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.*

Commercial Language:JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are OpenClinica, GS General Electric Vivid-S6,

1) Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.

*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.*

If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

*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).