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Title:

Measurement of tissue oxygenation using near-infrared spectroscopy in patients undergoing hemodialysis

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KEYWORDS:

tissue oxygenation, hemodialysis, near-infrared spectroscopy, regional oxygen saturation, intradialytic hypotension, hypoxia, diabetes mellitus

SUMMARY:

We present a protocol to measure regional oxygen saturation (rSO_2) in hemodialysis (HD) patients by using a near-infrared spectroscopy monitor. The rSO_2 value is an index of tissue

oxygenation. This noninvasive and real-time monitoring could be useful for confirming changes in organ oxygenation during HD.

ABSTRACT:

Near-infrared spectroscopy (NIRS) has recently been applied as a tool to measure regional oxygen saturation (rSO_2), a marker of tissue oxygenation, in clinical settings including cardiovascular and brain surgery, neonatal monitoring and prehospital medicine. The NIRS monitoring devices are real-time and noninvasive, and have mainly been used for evaluating cerebral oxygenation in critically ill patients during an operation or intensive care. Thus far, the use of NIRS monitoring in patients with chronic kidney disease (CKD) including hemodialysis (HD) has been limited; therefore, we investigated rSO_2 values in some organs during HD. We monitored rSO_2 values using a NIRS device transmitting near-infrared light at 2 wavelengths of attachment. The HD patients were placed in a supine position, with rSO_2 measurement sensors attached to the foreheads, the right hypochondrium and the lower legs to evaluate rSO_2 in the brain, liver and lower leg muscles, respectively. NIRS monitoring could be a new approach to clarify changes in organ oxygenation during HD or factors affecting tissue oxygenation in CKD patients. This article describes a protocol to measure tissue oxygenation represented by rSO_2 as applied in HD patients.

INTRODUCTION:

Near-infrared spectroscopy (NIRS) has been used to evaluate regional oxygen saturation (rSO_2), a marker of tissue oxygenation, especially cerebral oxygenation in various clinical settings[1-3] and has recently been applied to patients undergoing hemodialysis (HD)[4-11]. Cerebral rSO_2 is reportedly associated with cognitive function in patients undergoing HD or those with non-dialyzed chronic kidney disease (CKD)[11,12]. However, thus far, the use of NIRS monitoring has been limited in patients with CKD.

As NIRS monitoring is real-time and noninvasive, we assessed its usefulness as a monitoring device in patients undergoing HD. Although NIRS is mainly used to measure cerebral rSO_2, we also investigated rSO_2 values in other organs during HD. Specifically, the rSO_2 measurement sensors were attached to the forehead, the right hypochondrium and the lower legs to evaluate rSO_2 in the brain, liver and lower muscles, respectively. The results showed that NIRS monitoring could be a new approach to clarify changes in organ oxygenation during HD or factors affecting tissue oxygenation in CKD patients.

To date, continuous monitoring was performed during HD, blood volume monitoring, central venous oxygen saturation, thoracic admittance and electronic stethoscope-guided estimated

blood pressure (BP) in clinical settings[13-15]; however, there are limitations for the prediction of hypotension or the wide use of devices. In contrast, the new noninvasive approach here could provide real-time information on intradialytic oxygen dynamics in individual organs. Therefore, this monitoring method may allow the detection of transient organ ischemia in the early phases of intradialytic hypotension and may also permit the safe performance of HD. This article describes a protocol to measure tissue oxygenation represented by rSO_2, as applied in patients undergoing HD.

PROTOCOL:

All participants provided written informed consent. The study was approved by the Institutional Review Board of the Saitama Medical Center, Jichi Medical University, Japan (RIN 15–104).

1. Device for the monitoring of rSO_2

1.1. Prepare a NIRS device for measuring tissue oxygenation. This device has four channels and can perform measurement in up to four organs at the same time.

1.2. Prepare a measurement sensor for NIRS monitoring, to evaluate rSO_2 values in each organ via transmitting near-infrared light at two wavelengths of attachment.

2. Attaching the measurement sensor

2.1. Allow each patient to rest in a supine position for at least 5 minutes before HD.

2.2. Attach measurement sensors to the forehead, the right hypochondrium and lower legs to evaluate rSO_2 in the brain, liver and lower leg muscles, respectively.

2.3. Monitoring of cerebral oxygenation

2.3.1. Attach measurement sensors to the forehead of the dominant hemisphere.

2.4. Monitoring of hepatic oxygenation

2.4.1. Prepare echocardiography to measure the depth to the patients' liver from the body surface. Confirm that this measurement is within 20–30 mm from the body surface. Next, attach the measurement sensors to the right hypochondrium.

NOTE: In this device, rSO_2 values should be obtained in deep tissue 20–30 mm from the body surface. In some instances, the liver may be located in more than 30 mm from the body surface due to the presence of thick subcutaneous fat.

2.5. Monitoring of muscle oxygenation

2.5.1. Attach measurement sensors to the right or bilateral lower legs.

2.6. Sensor connection and powering the device

2.6.1. Connect each sensor to the leads from the device. Next, turn on the device, and start measuring oxygenation.

3. Puncturing the dialysis shunt and starting monitoring

3.1. Puncturing the dialysis shunt

3.1.1. Puncture the patients' dialysis shunt to start HD therapy. At this time, measure BP using a digital blood pressure monitor equipped with the dialysis machine and collect blood samples using syringes.

3.2. Start monitoring

3.2.1. After starting HD therapy, start monitoring the tissue oxygenation of the three organs: the brain, liver and lower leg muscle.

3.3. Monitoring of rSO_2 during HD

3.3.1. Observe changes in rSO_2 values of each organ and measure BP regularly in addition to the usual monitoring performed during HD therapy including heart rate, venous pressure and blood volume. Confirm the attachment area and connection between the sensors and leads.

REPRESENTATIVE RESULTS:

Cerebral rSO_2 values before HD were lower than those in healthy subjects and cerebral rSO_2 in HD patients with diabetes mellitus (DM) were lower than those in HD patients without DM (**Figure 1**)[16]. Furthermore, although tissue oxygenation continues without a decrease of BP during HD, we incidentally observed changes in cerebral and hepatic rSO_2 due to intradialytic hypotension (**Figure 2**). Due to the continuous monitoring, the changes in tissue oxygenation were observed more quickly than by intermittently monitored BP. Data were expressed as means ± standard error. The analysis of variance for non-paired values was used to compare three groups.

FIGURE AND TABLE LEGENDS:

Figure 1. Comparison of cerebral rSO_2 before HD among HD patients with diabetes mellitus (n = 27), HD patients without diabetes mellitus (n = 27) and healthy subjects (n = 28). The patients included 38 men and 16 women with mean age of 67.7 ± 1.2 years and HD duration of 6.5 ± 1.9 years. The causes of chronic kidney disease were DM (27 patients), chronic glomerulonephritis (14 patients), nephrosclerosis (4 patients), polycystic kidney disease (4 patients), and other (5 patients). The error bars indicate the standard error. The data were based on and the figure has been modified from a previous report[16]. DM; diabetes mellitus, HD; hemodialysis, rSO_2; regional oxygen saturation

Figure 2. Changes in cerebral and hepatic rSO_2 in a patient with acute intradialytic hypotension. BP; blood pressure, h; hour, rSO_2; regional oxygen saturation, UFR; ultrafiltration rate

DISCUSSION:

NIRS monitoring has been mainly used to evaluate cerebral rSO_2, especially in cardiovascular or cerebrovascular surgeries, which require extracorporeal circulation. During extracorporeal circulation including HD therapy, some organs could show relative ischemia[7,17,18]; however, it remains unclear whether tissue oxygenation becomes low or not. Muscle cramps or abdominal pain during HD could be one of the prodromal symptoms of intradialytic hypotension via organ hypoperfusion. However, in HD therapy, there is currently no method for the real-time evaluation of tissue oxygenation. Therefore, we focused on using this monitoring device to evaluate organ oxygenation using the protocol described above. This protocol is noninvasive for HD patients and is useful for confirming changes in tissue oxygenation in real-time.

As shown in **Figure 1**, cerebral rSO_2 in HD patients with DM was lower than that in patients without DM. Furthermore, higher vascular calcification was associated with lower cerebral oxygenation[19]. Thus, micro- and macro-vascular disorders could be associated with impairment of cerebral oxygenation. Furthermore, cerebral rSO_2 was relatively maintained constant within 60-150 mmHg in HD patients[4]. However, in intradialytic hypotension, an acute decrease in BP could lead to changes in organ oxygenation (**Figure 2**). Before observing changes in rSO_2 values by this protocol, we could not confirm the influence of tissue oxygenation during HD. Besides continuous arterial pressure monitoring, BP is generally evaluated intermittently. In contrast, the continuous monitoring by NIRS might be able to detect changes in organ oxygenation before being detected by changes in BP during HD. Thus, we could observe the state of hypoxia ahead of confirming the lowering of BP. In addition to changes in BP, blood transfusion, low-density lipoprotein apheresis and ultrafiltration might cause changes in organ oxygenation such as the rSO_2 of the lower-legs[20-22]. Therefore, we should pay attention to acute changes in organ oxygenation during HD.

This protocol has several limitations. First, cerebral rSO_2 could be only measured from the forehead; however, it is difficult to perform this evaluation in the posterior brain circulation. As the measurement sensors are a seal type, their sensors could be fixed on the hair. Next, measurement of hepatic rSO_2 requires confirmation of the subcutaneous fat thickness. In patients with obesity, the measured rSO_2 might be not accurate, because the near-infrared lights could not reach target organs. Third, rSO_2 values might be affected by body motion or position (i.e., supine and seated positions). Therefore, during HD, patients should be measured while in their beds and in the same position, as possible.

Furthermore, the rSO_2 values measured in this protocol represents mixed venous saturation, which reflects tissue oxygenation in venous (70–80%), capillary (5%), and arterial (20–25%) blood[23]. Therefore, changes in rSO_2 values do not necessarily parallel changes in percutaneous oxygen saturation[24,25]. Thus, the measured rSO_2 values should be carefully interpreted. Furthermore, this protocol is easy to perform and noninvasive for patients if NIRS monitoring device is available. Therefore, this method would provide widely general versatility. We hope that this NIRS monitoring would be equipped with dialysis machines as a dialysis monitor in the future.

In conclusion, we have described a protocol for the measurement of tissue oxygenation by NIRS in patients undergoing HD. This monitoring during HD might provide new findings regarding changes in tissue oxygenation affected by HD therapy.

ACKNOWLEDGMENTS:

We thank the dialysis staffs and members of the department of nephrology in Saitama medical center of Jichi Medical University. We would like to thank Editage (www.editage.com) for English language editing.

DISCLOSURES:

No conflicts of interest.

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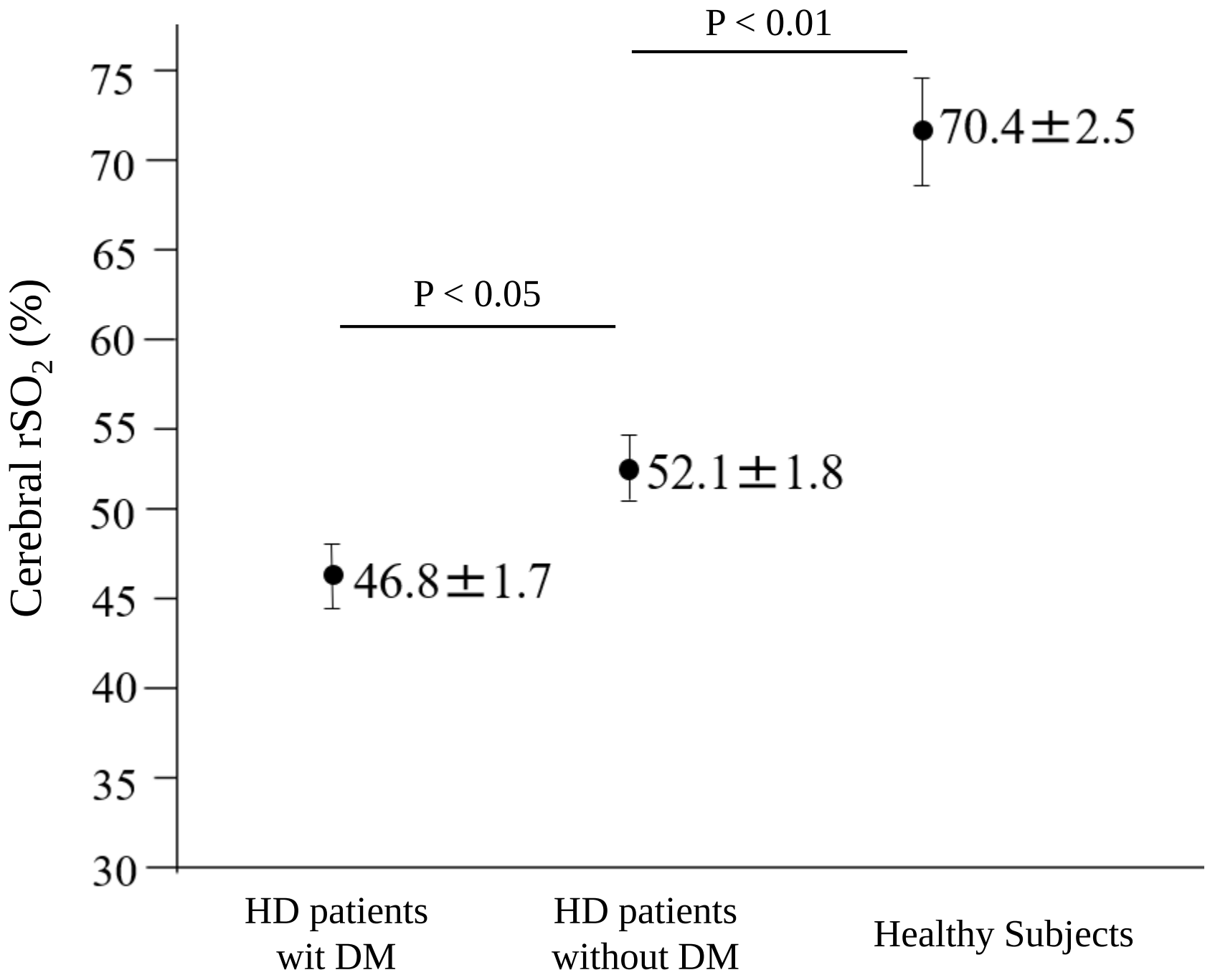

Figure 1. Ito K, et al.

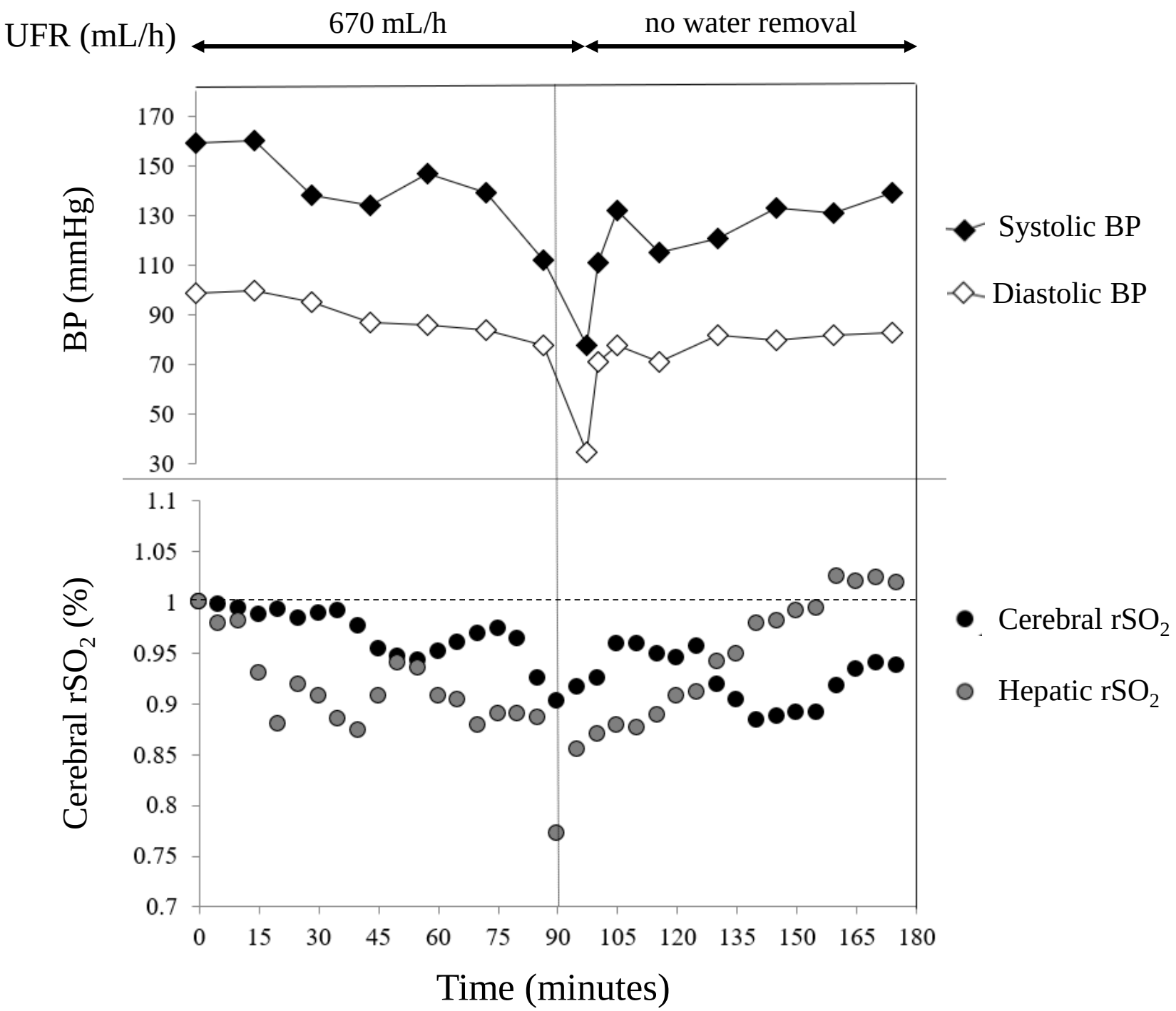

Figure 2. Ito K, et al.

Name of Material/Equipment	Company	Catalog Number	Comments/Description
DBB-100NX	Nikkiso	DBB-100NX	Dialysis machine
INVOS 5100c	Covidien Japan	INVOSTM 5100c	tissue oxygenation device
SOMASENSER	Covidien Japan	CV-SAFB-SM/INTL	NIRS sensor

Response to Reviewers' comments

We appreciate your careful review of our manuscript and hope that we have satisfactorily addressed each of the issues you have raised.

Comment 1(Editorial Comments)
The manuscript will benefit from thorough language revision as there are a number of grammatical errors throughout. Please thoroughly review the manuscript and edit any errors.

Reply 1(Editorial Comments)
Thank you for your comment. We have proofread our manuscript to correct grammatical errors.

Comment 2(Introduction)
Please expand your Introduction to include the following: The advantages over alternative techniques with applicable references to previous studies; Description of the context of the technique in the wider body of literature; Information that can help readers to determine if the method is appropriate for their application.

Reply 2(Introduction)
Thank you for your comment. We have expanded the introduction to include the other techniques using continuous monitoring during HD. Additionally, we have included the strengths and our expectations of NIRS monitoring, although we could not mention the advantages because there are no reports comparing between NIRS monitoring and other continuous monitoring in HD. We hope that we have satisfactorily addressed the issues that you have raised.

Comment 3 (Protocol Language)
The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief "Note" at the end of the step (please limit notes). Please re-write your ENTIRE protocol section accordingly. Descriptive sections of the protocol can be moved to Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study.

Any reporting should be moved into the representative results.

Reply 3 (Protocol Language)
Thank you for your comment. We have corrected the protocol to use imperative sentences as possible. Additionally, we added a brief “Note” to a part of the protocol.

Comment 4 (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.
Define error bars in Fig 1.

Reply 4 (Protocol Language)
Thank you for your comment. We added our protocol of study method in as much detail as possible. Our protocol is very easy; therefore, the length of protocol is short compared to those for basic research. Additionally, in Figure 1, we added that the error bar indicated the standard error. We hope that we have satisfactorily addressed the issues you have raised.

Comment 5 (Discussion)
JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Reply 5 (Discussion)
Thank you for your comment. We added several sentences in the discussion section regarding the limitations, important points and future vision. We hope that we have satisfactorily addressed the issues you have raised.

Page 8, lines 201-203.
“Third, rSO_2 values might be affected by body motion or position; i.e. supine and seated positions. Therefore, during HD, patients should be measured while in their beds and in

the same position, as possible."

and

Page 9, lines 208-211.
"Furthermore, this protocol is easy to perform and non-invasive for patients if NIRS monitoring device is available. Therefore, this method would provide widely general versatility. We hope that this NIRS monitoring would be equipped with dialysis machines as a dialysis monitor in the future."

Comment 6 (References)
1) Please make sure that your references comply with JoVE instructions for authors. Citation formatting should appear as follows: (For less than six authors, list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. Volume (Issue), FirstPage – LastPage, (YEAR).]
2) Please spell out journal names.

Reply 6 (References)
Thank you for your comment. We apologize and have made sure to comply with the JoVE instructions for authors. We corrected the reference style according to instructions for authors.

Comment 7 (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 INVOS 5100c (Covidien).
1) 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.

Reply 7 (Commercial Language)
Thank you for your comment. We deleted "INVOS 5100c (Covidien)" from the revised manuscript and corrected all commercial products to generic term.

Comment 8 (Table of Materials)

1) Please remove the registered trademark symbols TM/R from the table of reagents/materials.

2) Please sort in alphabetical order.

Reply 8 (Table of Materials)

Thank you for your comment. We corrected the figure legends, and remove the registered trademark symbols TM/R from the figure. In addition, we sorted the abbreviation in alphabetical order.

Comment 9

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]."

Reply 9

Thank you for your comment. In Figure 1, we re-used the previous data from reference 16 (PLoS One). PLoS One applies the Creative Commons Attribution license to various articles and other works. Therefore, we do not need to obtain permission to re-use the figure, as long as the author and original source are properly cited. We added a sentence to the figure legends. In Figure 2, we used original data, and the figure was not published previously. We hope that we have satisfactorily addressed the issues you have raised.

Page 7, lines 158-159.

“The data were based on and the figure has been modified from a previous report[16].”

Reviewers' comments:

Reviewer #1

General comment

We felt this was a unique report on the use of NIRS to assess tissue oxygenation in dialysis patients. The ability to assess in real time with minimal invasion is considered clinically useful. I wish to make this report better. Please then consider the following points

Reply to general comment

Thank you for your careful review of our manuscript. We corrected our manuscript as suggested by the reviewer, and therefore, we have satisfactorily addressed each of the issues you have raised.

Comment 1

Q1. What is the ultimate goal of this study? Confirmation of temporary organ ischemia due to dialysis treatment? Or the widespread use of NIRS patient monitoring? Would you like to add a description of your purpose?

Reply 1

Thank you for your comment. Our aims are to safely perform HD. To do so, we believe that new monitoring and approaches are needed and hope that NIRS monitoring could be sed to detect transient organ ischemia due to intradialytic hypotension. Thus, we hope that the use of NIRS for patients' monitoring will become widespread. We added the following sentence in the introduction.

Page3, lines76-83

"To date, continuous monitoring was performed during HD, blood volume monitoring, central venous oxygen saturation, thoracic admittance and electronic stethoscope-guided estimated blood pressure (BP) so on in clinical settings [13-15]; however, there are limitations for the prediction of hypotension or the wide use of devices. In contrast, our non-invasive new approach could provide real-time information on intradialytic oxygen dynamics in individual organs. Therefore, this monitoring method may allow the detection of transient organ ischemia in the early phases of intradialytic hypotension and may also permit the safe performance of HD."

Comment 2

Q2. Please specify why you chose the liver and lower legs. The heart may be difficult to assess because of its beating, but if the liver can be assessed, then the kidneys, spleen, and thyroid should also be assessed more generally (although they may be unnecessary in this report because of ESRD).

Reply 2

Thank you for your comment. We hoped to measure renal oxygenation. However, this measurement was difficult, as near-infrared lights of the measurement sensors could not reach the kidney. The light range is approximately 3 - 4 cm from the body surface. Therefore, we did not evaluate renal oxygenation. Thus, we performed measurements in the liver and lower legs. As suggested by the reviewer, we agree that the spleen could be a candidate for the measurement of oxygenation. Measurement of thyroid oxygenation is of less interest in HD patients. We hope that we have satisfactorily addressed the issues you have raised.

Comment 3

Q3. In relation to Q2. What was the reproducibility in the liver and lower legs ?

Reply 3

Thank you for your comment. The reproducibility in the liver and lower legs was acceptable. As shown in our previous reports (Ookawara S, et al. J Artif Organs. 2017, Ito K, et al. Nefrologia. 2018, Ito K, et al. Nefrologia. 2019, Minato S, et al. J Artif Organs. 2019.), rSO_2 values in the liver and lower legs could be evaluated. However, as there are few reports of rSO_2 in these regions, further studies are needed in HD patients.

Comment 4

Q4. Figure 1 appears to be comparative data for rSO_2 during dialysis according to the protocol, but in the non-dialysis Was it? I think that the rSO_2 base during non-dialysis is an important basic data and necessary. If it has not been measured, I think it needs to be explained.

Reply 4

Thank you for your comment. Figure 1 shows cerebral rSO_2 values before HD among HD patients with diabetes mellitus (n = 27), HD patients without diabetes mellitus (n = 27) and healthy subjects (n = 28); that is baseline rSO_2 data during non-dialysis. We apologize

for the inadequate figure legend and included the numbers of patients in Figure 1, as follows:

Page7, Lines152-154
“Comparison of cerebral rSO_2 before HD among HD patients with diabetes mellitus (n = 27), HD patients without diabetes mellitus (n = 27) and healthy subjects (n = 28).”

Comment 5
Q5. Three patients were included in the study: one diabetic dialysis patient, one non-diabetic dialysis patient and one control Isn't it? Or is the comparison based on an average of multiple cases?

Reply 5
Thank you for your comment. We apologized for not including the number of patients in Figure 1. We added this information in Figure 1.

Comment 6
Q6. In relation to Q5. How was patient selection carried out?

Reply 6
Thank you for your comment. In Figure 1, the selected patients were those who had provided informed consent and received HD therapy. These 54 consecutive patients did not require oxygen inhalation and had no cognitive impairment or no cerebrovascular disease.

Comment 7
Q7. In relation to Q5. Would you like to add a brief description of the patient's clinical background? I am concerned about the primary disease of ESRD, the presence of hypertension and the presence of heart failure etc..

Reply 7
Thank you for your comment. In Figure 1, all included patients were in a stable state an no patients had heart failure. We added information on including sex, mean age, HD duration and the primary disease of chronic kidney disease in the figure legends as follows:

Page7 Lines154-158

“The patients included 38 men and 16 women with mean age of 67.7 ± 1.2 years and HD duration of 6.5 ± 1.9 years. The causes of chronic kidney disease were DM (27 patients), chronic glomerulonephritis (14 patients), nephrosclerosis (4 patients), polycystic kidney disease (4 patients), and other (5 patients).”

Comment 8

Q8. In the discussion, the authors explain the possibility of organ ischemia from lower blood pressure. However, we can add a discussion of why the changes in rSO_2 appear before the changes in blood pressure?

Reply 8

Thank you for your comment. Changes in organ oxygenation could occur owing to changes in BP. However, in HD therapy, BP is usually monitored intermittently. In contrast, NIRS monitoring is continuous. Therefore, changes in organ oxygenation would be detected before changes in BP. We added the following sentence in the discussion section.

Page8, lines188-192

“Except continuous arterial pressure monitoring, BP is generally evaluated intermittently. In contrast, the continuous monitoring by NIRS might be able to detect changes in organ oxygenation before being detected by changes in BP during HD. Thus, we could observe the state of hypoxia ahead of confirming the lowering of BP.”

Comment 9

Q9. Authors showed 'During extracorporeal circulation including HD therapy, some organs could tend to be in state of ischemia, and it need to evaluate whether tissue oxygenation become lower or not.' in the discussion. Please indicate the references.

Reply 9

Thank you for your comment. As suggested by the reviewer, we added additional references on changes in organ oxygenation using NIRS during extracorporeal circulation including HD therapy.

Page8, lines173-174

“During extracorporeal circulation including HD therapy, some organs could show

relative ischemia [7,17,18] “

17 Imai S, Ookawara S, Ito K, Kiryu S, Tabei K, Morishita Y. Deterioration of Hepatic Oxygenation Precedes an Onset of Intradialytic Hypotension with Little Change in Blood Volume during Hemodialysis. *Blood Purification.* **45** (4), 345-346 (2018).

18 Cho AR, Kwon JY, Kim C, Hong JM, Kang C. Effect of sensor location on regional cerebral oxygen saturation measured by INVOS 5100 in on-pump cardiac surgery. *Journal of Anesthesia.* **31** (2), 178-184 (2017).

Comment 10 (Minor Concerns)

In Figure, abbreviations should have explanation.

There is no statistical explanation and the ethical handling. Aren't there necessary?

Reply 10 (Minor Concerns)

Thank you for your comment. As suggested by the reviewer, we added the abbreviations in the Figure and also added information on statistics and ethics in the Representative Results. We hope that we have satisfactorily addressed each of the issues you have raised.

Page6, lines144-148

“**Statistics and Ethics**

Data were expressed as means ± standard error. The analysis of variance for non-paired values was used to compare three groups. All participants provided written informed consent. The study was approved by the Institutional Review Board of the Saitama Medical Center, Jichi Medical University, Japan (RIN 15–104).”

Reviewer #2:

Comment 1

The measurement of tissue oxygenation is a very interesting topic that has not been properly explored in hemodialysis patients. It is established that tissue oxygenation is disturbed in kidney disease and mainly in diabetic with end stage kidney disease. It is not novel to record these differences. It would be interesting to look whether NIRS changes i) could predict intradialytic hypotension prior to symptoms or low blood pressure ii) could help to early recognition of diabetic angiopathy-it is known that the diabetic patients who start on dialysis will do very quickly angiopathic changes or may come into feet amputation quickly.

Reply 1

Thank you for your thoughtful comment. We are also interested in the prediction of intradialytic hypotension or the recognition of diabetic angiopathy. We think that continuous monitoring by NIRS might be able to detect intradialytic hypotension before it is indicated by low blood pressure, as reported previously (Imai S, et al. Blood Purif. 2018, Kitano T, et al. J Artif Organs. 2020). Figure 2 shows a case with intradialytic hypotension evaluated by NIRS. We hope to investigate this in future observational studies. Thus far, we have not investigated the detection of angiopathic changes or amputation in early phases. As suggested by the reviewer, we hope to observe angiopathic changes or amputation in the future.

Reviewer #3:

General comment

Manuscript Summary: The Authors present tissue oxymetry in various parts of human body (brain, liver, lower extremity muscles) in patients treated by chronic hemodialysis. They observed lower brain oxygenation in hemodialysis patients in comparison to healthy subjects. Diabetics had even lower values.

Major Concerns: None

Comment 1(Minor Concerns)

I think that the manuscript presents tissue oxymetry accordingly. I have only the following minor comments:

1. Please, describe the studied population in more detail. For example, heart failure was associated with lower tissue oxygenation in a recent study (Valerianova A et al. Physiol Res 2019;68:651-658.

Reply 1

Thank you for your comment. We apologize for the lack of information on the study population. Our recruited patients were in stable condition, and none had heart failure. We added the following sentence in the figure and table legends. Furthermore, we agree that the cerebral rSO_2 value would be low in patients with heart failure, as suggested by the reviewer. We also experienced that a case with acute heart failure with low cerebral rSO_2 (Minato S, et al. J Artif Organs. 2019 (In press)). We inserted this reference.

Page7, lines154-158

"The patients included 38 men and 16 women with mean age of 67.7 ± 1.2 years and HD duration of 6.5 ± 1.9 years. The causes of chronic kidney disease were DM (27 patients), chronic glomerulonephritis (14 patients), nephrosclerosis (4 patients), polycystic kidney disease (4 patients), and other (5 patients)."

And

Page13, lines300-302

"25 Minato S, et al. Continuous monitoring of changes in cerebral oxygenation during hemodialysis in a patient with acute congestive heart failure. *Journal of Artificial Organs.* (2019). (In Press)"

Comment 2 (Minor Concerns)

2. Hemodialysis patients with cognitive decline had lower brain oxygenation (Kovarova L., Nephron, 2018;139(2):113-119, Miyazawa H et al. PLoS One 2018;13(6):e0199366.). I would add this information because it considerably increases the clinical impact of brain oxymetry.

Reply 2

Thank you for your thoughtful comment. We agree that the association between cognitive impairment and cerebral oxygenation is important in CKD and HD patients. Therefore, as suggested by the reviewer, we added the indicated references in the introduction section.

Page 3, lines 66-67

"Cerebral rSO_2 is reportedly associated with cognitive function in patients undergoing HD or those with non-dialyzed chronic kidney disease (CKD)[11,12]."

11 Kovarova L, Valerianova A, Kmentova T, Lachmanova J, Hladinova Z, Malik J. Low Cerebral Oxygenation Is Associated with Cognitive Impairment in Chronic Hemodialysis Patients. *Nephron.* **139** (2), 113-119 (2018).

12 Miyazawa H, et al. Association of cerebral oxygenation with estimated glomerular filtration rate and cognitive function in chronic kidney disease patients without dialysis therapy. *PLoS One.* **13** (6): e0199366 (2018).

Comment 3 (Minor Concerns)

3. Figure 2: add muscle oxymetry into the graph

Reply 3

Thank you for your comment. Unfortunately, lower-leg rSO_2 was not measured in the case with intradialytic hypotension. Therefore, we could not show it in Figure 2. Regarding rSO_2 of the lower-leg muscles during HD, please refer to our previous reports (Ito K, et al. Neflorogia. 2018, Ito K, et al. Neflorogia. 2019, Kitano T, et al. J Artif Organs. 2020). We added these references in the discussion section as follows:

Page8, lines192-194

"In addition to changes in BP, blood transfusion, low-density lipoprotein apheresis and

ultrafiltration might cause changes in organ oxygenation such as the rSO_2 of lower-legs'[20-22]."

20 Ito K, et al. Blood transfusion during haemodialysis improves systemic tissue oxygenation: A case report. *Nefrologia.* **37** (4), 435-437 (2017).

21 Ito K, et al. Improvement of bilateral lower-limb muscle oxygenation by low-density lipoprotein apheresis in a patient with peripheral artery disease undergoing hemodialysis. *Nefrologia.* **39** (1), 90-92 (2019).

21 Kitano T, et al. Changes in tissue oxygenation in response to sudden intradialytic hypotension. *Journal of Artificial Organs.* **23** (2), 187-190 (2020).

Aug. 30, 2020.

The Review Editor, Vineeta Bajaj, Ph.D.
JoVE Editorial Office,

Dear Vineeta Bajaj

The submitted is our original manuscript and figures for the paper entitle
"Measurement of tissue oxygenation using near-infrared spectroscopy in patients undergoing hemodialysis".
We are submitting this for review as our revised manuscript.

Thank you for your attention in this matter.

Sincerely yours,

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