PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol for a prospective cohort study on the feasibility of application of nutritional ultrasound in the diagnosis and follow-up of patients with nutritional risk at hospital discharge: Study on body composition and function (DRECO).
AUTHORS	García Almeida, José Manuel; Bellido, Diego; De Luis, D.A; Guzmán Rolo, Germán; Olveira, Gabriel

VERSION 1 – REVIEW

REVIEWER	Nakanishi, Nobuto Tokushima University Hospital, Emergency and Critical Care Medicine
REVIEW RETURNED	18-Jul-2023

GENERAL COMMENTS	Thank you for the opportunity for this review. This is an interesting article, but I have to say this study is not very novel. Major revision is needed if you want to publish it as study protcol. Method statement is not clear. Especially ultrasound. What mucle do you measure, How do you measure? Please write the protcol. Who do you measure, how many times for one measurement. Because ultrasound depends on the examiners skill, it is important state the medhod clearly (PMID: 34202957). How do you encure the inter and intra observer correlation for ultrsound measurement? How do you excled patients with edema in bioelectrical impedance analysis. Because it is affected by fluid balance, you have to state how you deal data of patients with fluid balance change. You can see the demerit of bioelectrical impedance analysis in the following artice (PMID: 31890223) For the follow up, why did you choose TUG and grip strength tests? You need to cite some paper for the validity of your test choice. Why you do not measure SF-36 or some ADL? How did you choose 3 month and 6 mongh for study follow up. If you plan some subanalysis, you have to mention that in this protcol. You need to discuss more about your protcol. How and why you choose the outcme measurment? What is the novelty of your research? What is the difference of your research from the other

	research. Otherwise, it is meaningless to publish this protcol paper. Your citation is limitted. You can cite more research with more discussion.

REVIEWER	Ryan, Aoife University College Cork School of Food and Nutritional Sciences, School of Food & Nutritional Scienes
REVIEW RETURNED	03-Oct-2023

GENERAL COMMENTS	Methodology Can the authors please provide further information required on the training attended by the physicians and staff undertaking ultrasound measurements – how are they minimising risk of inter-variability? How are they ensuring that practice is the same across all 25 sites? Do they require refresher sessions over the course of the study? Is there any quality control? Appears that different researchers will be taking the measurements at 3 months and 6 months compared to baseline.This is a concern regarding the intra observer variability and reliability of the results at the end of the study. Patient and Public Involvement Please address the reasons why PPI is not involved in this trial – no specific reasons given Dissemination / Use of Thresholds Following standardisation Whilst a secondary outcome – how do you suggest that you will present the thresholds for translation into clinical practice e.g. centiles (like Dodds 2014 for HGS)?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Nobuto Nakanishi, Tokushima University Hospital

Comments to the Author:
Thank you for the opportunity for this review. This is an interesting article, but I have to say this study is not very novel. Major revision is needed if you want to publish it as study protocol.

1. Method statement is not clear. Especially ultrasound. What muscle do you measure, how do you measure? Please write the protocol. Who do you measure, how many times for one measurement. Because ultrasound depends on the examiner's skill, it is important state the method clearly (PMID: 34202957).
Thank you for the good comment. In order to answer your doubts, we have included the next paragraphs:
"US accuracy highly depends on the skills of the technician. Point training using rectus femoris phantom have shown to improve the accuracy of measurements. (20) Before starting the study, a training session was held. All study participants were required to attend, and they had the opportunity

to practice with the same ultrasound machine that was going to be used in the study in phantom patients. Besides, several videos explaining detailed measurements technique were recorded. These videos were proactively shared with all researchers and available anytime at the study on-line electronic data capture (EDC) platform.
Beyond, once the study finishes, all DICOM images gathered will be analysed to develop a semi-automated algorithm that helps diagnose the patient's nutritional status. Subsequently, once the algorithm is available, the individual and manual US measurements will be contrasted with the data showed by the automatic algorithm, thus minimizing the inter and intra observer correlation. This work will have its own analysis and publication plan."
"With the patient lying supine with knees extended and relaxed, ultrasound measurements of unilateral (right side) quadriceps rectus femoris is performed at each participating center by an experienced medical sonographer blinded to the clinical data and other results of nutritional assessment. The acquisition site is located two-thirds of the way along the femur length, measured between the anterior superior iliac spine and the upper edge of the patella. The transducer is placed perpendicular to the long axis of the thigh with excessive use of contact gel and minimal pressure to avoid compression of the muscle. All parameters are taken as an average of three consecutive measurements in the dominant leg. We measure the transversal axis of the cross-sectional area (CSA) in cm^2, the X-axis and Y-axis in mm, which corresponded to the linear measurement of the distance between the muscular limits of the rectus femoris (lateral and anteroposterior), the X-axis/Y-axis ratio, and the total fat tissue in mm. All US parameters were also standardized divided by height squared (in cm^2 for rectus femoris). The DICOM images of the QRF ultrasounds will be kept for later analysis."
"The second component of nutritional ultrasound is the evaluation of fat at the level of the abdominal wall. (21) The location of the measurement point is set at the midpoint between the xiphoid appendix and the navel on the midline. The patient remains in a supine position in a situation of relaxation and the image is taken during the unforced expiration, in a transverse plane using the same linear probe perpendicular to the skin. In the cross-section, the anatomical structures that are visualized are ordered from the most superficial layer corresponding to the epidermis, followed by the layer of subcutaneous, superficial, and deep adipose tissue. Then the two muscles of the anterior rectum of the abdomen that join in the central part in the linea alba are identified. (21) We measure both total and superficial subcutaneous adipose tissue and the pre-peritoneal visceral adipose tissue. The DICOM images of the abdominal ultrasounds will be kept for later analysis."
We have included the reference (PMID: 34202957) and we mention the importance of phantom systems. "Point training using rectus femoris phantom have shown to improve the accuracy of measurements. (20)"

2. How do you ensure the inter and intra observer correlation for ultrasound measurement?
Thank you for the good comment. In each centre the same researcher performed the different ultrasounds, and in addition a central control centre formed by an independent radiologist will check the validity of all the US images.
We include the next paragraph, too: *"Beyond, once the study finishes, all DICOM images gathered will be analysed to develop a semi-automated algorithm that helps diagnose the patient's nutritional status. Subsequently, once the algorithm is available, the individual and manual US measurements will be contrasted with the data showed by the automatic algorithm, thus minimizing the inter and intra observer correlation. This work will have its own analysis and publication plan".*

3. How do you exclude patients with edema in bioelectrical impedance analysis. Because it is affected by fluid balance, you have to state how you deal data of patients with fluid balance

change. You can see the demerit of bioelectrical impedance analysis in the following article (PMID: 31890223).

Thank you for the good comment. In order to answer your doubts, we have included the next paragraphs and the reference (PMID: 31890223). Moreover, our study is realized in No-UCI patients, this is an exclusion criteria, and fluid balance is more accuracy in our hospitalized patients.

"Total body BIA (50-kHz frequency) (Tanita BC-420MA BIA analyzer, Tanita Corporation, Arlington Heights, IL, USA) was used to determine phase angle (degrees), total body water (%), fat mass (kg), lean mass (kg), body cell mass (kg), and appendicular skeletal muscle mass (ASMM) (kg).
Since interval fluid balance is more sensible to the change of edema, bioelectrical impedance analysis can be affected in edematous patients. (22) Therefore, extreme phase angle values and/or non-coherent reactance/resistance ratios will be discarded, as a control measure, to detect patients with edema and fluid balance change.

4. For the follow up, why did you choose TUG and grip strength tests? You need to cite some paper for the validity of your test choice. Why do you not measure SF-36 or some ADL?

Thank you for the good comment. We use dynamometry to carry out a diagnosis of suspected sarcopenia and the time up and go test to categorize the severity of sarcopenia, following the guidelines (EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis) (this reference has been included), both are tests used in clinical practice, however the quality-of-life tests, are not used in normal clinical practice. We have also included the following paragraphs:

"2.4.3 Timed Up and Go test (TUG)

The TUG test was used to assess functionality. A coloured tape was marked 3 m away from an armless chair in which participants were sitting. Participants were asked to walk 3 m, turn around the marked tape, and return to the chair as fast as they could. A timer was set as soon as the patient stood up from the chair and was stopped when the patient was seated again. At least one practice trial was performed before the test. Being that a TUG-score of ≥ 20 s is identified as a cut-off point for severe sarcopenia, TUG was considered in this study. (23)

2.4.4 Handgrip strength test

Handgrip strength was determined using the Jamar dynamometer (J A Preston Corporation, New York, NY, USA). The dominant hand was tested. Three measurements of both media and maximum value were taken. The American Society for Parenteral and Enteral Nutrition has included the assessment of grip strength by dynamometer as one of the six criteria to define malnutrition. (24) In this study, the cut-off values defined for the Spanish population will be considered. (14)
Although some quality-of-life test, such as SF-36 or ADL test (activities of daily living), were initially considered in the study protocol, they were finally rejected because, in real clinical practice, these tests are not used with the patient profile included in this study."

5. How did you choose 3 month and 6 months for study follow up.

Thank you for the good comment. In order to answer your doubts, we have included the next paragraphs:

"A follow-up period of 6 months was established since it is common clinical practice in these patients, and with the aim of making the results more generalizable.
It is planned that the same physician attends the three visits to the patient (baseline, 3 and 6 months), to minimise the interpersonal variability in the measurements.

6. If you plan some subanalysis, you have to mention that in this protocol.

No intermediate subanalyses are proposed during the protocol.

7. You need to discuss more about your protocol. How and why, you choose the outcome measurement? What is the novelty of your research What is the difference of

your research from the other research. Otherwise, it is meaningless to publish this protocol paper.

Thank you for the good comment. In order to answer your doubts, we have included the next paragraph:

"This study stands out for the use of several morphofunctional assessment techniques in patients with disease-related malnutrition in real clinical practice. Beyond its large sample, it is the first study with this design, as a real-world study, to evaluate the feasibility of nutritional ultrasound."

"This protocol will be realized in a sample of patients at risk of malnutrition, which will generate very interesting results for routine clinical practice and nutritional care in these patients, easily generalizable and free to use with publication."

8. Your citation is limited. You can cite more research with more discussion.

Thank you for your good comment. We have included four new references in order to improve the discussion.

- *Nakanishi N, Inoue S, Tsutsumi R, Akimoto Y, Ono Y, Kotani J, Sakaue H, Oto J. Rectus Femoris Mimicking Ultrasound Phantom for Muscle Mass Assessment: Design, Research, and Training Application. J Clin Med. 2021 Jun 20;10(12):2721. doi: 10.3390/jcm10122721. PMID: 34202957; PMCID: PMC8235438.*
- *Nakanishi N, Tsutsumi R, Okayama Y, Takashima T, Ueno Y, Itagaki T, Tsutsumi Y, Sakaue H, Oto J. Monitoring of muscle mass in critically ill patients: comparison of ultrasound and two bioele ctrical impedance analysis devices. J Intensive Care. 2019 Dec 16;7:61. doi: 10.1186/s40560-019-0416-y. PMID: 31890223; PMCID: PMC6916000.*
- *Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA, Schneider SM, Sieber CC, Topinkova E, Vandewoude M, Visser M, Zamboni M; Writing Group for the European Working Group on Sarcopenia in Older Proceople 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 2019;48(4):601. doi: 10.1093/ageing/afz046.*
- *White JV, Guenter P, Jensen G, Malone A, Schofield M, Academy of Nutrition and Dietetics Malnutrition Work Group, et al. Consensus statement of the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition: Characteristics recommended for the identification and documentation of adult malnutrition (undernutrition). J Acad Nutr Diet 2012;112(5):730-8.*

Reviewer: 2

Dr. Aoife Ryan, University College Cork School of Food and Nutritional Sciences

Comments to the Author:

Methodology

1. Can the authors please provide further information required on the training attended by the physicians and staff undertaking ultrasound measurements – how are they minimising risk of inter-variability?

Thank you for the good comment. In order to answer your doubts, we have included the next paragraphs:

"US accuracy highly depends on the skills of the technician. Point training using rectus femoris phantom have shown to improve the accuracy of measurements. (20) Before starting the study, a training session was held. All study participants were required to attend, and they had the opportunity to practice with the same ultrasound machine that was going to be used in the study in phantom patients. Besides, several videos explaining detailed measurements technique were recorded. These

videos were proactively shared with all researchers and available anytime at the study on-line electronic data capture (EDC) platform.
Beyond, once the study finishes, all DICOM images gathered will be analysed to develop a semi-automated algorithm that helps diagnose the patient's nutritional status. Subsequently, once the algorithm is available, the individual and manual US measurements will be contrasted with the data showed by the automatic algorithm, thus minimizing the inter and intra observer correlation. This work will have its own analysis and publication plan".

2. How are they ensuring that practice is the same across all 25 sites?
Thank you for the good comment. In order to answer your doubts, we have included the next paragraphs:
"US accuracy highly depends on the skills of the technician. Point training using rectus femoris phantom have shown to improve the accuracy of measurements. (20) Before starting the study, a training session was held. All study participants were required to attend, and they had the opportunity to practice with the same ultrasound machine that was going to be used in the study in phantom patients. Besides, several videos explaining detailed measurements technique were recorded. These videos were proactively shared with all researchers and available anytime at the study on-line electronic data capture (EDC) platform."

3. Do they require refresher sessions over the course of the study?
Thank you for the good comment. In order to answer your doubts, we have included the next paragraphs:
"Throughout the entire study, monthly meetings are hold with all participants on Thursdays at 8:30 a.m., and on Fridays at 8:30 a.m. with the study's central committee. The objective of these meetings is to monitor the status of the study at each participating center, to resolve doubts, and to make sure that all techniques and measurements are properly made according to previous training. "

4. Is there any quality control?
Thank you for the good comment. In each center the same researcher performed the different ultrasounds, and in addition a central control center formed by an independent radiologist will check the validity of all the images.
We include the next paragraph, too. *"Beyond, once the study finishes, all DICOM images gathered will be analysed to develop a semi-automated algorithm that helps diagnose the patient's nutritional status. Subsequently, once the algorithm is available, the individual and manual US measurements will be contrasted with the data showed by the automatic algorithm, thus minimizing the inter and intra observer correlation. This work will have its own analysis and publication plan".*

5. Appears that different researchers will be taking the measurements at 3 months and 6 months compared to baseline.
Thank you for the good comment. In each center the same researcher performed the different ultrasounds. And a paragraph has been included.
". A follow-up period of 6 months was established since it is common clinical practice in these patients, and with the aim of making the results more generalizable.
It is planned that the same physician attends the three visits to the patient (baseline, 3 and 6 months), to minimise the interpersonal variability in the measurements".

6. This is a concern regarding the intra observer variability and reliability of the results at the end of the study.
Thank you for the good comment. In each centre the same researcher performed the different ultrasounds, and in addition a central control centre formed by an independent radiologist will check the validity of all the images.

We include the next paragraph *"Beyond, once the study finishes, all DICOM images gathered will be analysed to develop a semi-automated algorithm that helps diagnose the patient's nutritional status. Subsequently, once the algorithm is available, the individual and manual US measurements will be contrasted with the data showed by the automatic algorithm, thus minimizing the inter and intra observer correlation. This work will have its own analysis and publication plan."*

7. Patient and Public Involvement Please address the reasons why PPI is not involved in this trial – no specific reasons given

Thank you for the good comment We include the next paragraph:

*"**3.6. Patient and Public Involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.*"

8. Dissemination / Use of Thresholds Following standardization.Whilst a secondary outcome – how do you suggest that you will present the thresholds for translation into clinical practice e.g. centiles (like Dodds 2014 for HGS)?

Thank you for the good comment We include the next paragraph.

"The thresholds for translation into clinical practice will be presented as cut-off points that will be estimated by AUC ROC curves. Centiles will be also considered."

VERSION 2 – REVIEW

REVIEWER	Nakanishi, Nobuto Tokushima University Hospital, Emergency and Critical Care Medicine
REVIEW RETURNED	23-Oct-2023

GENERAL COMMENTS	Thank you for the polite and detailed response. This manuscript has improved greatly. I completely agree with this publication.