors,

Renske Wiersema

Iwan CC van der Horst

Editorial comments:

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

Please simplify and reduce the length of the title (~ 150 characters maximum)

Response: we thank you for this comment, we have shortened the title.

Protocol Detail: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

1) Line 194: Please cite a reference for GCS. Unclear what is done here and what we would film.

2) Line 195–197: Unclear what is done here and what we would film.

3) Line 253: Unclear how strain measurements are obtained. Mention any button clicks and menu selections.

Response: we have clarified the text in these lines. We have added more specific details to further explain the protocol. We have also added a reference for the GCS. The strain measurements are part of a substudy and we have chosen to leave it out of this manuscript for now.

Protocol Numbering: Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

Response: we have adjusted the numbering.

Protocol Highlight: If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE's instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

2) Some of your shorter protocol steps can be combined so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

3) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

4) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.

5) Notes cannot be filmed and should be excluded from highlighting.

6) I suggest un-highlighting portions of Section 1, 6, and 7 due to low filmable content.

Response: we have adjusted the highlighting.

Figures: Each individual figure should fit within 1 page. Please re-scale Fig 3 to meet this requirement.

Figure/Table Legends: Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.

Response: we have reformatted all the figures.

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Response: we have removed all commercial names.

Please define all abbreviations at first use.

Response: we have defined all abbreviations at first use.

Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

Response: we have used standard abbreviations.

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Response: we have no figures that have previously been published.

Reviewer Comments to Author:
Reviewer: 1
Comments to the Author

Overall:
‘’However, no information on the statistical analyses nor the patients' treatment is given. The discussion emphasizes on the used methods including their limitations and the purpose of the study design. You might consider to give more information how you plan to interpret the data why the gained insight is valuable. Generally, I'm uncertain, why you choose to publish the study protocol at this point. Even though, patients have been included already, no data, exempt for a single example patient, is being presented. The study seems well planned, however I have some concerns regarding the statistical analyses and the interpretation of the data. Overall the reason for publication at this stage should be made more clearly.’’

Response: we agree with this statement and have clarified the purpose of publishing this protocol now, which does not include a statistical plan as it concerns the purpose of a data registry and protocol on itself. This applies to more of your comments, which has urged us to make significant changes in the introduction and results. We have also edited the title of the manuscript to clearly demonstrate the purpose of publishing the study protocol.

Specific comments:
1) Your case report forms (CRF) give a detailed description of the collected data and include a comprehensive assessment of the hemodynamic status. However, it is unclear, which biochemical variables are collected. Are only the CRF parameters included or do you also record other blood/serum values, e.g. haematocrit, creatinine (see the example patient) or uric acid as parameters for volume status and renal function (as stated under 6.1)? Which values are being measured?

Response: thank you for this comment. We collect multiple biochemical variables and have added an extra supplement (table 3) stating the list of biochemical variables we gather.

2) Your study is observational, and the treatment of patients is not affected by study participation. In order to get a better of the understanding of the collected data, it would be interesting to get insight on the standard care, especially fluid and vasoactive treatment (e.g. type of fluid/medication, basic volumes etc.). As some data is obtained apart from standard care, is this information provided to the attending physician?

Response: fluids and vasoactive medications are given as part of standard care. The measurement performed in our study are not provided to the attending physician. We however estimate and know from experience that physicians perform similar investigations, but more subjectively.

3) It is unclear, how the data will be used as there is no information on statistical analyses. How do you plan to analyse and interpret the data? In the study protocol on clinicaltrials.gov you state that prediction of acute kidney injury, short-term organ deterioration and 7-day mortality are secondary objectives, however these are hardly mentioned in the protocol. In order to create useful and high quality data, the outcome parameters and research questions should be defined ahead of time. Also, there is no information how many patients you plan to include. Do you include all unplanned ICU patients and only exclude those, who fall under the exclusion criteria? The inclusion criteria are mentioned several times, yet I could not find inclusion criteria.

Response: we thank you for this comment, so we may clarify this part in our manuscript. We have added the inclusion criteria. We have further clarified the objectives of this manuscript and also of the purpose of publishing this manuscript:to provide a research structure and evaluate our protocol.

4) In the introduction, you state that a high risk-profile for developing venous congestion was successfully identified based on different variables. How was this derived from your data set? This claim should be supported by numbers and statistics as it is not picked up throughout the manuscript. In your manuscript you state that 60 patients have already been included in the study, however no such data is presented.

Response: we have deleted this sentence from our manuscript. We meant to state that our preliminary results show that the protocol is feasible and that examples of our hypothesis could be seen. The overall goal of our protocol, not of this manuscript, is to identify this high risk profile with sufficient power and proper statistical analysis according to our statistical analysis plan, which will be published later on.

5) In the protocol, several aspects are marked as "NOTES". Please explain the purpose of this label for the instructions.

Response: the notes are meant for the format of the JoVE, and we were asked to provide certain (unfilmable) comments in this format.

6) There seems to be a formatting issue with the manuscript as figure 1 and 4 are listed under supplementary data. This should be fixed in order to avoid confusion. Also the footer lines states that there are only 6 pages in the manuscript (in my version it adds up to 15) and that is was already revised in November 2017.

Response: we worked in our own document and later transferred it to the JoVE template, this might have caused the mentioned issues. We will make sure these are fixed upon re-submission.

Reviewer #2:

I believe the rationale of the project is absolutely justified and tackle an important issue in critically ill patients. However, I don't fully understand the underlying direction of the present manuscript. The authors seem to pursue multiple goals:

1. To publish a protocol for this study describing its design and practical implementation

2. To describe their multisystem ultrasound assessment of venous congestion in critically ill patients

3. To describe how a group of student-investigators supported by experts can lead a large observational cohort study with detailed echographic examinations.

4. To demonstrate the feasibility of the study by presenting its implementation so far and a model case.

Unfortunately, I feel that none of these goals are met with the present manuscript. I think the authors should consider choosing one of these aspects to focus on. Please consider the following:

Multiple elements of study design that are of primary importance are not presented. The primary outcomes such as "Short term organ failure" in the clinicaltrial.gov entry is not precisely defined. Sample size estimation is not reported. What is the planned statistical analysis? This is important: consider 4 organs assessed (kidney, heart, IVC, lung) assessed at 3 timepoints and interpreted alone or in combination and 3 clinical outcomes (short term death, long term death, organ failure). This could result potentially in very high number of statistical tests inflating the risk of Type 1 error. While I understand the exploratory nature of the study, a priori hypothesis should be stated clearly or at least how the issue of multiplicity testing should be approached. Furthermore, I suppose they plan to adjust to other variables representing the severity of illness. Also, how will missing values will be handled should be detailed.

Response: thank you for this excellent comment. We have re-written the manuscript extensively with one specific goal: to present the protocol, its design, practical implementation and feasibility (goal 1 and goal 4 in your comment). Therefore we have decided to diminish the focus on venous congestion and AKI. We are currently writing the statistical analysis plan, as we have done for the SICS-I.[1] *We plan to describe goal 2 in your comment in a future design paper with a detailed statistical analysis plan and goal 3 after completion of the study.*

-Please consider the implications of this statement in the introduction of the manuscript: "Based on the findings in 60 patients examined thus far, the use of repeated measurements of a broad array of clinical variables seems to provide a more detailed impression of signs for venous congestion compared to CVP or fluid balance only. For example, a high-risk profile for developing venous congestion and acute kidney injury (AKI) during ICU stay was successfully identified based on the changes in administered vasoactive agents and in the observed rise in fluid balance, venous impedance index and CO. Table 1 presents the most relevant set of variables of an anonymous patient." This statement is inappropriate in the context of this manuscript. It is either based of undisclosed, very preliminary analysis of partial data or on a subjective assessment of the investigators.

Response: this statement has been removed from our manuscript. We meant to state that our preliminary results show that the protocol is feasible and that examples of our hypothesis could be seen. The overall goal of our protocol, not of this manuscript, is to identify this high

risk profile with sufficient power and proper statistical analysis according to our statistical analysis plan, which will be published later on.

-The authors should consider how excluding patients who have been discharged to the ward will potentially introduce a bias. (patients with a less severe illness will have missing exams).

Response: we thank you for this comment and are aware of this bias. We have not included it in our discussion as we aim to start performing ultrasonography on the ward soon.

-The consent process is ambiguous. Informed consent seems to be deferred, however the ability to consent is an inclusion criteria. Do the patient must at least be able to consent to have the ultrasound exam performed on them to be included? Only 13 patients were considered unable to provide informed consent according to the flowchart so I suspect the inclusion criteria refer to the clinician impression of the potential ability of the patient in the future to provide informed consent, despite the possibility to obtain it from the family??: "obtain family consent if family members are reachable. NOTE: If no family members are present and patient consent cannot be obtained, images collected can be used without express consent, under UMCG regulations for observational measurements per 1st of January 2016" In addition, what about the patient presented as a case?: "Patient X was admitted after having been found with impaired consciousness….Within 3 hours after admission the first clinical examination was performed. During this examination the patient was sedated, intubated and was on vasopressors." This is rather confusing and there is probably a clearer way to present this.

Response: we agree with your comment and have reported more clearly how our consent process takes place.

-Some elements of the flowchart are confusing. I don't understand the 7 patients who were "Already included" while being screened for eligibility. It would be important to know how many patients had the 'full-set' of ultrasound examination. If I understand the flowchart correctly, less than 50% of patients included in the cohort underwent the first ultrasound assessment and a significant proportion might not had repeated assessments.

Response: we have rearranged our flowchart to clarify the inclusion and exclusion in our data thus far. The previous flowchart showed our substudy (SOCCS)[2], which performs clinical examination 1 in all patients, also those who are not eligible for repeated measures. We figure this might lead to confusion and have thus removed this step from our flowchart.

- As shown by the low number of patients lost due to logistical reasons" The 16 patients not included for logistic reasons would have potentially represented 21.1% (16/ (60+16)) of your cohort if they would have been included. Consequently, I am not sure we can consider this to be a low number. An analysis of the logistic reasons would have been interesting.

Response: we removed the term: logistic reasons and replaced it by specific reasons, which include mainly patients that were continuously resuscitated and thus we had no possibility to perform ultrasound for research within the first 24 hours of admission.

-Figure 7 c: I am not sure the nadir of renal venous flow is adequately measured on this image because the measurement is taken at the limit of the venous waveform.

Response: this measurement considers the venous impedance index which has to be measured at the highest and lowest point of the venous flow curve. Otherwise, it is not possible to correctly calculate the venous impedance index.

-The renal Doppler is performed in the hilum of the kidney which may underestimate the renal resistive index and overestimated the alterations of intra-renal venous flow. Please note that previous literature has assessed intra-renal venous flow at the corticomedullary

junction.(1, 2) I realize this may be difficult to perform using point-of-care ultrasound in critically ill patients.

Response: we apologize, as we have not described this correctly. We aim to perform a vascular measurement at the corticomedullary junction in every patient in the same way. We have rewritten and clarified this in our manuscript. The reason we have described it as at the hilum of the kidney is that it appeared quite difficult to perform an adequate vascular measurement at the same part of corticomedullary junction each time, and therefore we aimed to perform the measurement at least in the center of the kidney (near the hilum). Reasons for this are that it concerns critically ill patients in whom it is difficult to visualize the kidney in such a way that a reproducible measurement is achieved. Patients cannot be easily turned at their left or right side, and measurements are often more difficult because of lines/other material present outside of the patient.

-I think there is an opportunity to describe how your experience with SICS-I study shaped the design of this cohort study. I see that the previous study was more developed as a database to be used for multiple sub-studies.(3)

Response: we agree with your comment and will include more information about SICS-I. The SICS-II has similar possibilities for substudies.

-If the goal is to assess the feasibility of the ultrasound measurements by trainees. I would suggest presenting the results of the validation of the images performed by the done by the independent experts so far. Additionally, a clear plan should be outline for inter-observer variability assessment. This point is raised in the concerns but is not addressed. Even if all investigators does not undergo a complete comparison with each other, there should be a practical way to partially assess the variability of measurements and there should be a plan to implement it in the study. The fact that this study is performed by a large group of trainees offer a unique opportunity to explore those issues.

Response: we have added the results of our validation thus far. We plan to assess inter – observer variability using the Intraclass Correlation Coefficient (ICC). As mentioned previously, this is part of our statistical analysis plan which will be published later on.

Minor corrections

P1 Line 40: may detect early

P1 line 44: In its sequel, the Simple Intensive Care Studies II (SICS-II), all patients admitted to the ICU will be screened to identify those that may be at risk for fluid overload.

P2 line 68 peripheral: edema

P1 line 69: This possible elevated "afterload" in the venous system may contribute to diminished end-organ perfusion followed by short-term organ failure.

Please reformulate, would suggest reduction of the a-v gradient.

Response: we have rewritten these parts of our manuscript.

Page 6 line 186: What is the value of determining the skin temperature on the dorsum of the foot by placing an additional temperature sensor on the middle of the dorsum and connecting it to the monitor?

Response: we have included this measurement to assess the delta temperature, which provides information on circulation from physical examination.

Page 6, line 195: The authors mentioned that they "Estimate the patients' survival in hospital, 6 months survival and ability to return to their original residence based on the results from this physical examination." How was this done? Were there some guidelines? Charts? Or just intuition and gut feeling?

Response: we have clarified this part in our manuscript. This educated guess is based on intuition and gut feeling, and nurses and doctors are asked to do the same. This is also one of our sub studies which investigates the differences and accuracy in predicting mortality with gut feeling and basic information only.[3]

Table 2: not sure exactly to understand, it was splitted in page 27 and 28. They most likely go together.

Response: this is a mandatory table with the products we used. We will reformat this table.

Page 7: I was very impressed that the authors were measuring strain. They mentioned "If the ECG signal is present and the heart rate is regular, obtain strain measurements of the left and right ventricular walls and of the septum wall"

How do you interpret abnormal strain but normal function n 2D exam? Anything done?

Response: strain analysis is one of our substudies, which we have removed from this manuscript for the purposes we aim to clarify. We do perform strain in a subset of patients. We take the images 2D in high frames per second so that these images can be measured in our CoreLab later on under direct supervision of our experts, as these are complicated measurements.

Page 8, line 305: The authors mentioned "Press the "Color" button to get a color Doppler image of the kidney and determine flow in the renal vasculature

How is it reported using color? I only know how to report it with PW Doppler.

Response: it is true that PW Doppler is used to obtain the flow characteristics. However, color Doppler is used to identify the specific vascular structure. Therefore, it is the combination of color Doppler and PW Doppler which is used to obtain the flow characteristics.

Line 307: It is mentioned: " Place the cursor over any artery at the renal hilum where Doppler flow is clearly visible using the trackball."

In order to calculate resistance index, is it the good place or should it be at the cortico-medullary junction? The editorial that you refer from Tang (4) is the result of an article by Iida (1). That article should be quote. In that study renal Doppler measurements were made at the cortico-medullary junction and not at the origin of the main renal artery or vein. It seems however that in Figure 7, the measurements were made at the appropriate location. Also, add in the core text when a figure is related to the text.

Response: we have changed our description of our method in the manuscript as mentioned in a previous comment. We will add the article from Lida and refer to the image in text.

Page 10, Line 393 7. Kidney length and blood flow: how is it reported with color?

Response: kidney length is measured from the 2D image obtained earlier in the protocol, we have clarified this sentence. As to the blood flow: see previous comment, as we use color to identify the vascular structure to then use PW for the analysis and measurements.

Page 11, line 406: define DRRI and also every abbreviation when used the first time such as VII also.

Response: we have defined every abbreviation at first use.

Page 11, line 421: The author mentioned: "Register the blood gas analysis values, general serum variables, serum renal variables" Please document those variables.

It gives the reader the impression that the authors are hunting for data and not clearly to confirm hypothesis or their main objectives unless the objectives are just to describe a protocol.

Response: we have a predefined list that should be gathered as they provide necessary information concerning patient illness and AKI. We have reformulated this sentence and added table 3, which lists all variables that are recorded.

In terms of Figure: I see Figure 2, 5, 6 and 7. Figure 1 3 and 4 are not on the pdf.

Page 12, line 464: I cannot comment on the preliminary results.

Response: we are sorry that these figures were not viewable. We have rearranged our results and reformatted our figures.

Patient X, 52 year old female

In that example, would have been nicer to put it like I did below instead of using 3 separate pages.

Response: if you are referring to the table, we have reformatted it into a single page.

The data are interesting, but the interpretation would be help by knowing how much fluid the patient received. It seems that the patient went into a distributive shock with worsening renal dysfunction. The author might consider showing the data in a systematic view as mentioned before.

Response: we estimate that the fluid balance that was presented indicated the amount of fluid administered. We have rearranged the table so that the data is systematically displayed.

On top, neuro condition, delirium for instance? Then respiratory system: history (on ventilator), exam and labs then cardiac etc. Would be nice to see the creatinine close to the renal Doppler for instance.

Response: we have rearranged the results and hope it is clarified.

I did not see any strain measurement? Would be better for the reader to show that you can get all the measurements that you mention.

Response: strain images were obtained in this patients but are analyzed in our Corelab later on as mentioned in a previous comment, as this concerns a new substudy.

Page 13 line 488: Figure and legends should be after the discussion

Response: we have added everything according to the JoVE template, which determines the order.

Page 13, line 512: how the competency of the student-researcher evaluated?

Response: by validating the measurements the quality of the variables is assessed. Furthermore, student researchers are trained extensively before being allowed to independently conduct the clinical examinations.

How is the information obtained by the student-researcher transmitted to the doctor in charge of the patient? Are those clinicians taking care of the patient trained in bedside ultrasound?

Response: the measurement performed in our study are not provided to the attending physician. We however estimate and know from experience that physicians perform similar physical examination investigations, but more subjectively.

Measuring agreement between independent observers? Or between trained students before the study? It would be important however to determine the feasibility of such an approach and to document how often every single of your measurement can be obtained.

Response: we have added some information about our planned analysis regarding this agreement in the representative results of our manuscript.

Finally, other ultrasound measurements susceptible to add information about venous congestion such as optic nerve sheath (5), transcranial Doppler, arterial and venous vascular examination, spleen and portal Doppler(6) determination are not included in the protocol.

Response: we thank you for this comment, as these are very interesting and important suggestions. We will mention and consider to perform these in further studies, but as we have now clarified the purpose of our manuscript, we will not mention them now.

In summary this is a very interesting and ambitious approach in integrating bedside ultrasound to formal history and physical examination in a large trainee-based cohort study. I believe this manuscript would greatly benefit of a more focused approach as described before. I would suggest the authors to concentrate on one main goal, whether it is publishing a complete protocol including a statistical plan, describing/proposing a multisystemic ultrasound assessment or describing the methodological challenges to performing a large-scale study with a team of trainees supervised by more experienced ultra-sonographers. The latter element is particularly interesting, and I believe would add substantially to the available literature.

Response: we are very grateful for your extensive review and have taken all of your comments into account. We have rewritten the manuscript with a clarified purpose and believe that the manuscript, thanks to your comments, has substantially improved.

1. Wetterslev, J. *Statistical analysis plan Simple Intensive Care Studies-I DETAILED STATISTICAL ANALYSIS PLAN (SAP) 1. Administrative information 1.1. Title, registration, versions and revisions*. at <https://clinicaltrials.gov/ProvidedDocs/24/NCT02912624/SAP_000.pdf>.

2. I.C.C. van der Horst Simple Observational Critical Care Studies - Full Text View - ClinicalTrials.gov. at <https://clinicaltrials.gov/ct2/show/NCT03553069?term=SOCCS&rank=1>.

3. Lipson, A.R., Miano, S.J., Daly, B.J., Douglas, S.L. The Accuracy of Nurses' Predictions for Clinical Outcomes in the Chronically Critically Ill. *Research & reviews. Journal of nursing and health sciences*. **3** (2), 35–38 (2017).