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Early Detection of Muscle Weakness and Functional Limitations in the Critically Ill: A Retrospective Evaluation of Bioimpedance Spectroscopy

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TITLE: Early detection of muscle weakness and functional limitations in the critically ill: a retrospective evaluation of bioimpedance spectroscopy

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ABSTRACT:

Background: The potential for bioimpedance spectroscopy (BIS) to identify muscle weakness and functional limitations in critical illness is unknown; this study aimed to determine association of BIS with strength/function and differences between three intensive care units (ICUs). *Methods:* A retrospective post-hoc analysis of BIS, strength and functional data. Adults who required ≥48-hours of mechanical ventilation were eligible. Measures of body composition included: the proportion (%) of total body water (TBW), fat mass (FM), and fat free mass (FFM). The medical research council sum-score (MRC-ss) and physical function in ICU test score (PFIT-s) were used for strength and functional assessments. Non-parametric cross-sectional analyses were done at enrollment (≤48-hours of admission: site-A, site-C) and awakening from sedation (site-A, site-B). Raw impedance variables

including 50kHz phase angle (PA) and impedance ratio (IR) were available from site-A and site-B. *Results:* Participants were 135 adults (site-A n=59, site-B n=33, site-C n=44), with a median [IQR] age of 59[50-69] years. At enrollment TBW%, FM% and FFM% was similar between site-A and site-C (p>0.05); pooled data were not associated with MRC-ss at awakening, or, MRC-ss/PFIT-s at ICU discharge. At awakening there was less TBW%, less FFM%, and greater FM% at site-B versus site-A (p≤0.001), but no associations with MRC-ss/ PFIT-s when using pooled data. Trends with pooled data of a lower PA and higher IR being associated with awakening MRC-ss were confirmed within site-B (PA rho=0.70, p≤0.001; IR rho=-0.79, p≤0.001). *Conclusion:* Site-by-site data suggests that raw impedance variables might be useful for screening weakness and poor function.

CLINICAL RELEVANCY: Bioimpedance spectroscopy is a non-invasive bed-side method for body composition analysis with a range of potential applications in the critically ill. This study addresses the proof-of-concept that raw impedance variables (which do not rely on prediction algorithms or body weight measurement) may be a proxy for skeletal muscle health in the form of strength and function. This adds to existing understanding of body composition variables for functional status in other populations, and prognostication in the critically ill.

BACKGROUND:

Skeletal muscle health is vitally important for physical function. In people with a critical illness, the loss of muscle mass is rapid[1,2], which contributes to intensive care unit acquired weakness[3], reduced physical function[4] and prolonged disability for some patients[4,5]. Methods to measure muscle strength and physical function are volitional (effort-dependent); patients must be alert to achieve reliable results[6]. Portable, non-invasive, bed-side methods for assessing lean tissue are needed, not least because of the metabolic functions of lean tissue,[7] but also the prognostic value, as computerized

tomography (CT) and dual-energy x-ray absorptiometry (DXA) measures of lean tissue loss in the critically ill have been associated with increased morbidity and mortality[8,9], longer length of stay[8] and decreased long term physical functioning[10].

Bioimpedance is a non-invasive bed-side method for whole-body composition analysis that has great potential for assessing lean tissue in the clinical setting. There are challenges to validation studies, but multi-frequency and spectroscopy approaches have been validated in a number of clinical populations[11,12] for estimates of compartment fluid volumes (extra-cellular water (ECW) and intra-cellular water (ICW)) and masses (fat mass (FM) and fat free mass (FFM)). While the relationship between BIS parameters and strength/function is yet to be established in acutely hospitalized groups,[13] estimates of compartment volumes have been used to determine fluid and hydration status in the critically ill,[14,15] including ECW:ICW[16] which is thought to reflect the exchangeable sodium/potassium ratio (Na/K) and has been reported to be approximately 0.7 on average in healthy males.[17] For the critically ill, inherent inaccuracies and challenges of whole-body reference techniques like multiple dilution and DXA limits the application of bioimpedance prediction algorithms that often also require an accurate body weight. This may be the reason that research in critical illness has focused on understanding the potential utility of raw impedance variables,[12] including bioimpedance vector analysis,[18,19] 50 kHz phase angle (PA) and the impedance ratio (IR) at 200 kHz/5 kHz. The impedance ratio is a potential marker for nutrition status and/or disease severity, with values approaching 1.0 suggesting worse outcomes, and values greater than 1.0 suggesting device error.[11] Phase angle is calculated from the arctangent of the ratio of reactance (Xc) to resistance (R) at 50 kHz. It is hypothesized to reflect cell membrane integrity and FFM, and has been suggested to represent both the amount and quality of soft tissue as it has been correlated with muscle strength (handgrip) and other functional indices.[11,20-22] As in other diseases, phase angle may be a prognostic

indicator for nutrition status[23] and mortality in the critically ill, including an observation that day one phase angle values <3.49° contributed to a model of 28-day mortality prediction.[20,24]

Despite some practical appeal and interest in the clinical significance of bioimpedance techniques at the bed-side[25], no study has sought to establish a relationship between BIS derived variables and early muscle strength and physical function in the critically ill. Only one study by Kuchnia and colleagues has sought to relate bioimpedance parameters to the muscularity of critically ill patients.[21] They reported c-indexes from receiver operating curves for phase angle (sample mean (SD), 4.34 ± 1.40) and impedance ratio (0.85 (0.04)) predicted low muscularity (defined as cross sectional area <110 cm^2 for females and <170 cm^2 for males on CT at the level of the 3rd lumbar vertebrae) to be 0.78 and 0.76 respectively. The relevance of low muscle quality and quantity as derived from CT is evident in its association with mortality in patients who are mechanically ventilated.[9,26]

The objectives of this study were: (1) to describe and compare BIS derived parameters alongside measures of strength and function, in critically ill patients at different Australian sites, and (2) to determine associations between BIS derived parameters with measures of muscle strength and physical function. We hypothesized that there would be associations between BIS parameters that are reflective of lean tissue and actual muscle performance.

METHODS:

Study design, setting, participants

This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[27] It was a retrospective post-hoc analysis of prospectively collected data in the form of a multi-centre observational study, conducted across three Australian

tertiary intensive care units (ICU's): the Royal Melbourne Hospital, Melbourne, Victoria (site-A), Flinders Medical Centre, Bedford Park, South Australia (site-B) and the Royal Brisbane and Women's Hospital, Brisbane, Queensland (site-C). Data came from four independent prospective studies: one longitudinal (site-A, August 2012 – February 2014),[13] two cross-sectional (site-B: November 2009 to March 2010 and November 2010 to December 2011)[19,28] and one double blinded randomized controlled trial (site-C, December 2010 – August 2012).[29] As per the original publications, each study had institutional ethical approval including procedures for informed consent.

We identified participants from the original studies who were ≥18 years of age, required mechanical ventilation ≥48 hours, were able to ambulate at least 10 meters (+/- aids) within the three months prior to ICU admission, and, had BIS data. The original inclusion and exclusion criteria are outlined in Table S1. Two studies recruited participants with sepsis.[28,29] Commonly patients with severe burns, acute neurological insults, multi-trauma/inability to assess multiple limbs, or a directive for end of life care were excluded.

Demographic data and clinical care

Participants were characterized by age, sex, type of ICU admission, severity of illness in the first 24-hours of admission (Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA)) and comorbidities (Charlson Comorbidity Index, (CCI).[30] Estimated energy requirement (EER) based on 25kcal/kg of actual body weight or ideal body weight (for overweight participants up to a body mass index (BMI) of 32kg/m^2) was calculated;[31] if the BMI was >32 kg/m^2, an adjusted weight (ideal body weight + 25% of actual body weight - ideal body weight) was used.[13] Nutritional risk was described with the modified Nutritional Risk in Critically Ill

(NUTRIC) score.[32] Outcome characteristics included days of mechanical ventilation, ICU and hospital length of stay, hospital mortality, and discharge destination.

It was not possible to consistently describe standard nutrition and physiotherapy care across sites, other than nutrition was delivered with the use of standard nutrition protocols and in all cases, patients received either enteral and/or parenteral nutrition for a period of their ICU stay. Physical therapies were provided by ICU physiotherapists, which could have involved in or out of bed mobility/exercise. This was except for 26 participants in the intervention group at site-C who underwent an earlier, more targeted and regular physiotherapy, which included electrical muscle stimulation.[29]

Procedure

Bioimpedance data were collated at two cross-sectional time points: enrollment, which was within 48 hours of ICU admission (data available from site-A and site-C); and awakening (data available from site-A and site-B). Awakening was defined as the first day patient's passed screening on two consecutive evaluations according to the De Jonghe comprehension criteria (site-A)[3] or the attention screening component of the cognitive assessment method for ICU (CAM-ICU, site-B).[33] Briefly, the De Jonghe method required a correct response to at least 3 of 5 orders (specified one or two stage commands) and the attention screening examination required at least 8 correct responses out of 10, in a visual/auditory recognition sequence. Muscle strength and physical function were assessed at various combinations of clinical milestones at each site, which covered awakening, ICU discharge and hospital discharge.

Bioimpedance analysis

Site-by-site procedures related to the preparation, testing conditions and considerations for whole body BIS measurements[11] are outlined in Table S2. To summarize, participants' actual body weight was mostly used and measured to the nearest 0.1 kg. Site-A calculated predicted height using ulna length, while site-B and site-C measured supine body length. BIS was performed using the same model of device at all sites (SFB7, AU/NZ, Impedimed Limited, Pinkenba, Australia). Participants were supine with a pillow supporting the head and medial surfaces of the limbs abducted so as resting away from and not touching the body. Device specific gel electrodes (dual or single tab, ImpediMed Limited, Pinkenba, Australia) with the same proximal-distal spacing were applied in an ipsilateral tetra-polar configuration with reference to anatomical landmarks on the dorsum of preferably the right hand and foot.[11] In all sites, the Impedimed Bioimp software (Pinkenba, Australia) was used to analyze BIS spectral data with correction for time delay and no data rejection limits. Spectral data were fit to the Cole model, which generated Cole model terms that were then applied to the default algorithm and resistivity constants to generate BIS-derived whole-body volumes/masses: total body water (TBW), ECW, ICW, FFM, and FM. The relative volume of ECW and ICW was expressed as a percentage of TBW and as ECW:ICW. The relative volume/mass of TBW, FFM and FM was expressed as a percentage of body weight. Raw impedance/Cole model variables were available from site-A and site-B. Raw impedance variables included 50 kHz phase angle and 200/5 kHz impedance ratio. Cole model variables included: cell membrane capacitance (*C*m, indicative of the retention capacity of membrane potential gradient and the depolarization reactivity of cell membranes), characteristic frequency (*fc*, the frequency at which maximal reactance occurs in the spectral data, suggested to reflect the heterogeneity in the density of the tissue and is around 30-40kHz in healthy individuals)[34] and R_∞:R_0 (reflective of the ECW resistance:TBW resistance).[11]

Muscle strength and physical function

Global muscle strength was graded with the Medical Research Council sum score (MRC-ss), where higher scores represent better strength. A score of <48 out of 60 was used to classify the presence of ICU-acquired weakness.[35] Awakening MRC-ss scoring was done on the same day as BIS at site-B and within three days at site-A. The Physical Function in ICU test-scored (PFIT-s) was used to quantify physical function in which participants are rated on sit to stand level of assistance, marching on the spot cadence, and both shoulder flexor and knee extensor strength.[36] Scored out of 10, high scores indicate higher (better) physical function. At site-B, right sided hand-grip strength was additionally measured by dynamometry[6] and physical function with the DeMorton Mobility Index.[37]

Study size and statistical analyses

The sample size was limited by the size of the original studies. Data were analyzed using SPSS (v.25, SPSS, Chicago, IL). Descriptive statistics are presented as median [inter-quartile range (IQR)] or frequencies, n (%). Differences between study cohorts were assessed with Kruskal-Wallis tests and contingency tables (Phi and Cramer's V) according to variable type. Site-to-site comparisons at enrollment and awakening were made with the Mann-Whitney test, and statistical significance at $p<0.05$ (Objective 1). Associations of BIS variables with measures of strength or function were made with spearman's rho using all available data, and, site-by-site; due to multiple comparisons statistical significance was determined as a Bonferroni corrected $p\leq0.001$ (Objective 2). For context with existing work, associations of BIS variables with ICU and hospital LOS, the CCI, NUTRIC score and hospital mortality were also analyzed.

RESULTS:

Demographic and clinical data

Of 148 potentially eligible participants (Figure 1), 135 were included, with mainly medical admissions (n=105, 78%). There were no significant differences between study cohorts for age, sex or illness severity such that for the whole sample the median [IQR] age was 59 [50-69] years, with 77 (57%) male participants, and APACHE II score of 22 [17-27]) (Table 1). All nutritional markers were similar across the studies (weight, height, BMI, EER and NUTRIC score, p values >0.05) and all sites commenced nutrition within 1 [1-2] day. The more prolonged duration of mechanical ventilation, sedation days and tracheostomy incidence at site-B is reflective of the original study inclusion criteria of five days minimum mechanical ventilation (rather than 48 hours). Site differences in PFIT-s at ICU discharge (p=0.007) were such that physical function was best in the cohort at site-A (5.9 [4.9-8.6]), followed by site-C (5.2 [3.9-7.1]) and lowest at site-B (4.9 [3.9-5.4]).

Comparison of BIS data between sites (objective 1):

BIS data were available at enrollment from site-A and site-C for a total of 103 participants (Figure 1, Table 2), which was measured as per protocol on ICU day 2 [1-2]. The compartment masses (FFM/FM) and volumes were similar across sites, except that participants at site-A had a marginally greater proportion (%) of ICW compared to site-C (p=0.049). The ECW:ICW was also comparable between sites.

BIS data were available at awakening from site-A and site-B for a total of 80 participants (Figure 1, Table 2). As there were no significant differences in any BIS variables between the two site-B cohorts (p≥0.18),[19,28] data are presented together as a site. Patients at site-B had less TBW (L/%), less FFM

(kg/%) and thus more FM (kg/%) than patients at site-A (p≤0.001). Participants at site-B had lower volumes of both ICW (L) and ECW (L) than site-A, but proportionally patients at site-A had a lower (closer to normal) ECW:ICW (p=0.043). In terms of other BIS generated variables, there were no significant differences between sites in phase angle, impedance ratio, or characteristic frequency (p≥0.22), but site-B participants had a higher R_∞:R_0 (p=0.047), and lower cell membrane capacitance (p≤0.001) than site-A (Table 5).

Associations between BIS data and measures of muscle strength and function (objective 2):

Measurements at enrollment

On analysis of BIS predicted variables at enrollment, there were no significant associations with MRC-ss at awakening (data available from site-A only), or, MRC-ss/PFIT-s at ICU discharge (pooled site-A and site-C) (Table 3). On site-by-site analysis at site-C, greater FFM (kg) was weakly associated with MRC-ss (rho=0.33, p=0.047) and PFIT-s (rho=0.34, p=0.040) at ICU discharge. In terms of raw impedance and Cole model-derived data that were available from site-A, there were no significant associations between variables at enrollment with MRC-ss or PFIT-s down the recovery track (p≥0.11, Table S3).

Measurements at awakening

At awakening, there were no significant associations between BIS predicted variables and MRC-ss/PFIT-s when using pooled data (Table 4). Yet on site-by-site analysis at site-B, greater handgrip strength was associated with more ICW (L) (rho=0.61, p≤0.001) and there were trends with more TBW (L/%) and FFM (kg/%) (all p=0.003). In terms of pooled raw impedance data (site-A and site-B), there were trends for a higher phase angle (rho=0.31, p=0.013) and lower impedance ratio (rho=-

0.29, p=0.020) at awakening to be associated with greater MRC-ss also at awakening, and, higher awakening phase angle to be associated with PFIT-s at ICU discharge (rho=0.26, p=0.048) (other raw variables p≥0.07). However site-by-site analysis of raw impedance/Cole model data, there were no significant associations within site-A, while at site-B higher phase angle and cell membrane capacitance, and, lower impedance ratio, characteristic frequency, and R_∞:R_0 were associated with greater muscle strength at awakening (MRC-ss: rho≥0.70, p≤0.001; handgrip rho≥0.61, p≤0.001) (Table 5). There were no significant associations between awakening raw impedance/Cole model variables, and physical function on the PFIT-s, but a higher phase angle was associated with better physical function on the DEMMI at both ICU discharge (rho=0.64, p=0.011) and hospital discharge (rho=0.60, p=0.012). There were also relationships between awakening impedance ratio, R_∞:R_0, and cell membrane capacitance with DEMMI scores at hospital discharge (rho≥0.56, p≤0.031).

Associations with other outcomes

There were trends for a lower (closer to normal) ECW:ICW at enrollment to be associated with shorter ICU LOS (rho=0.27, p=0.006) and hospital LOS (rho=0.23, p=0.018) when site-A and site-C data were pooled (Table S4). Cross-sectional measurements at awakening similarly showed a lower ECW:ICW to be associated with shorter ICU LOS (rho=0.44, p≤0.001) and hospital LOS (rho=0.51, p≤0.001), along with a higher FFM (kg) (pooled site-A and site-B data, Table S4). Awakening ECW:ICW was furthermore associated with hospital mortality (eta^2 0.93, small effect size), particularly reflected in site-B data with an eta^2 0.368 (large effect size).

In terms of raw impedance/Cole model variables, at enrollment there were trends for lower phase angle and cell membrane capacitance, with higher R_∞:R_0, impedance ratio and characteristic frequency to be associated with ICU LOS (rho≥0.35, p≤0.007) (Table S3). Cross-sectional

measurement at awakening similarly showed lower phase angle and cell membrane capacitance, with higher R_∞:R_0, impedance ratio and cell membrane capacitance to be associated with prolonged ICU LOS (rho≥-0.50, p≤0.001) and hospital LOS (rho≥-0.54, p≤0.001) (Table S5). There were trends for a lower phase angle and impedance ratio to be associated with greater nutritional risk (NUTRIC) and comorbidity (CCI). The strength of correlations at site-B were consistently greater than site-A for all variables.

DISCUSSION:

This is currently the largest study to relate BIS parameters to volitional muscle strength and physical function in survivors of a critical illness. BIS derived whole body volumes/masses at enrollment were not significantly associated with later strength/functional outcomes, other than absolute (kg) FFM at site-C which may have been overestimated. Yet there was a signal from raw impedance/Cole model data at awakening of a relationship between phase angle and impedance ratio with concurrent muscle strength, and physical function at ICU discharge. This suggests that raw impedance and Cole model variables may perform better than estimated volumes/masses and be developed in the future as an indicator of volitional strength/function.[12] This proof of concept was most evident at site-B that sampled long-stay patients with sepsis, where there were higher correlation coefficients (than site-A) which reached statistical significance for a range of variables; possibly due to sampling, fluid management and protocol differences.

The value of BIS as an early indicator of recovery may be when patients are unstable, or when effort-dependent strength and functional testing is not possible (for example due to cognition changes or environmental conditions). Limitations to volitional tests have been recognized, and a research agenda has been described that outlines the value of evaluating the diagnostic utility of non-volitional tools.[36] To date the focus on non-volitional tools for lean tissue evaluation has been on

musculoskeletal ultrasound (US) for single muscle groups,[2] which like BIS requires further investigation as to the clinical relevance.[12,38] Comparatively, BIS may be less accessible at the point-of-care than US but also requires less training for use. Lean tissue parameters are infrequently estimated with both BIS and US in the critically ill[28], but these methodologies could be complimentary in studies that integrate exercise and nutrition therapy,[38] especially with use of raw impedance data and where outcomes like nutritional risk and length of stay are of interest. With any future use of BIS, consideration should be given to the rigor of procedures. As suggested by site differences in our data, this is particularly important if planning multi-site studies where fluid management practices and known sources of error (with incorrect limb positioning and obesity potentially being the most influential)[11] could be variably introduced.

We considered cohort, methodological and BIS procedural discrepancies as reasons why there were differences in body composition between sites, and, associations within some sites but not others. Site-A had the most liberal sampling criteria, while the studies at site-B and site-C predominantly recruited patients with sepsis, who due to systemic inflammation and multi-organ failure are expected to experience skeletal muscle wasting at a faster rate over the first week of critical illness than non-septic patients,[1] intra-cellular dehydration, and disrupted cell membrane integrity. The greater likelihood of significant lean tissue loss, and, difference in the number of days to awakening (median day 5 at site-A and day 15 at site-B, Table 2), is one explanation for why FFM was comparatively so low at site-B versus site-A.

This study also provided objective multi-site data on the fluid status of critically ill patients; our results demonstrated variable fluid status, like what has been shown with fluid management practices (volume and type) across ICUs.[39] It is important to understand the impact of fluid volume and

distribution on FFM as the outcome of interest for lean tissue. The SFB7 estimates FFM from TBW, using the assumption that FFM is normally hydrated at 73.2% and muscle is a water rich tissue.[11] Because the SFB7 algorithm uses Cole modeling to derive estimates of ECW and TBW, and from these derives ICW, the relatively independent ECW:ICW ratio is useful to consider as a reflection of hydration status. When ECW:ICW is elevated, estimates of FFM will likely be overestimated by BIS due to overhydration. The ECW:ICW was higher at site-C (enrollment) and site-B (awakening) than site-A (Table 2), suggesting overhydration and a risk of interpreting higher FFM at these site-C and site-B that sampled patients with sepsis. However, despite a higher ECW:ICW at site-B, bioimpedance vector analysis suggested that critically ill participants at site-B rather had less body water in comparison to both patients at site-A and healthy controls (Figure S1).[19,28] Research with segmental BIS in ageing would suggest relative expansion of ECW against ICW in the upper leg to be an independent explanatory factor for reduced knee extension force and gait speed, and thus an indicator of muscle quality.[40] However, there were no associations between ECW:ICW and measures of muscle strength or physical function in the present study. Still, ECW:ICW may be important for other outcomes like LOS and mortality, consistent with existing understanding of conservative fluid (and sodium) balance on mortality and other outcomes[41] keeping in mind that converse risks of dehydration (especially intra-cellularly) are not well understood in terms of mechanism and their impact on cognition[42] or function for survivors of a critical illness.

Associations with awakening BIS data were site specific, namely at site-B where FFM (%) and raw impedance variables were associated with awakening strength (MRC-ss and handgrip) and function (DEMMI) at both ICU and hospital discharge. Neither handgrip or the DEMMI have a ceiling effect at ICU/hospital discharge and therefore may have been more responsive to change at those timepoints than the PFIT-s.[43] Still, it is important to remember that this study analyzed associations that do not imply causation or prediction. Between site differences in methodology were such that awakening was

determined by different methods and strength/function was measured within a few hours of BIS at site-B, but within 72 hours at site-A. A difference of up to 72 hours between BIS and muscle testing is consistent with the timeframes in the study of Kuchnia et al[21] (used CT), but may be too long for clinical testing in the ICU. The main differences in BIS methodology were that site-B consistently used actual body weight and height, and, thoroughly prepared the skin prior to electrode application (Table S2). These procedural controls give confidence to the strength of associations found at site-B. The median phase angle and impedance ratio at site-B were 3.8° and 0.87 respectively, suggesting slightly worse physiology than the Kuchnia et al[21] cohort (mean phase angle 4.34° and impedance ratio 0.85). However, as the median characteristic frequency (top of the Cole plot) was 48 kHz, the standard 50kHz for reading phase angle may not have been appropriate. There may be disease specific determinants for phase angle[21] which for critical illness may include comorbidity. One of the co-variables that improved prediction of abdominal muscle cross-sectional area (phase angle predicted 20% of the variance) on CT was the CCI; there was a trend for phase angle at awakening at site-B to be associated with CCI in the present study (rho=-0.42, p=0.017, Table S5).

A strength of the study was the use of the same device and program for data processing, thus enabling comparison between studies.[12] Limitations of the study include the retrospective design meaning that we could not concurrently collect fluid balance at the measurement timepoints, and did not have repeated BIS at ICU discharge. Nutrition and physiotherapy practices were unable to be described in a standardized way across sites. Sites commenced nutrition within current international guideline recommendations,[31] however variations in regimes and nutrition delivery are likely to exist. Nutritional[44] and early mobility approaches[45] in research studies have not consistently made an impact on patient outcomes, and in the original site-C study, there was no difference in FFM between the intervention and control groups.[29] Also, we were not able to investigate associations between change in body composition over time with strength/function. In considering the age of the original

data and its relevance to the present, broadly in Australian ICUs there has been a trend to decrease fluid volumes[39] although sodium administration likely remains high;[46] there is a trend towards increasing protein provision with more conservative energy targets,[31,47] which may impact outcomes. As for mobility, the proportion of patients mobilized during ICU stay may be higher in Australia than some countries,[48] but is lower than others[49] possibly because translation of recommendations for early mobilization into practice is slow.[50]

Conclusions

The variables with the most promising data for investigation in future studies of BIS alongside muscle strength and physical function were raw impedance and Cole model variables that do not rely on an accurate body weight or prediction modelling. Standardization and documentation of both clinical care (like fluid therapy) and BIS methodology in multi-site studies will help improve data quality to progress this proof of concept forward.

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Table 1: Demographic and clinical data as median [interquartile range] or n(%), with differences between studies

Site	Site-A	Site-B	Site-B	Site-C	All participants	p value [a]
Study	Fetterplace et al[12]	Baldwin et al[19]	Baldwin et al[28 b]	Kayambu et al[29]		
n	59	13	19	44	135	
Demographics and clinical care						
Age (years)	60 [49- 69]	56 [43-80]	63 [50-72]	65 [53-69]	59 [50-69]	0.77
Male n (%)	32 (54)	7 (54)	11 (58)	27 (61)	77 (57)	0.90
ICU admission type Medical Surgical Trauma	 33 (56) 17 (29) 9 (15)	 11 (84) 1 (8) 1 (8)	 17 (89) 2 (11) 0 (0)	 44 (100) 0 (0) 0 (0)	 105 (78) 20 (15) 10 (7)	0.001
APACHE II	22 [17-28]	21 [14-23]	26 [20-32]	21 [16-26]	22 [17-27]	0.10
SOFA (admission)	10 [8-12]	9 [6-11]	10 [8-13]	11 [9-12]	10 [8-12]	0.040
CCI (age adjusted)	4 [2-5]	0 [0-4]	3 [2-5]	2 [0-4]	3 [1-5]	0.008
Mechanical ventilation (days)	4 [3-8]	12 [9-16]	10 [5-23]	7 [5-14]	7 [4-11]	≤0.001
Tracheostomy	5 (8)	7 (54)	8 (42)	0 (0)	20 (15)	≤0.001
Sedation duration (days)	4 [2-7]	9 [6-12]	8 [4-13]	1 [0-4]	4 [1-8]	≤0.001
Nutritional markers						

Weight (kg)	84.8 [71.7-102.0]	81.2 [64.1-98.3]	82.0 [71.4-96.5]	75.0 [68.0-98.8]	81.2 [70.0-99.7]	0.63
Height (cm)	170 [162-180]	170 [163-175]	177 [164-183]	171 [163-178]	171 [163-180]	0.57
BMI (kg/m^2) Underweight <18.5 Normal 18.5-24.9 Overweight 25-29.9 Obese >30	28 [25-33] 1 (1.7) 9 (15.3) 26 (44.1) 23 (39)	27 [22-34] 1 (7.7) 4 (31) 3 (23) 5 (39)	29 [24-31] 0 (0) 6 (32) 6 (32) 7 (37)	28 [23-34] 1 (2.3) 12 (27) 17 (39) 14 (32)	28 [25-33] 3 (2.2) 31 (23) 52 (39) 49 (36)	0.78 0.64
EER for 25 kcal/kg,	1910 [1738-2170	1875 [1602-2057]	1960 [1595-2198]	1875 [1703-2125]	1875 [1713-2125]	0.56
NUTRIC score (0-9)[c] Low score/risk (0-4) High score/risk (5-9)	5 [4-6] 21 (40) 31 (60)	4 [2-6] 8 (67) 4 (33)	5 [4-6] 5 (26) 14 (74)	5 [4-6] 18 (41) 26 (59)	5 [4-6] 52 (41) 75 (59)	0.20 0.17
Outcome characteristics						
Day (LOS) of awakening	4 [3-7]	13 [11-16]	15 [8-21]	N/A	7 [4-13]	≤0.001
ICU mortality	9 (15)	1 (8)	0 (0)	4 (9)	14 (10)	0.27
ICU length of stay (days)	5 [3-12]	20 [13-21]	17 [7-43]	9 [7-19]	8 [4-19]	≤0.001
Hospital length of stay (days)	14 [8-26]	28 [18-49]	36 [13-88]	42 [17-95]	23 [11-53]	≤0.001
Discharge						0.004

destination	29 (49)	10 (77)	6 (31)	32 (73)	77 (57)	
Home	14 (24)	2 (15)	10 (53)	2 (5)	28 (21)	
Rehabilitation	4 (7)	0 (0)	1 (5)	4 (9)	9 (7)	
Residential Care facility	12 (20)	1 (8)	2 (11)	6 (14)	21 (16)	
Deceased in hospital [d]						
Muscle strength and physical function						
ICUAW[e] at awakening						
Yes	23 (39)	N/A	8 (42)	N/A	31 (45)	0.19
No	27 (46)	N/A	11 (58)	N/A	38 (55)	
Not assessed [f]	9 (15)	13 (100)	0 (0)	44 (100)	-	
Score (0-60), median [IQR]	48 [35-54]	N/A	50 [45-60]	N/A	48 [38-56]	0.16
ICUAW[d] at ICU discharge						
Yes	10 (17)	N/A	N/A	15 (34)	23 (28)	0.043
No	37 (63)	N/A	N/A	21 (48)	60 (72)	
Not assessed [g]	12 (20)	13 (100)	20 (100)	8 (18)	-	
Median [IQR] score	53 [48-58]	N/A	N/A	50 [45-60]	52 [46-60]	0.87
PFIT-s at ICU discharge						
n (%)	48 (82)	0 (0)	15 (79)	36 (82)	99 (73)	
score (0-10), median [IQR]	5.9 [4.9-8.6]	N/A	4.9 [3.9-5.4]	5.2 [3.9-7.1]	5.4 [4.4-7.1]	0.007

APACHE II, acute physiology and chronic health evaluation II; BMI, body mass index; CCI, charlson comorbidity index; EER, estimated energy requirement; ICU, intensive care unit; ICUAW, intensive care unit acquired weakness; IQR, interquartile range; LOS, length of stay; n, number; N/A, not available; NUTRIC, nutritional risk in critically ill; PFIT-s, physical function in intensive care test scored; SOFA, sequential organ failure assessment.

[a] Differences between studies

[b] Sample includes 4 non-septic participants, not included in the original publication

[c] NUTRIC score missing n=7 (site-A), n=1 (study 1, site-B)[18]

[d] no difference in hospital mortality between sites p=0.55

[e] Intensive care unit acquired weakness defined by medical research council sum-scores <48/60.

[f] Not assessed at site-A due to death in ICU prior to awakening; not assessed at site-B[18] or site-C as not part of original data collection

[g] Not assessed at site-A due to death prior to ICU discharge (n=9), unable to follow commands (n=3); not assessed at site-B as not part of original data collection; not assessed at site-C due to ongoing coma or significant confusion (n=8)

Table 2: BIS derived body masses and volumes at enrollment and awakening

		Enrollment			Awakening[a]		
BIS variable		Site-A n=59	Site-C n=44	All available n=103	Site-A n=48	Site-B n=32	All available n=80
TBW	(L)	48.8 [42.2-56.1]	48.4 [41.7-58.6]	48.8 [42.2-57.0]	47.3 [39.3-55.7]	36.0 [26.8-42.2]	42.7 [33.8-49.9] [c]
	(%)	58.6 [52.8-65.8]	54.1 [48.0-66.2]	58.1 [50.8-65.8]	53.5 [49.6-65.4]	44.4 [39.0-49.0]	49.8 [44.6-58.4] [c]
ICW	(L)	26.5 [21.6-31.3]	26.0 [20.7-30.5]	26.4 [21.6-30.6]	26.3 [20.9-29.9]	20.2 [13.6-21.7]	22.0 [18.1-27.6] [c]
	(%)	53.2 [51.1-56.9]	52.2 [48.2-55.3]	52.7 [50.1.-55.7] [b]	53.4.2 [51.0-55.3]	51.4 [48.5-54.0]	52.7 [50.3-54.9]
ECW	(L)	22.5 [19.4-26.3]	22.9 [19.2-27.4]	22.5 [19.4-26.7]	21.1 [18.8-26.0]	16.8 [13.6-21.0]	20.0 [15.6-23.6] [c]
	(%)	46.8 [43.1-48.9]	47.1 [43.6-50.5]	47.0 [43.4-49.4]	46.6.4 [44.7-49.0]	48.6 [46.0-51.5]	47.3 [45.1-49.7]
ECW:ICW		0.89 [0.76-0.96]	0.91 [0.81-1.02]	0.90 [0.78-0.99]	0.87 [0.81-0.96]	0.95 [0.85-1.06]	0.90 [0.82-1.00] [c]
FFM	(kg)	67.7 [57.7-76.7]	63.4 [56.6-76.5]	66.4[56.9-76.7]	64.6 [53.7-76.1]	49.2 [36.7-57.6]	58.3 [46.2-68.2] [c]
	(%)	80.0 [72.1-89.8]	72.2 [65.5-88.1]	76.8 [70.1-88.7]	73.1 [67.7-89.3]	60.6 [53.2-67.7]	68.1 [60.9-79.8] [c]
FM	(kg)	17.5 [11.0-24.4]	16.9 [8.9-31.0]	17.3 [9.8-25.3]	19.4 [8.2-30.4]	31.8 [23.3-39.0]	25.4 [16.6-34.6] [c]

	(%)	20.0 [10.2-27.9]	25.1 [10.9-33.0]	21.3 [10.9-28.8]	26.9 [10.7-32.3]	39.4 [33.0-46.8]	31.9 [20.1-39.1] [c]

Data are presented as median [IQR]

BIS, bioimpedance spectroscopy; ECW, extracellular water; FFM, fat free mass; FM, fat mass; ICU, intensive care unit; ICW, intracellular water; kg, kilograms; L, liters; n, number; TBW, total body water; %, percentage.

[a] Measurements were made at site-A on ICU day 5 [5-5], and at site-B ICU day 15 [9-18]

[b] Significant difference between site-A and site-C for ICW (%) only p=0.049

[c] Significant difference between site-A and site-B for TBW (L/%), ICW (L), ECW (L), FFM (kg/%) and FM (kg/%) at p≤0.001; ECW:ICW p=0.043

Table 3: Associations between BIS derived body masses and volumes at enrollment with muscle strength and physical function

		Rho P value						
Measure		MRC-ss	MRC-ss			PFIT-s		
Timepoint	Enrollment	**Awakening**	**ICU discharge**			**ICU discharge**		
Site		site-A	site-A	site-C	All available	site-A	site-C	All available
n		n=50	n=47	n=36	n=83	n=48	n=36	n=84
TBW	(L)	-0.00 P=0.98	-0.20 P=0.18	0.28 P=0.12	0.02 P=0.86	-0.20 P=0.17	0.29 P=0.09	-0.02 P=0.85
	(%)	0.05 P=0.72	-0.08 P=0.59	0.02 P=0.93	-0.03 P=0.81	-0.01 P=0.95	0.12 P=0.50	0.08 P=0.47
ICW	(L)	0.01 P=0.97	-0.19 P=0.20	0.19 P=0.26	-0.01 P=0.97	-0.19 P=0.19	0.23 P=0.18	-0.02 P=0.87

	(%)	0.08 P=0.57	0.02 P=0.90	0.04 P=0.82	0.02 P=0.84	0.02 P=0.87	-0.00 P=1.00	0.06 P=0.60
ECW	(L)	-0.05 P=0.73	-0.19 P=0.19	0.23 P=0.17	0.02 P=0.84	-0.22 P=0.14	0.26 P=0.13	-0.02 P=0.84
	(%)	-0.08 P=0.57	-0.02 P=0.90	-0.23 P=0.18	-0.11 P=0.35	-0.02 P=0.87	-0.05 P=0.78	-0.06 P=0.62
ECW:ICW		-0.08 P=59	-0.02 P=0.90	0.02 P=0.90	0.02 P=88	-0.02 P=0.87	0.03 P=0.86	-0.03 P=0.81
FFM	(kg)	-0.00 P=0.98	-0.20 P=0.18	0.33 P=0.047	0.06 P=0.58	-0.20 P=0.17	0.34 P=0.040	0.01 P=0.90
	(%)	0.05 P=0.72	-0.08 P=0.59	0.19 P=0.26	0.04 P=0.74	-0.01 P=0.95	0.19 P=0.26	0.12 P=0.28
FM	(kg)	-0.03 P=0.83	0.06 P=0.71	0.15 P=0.37	0.07 P=0.54	0.00 P=0.98	0.01 P=0.95	-0.07 P=0.55
	(%)	-0.05 P=0.72	0.08 P=0.59	-0.06 P=0.93	0.03 P=0.81	0.01 P=0.95	-0.07 P=0.67	-0.06 P=0.59

BIS, bioimpedance spectroscopy; ECW, extracellular water; FFM, fat free mass; FM, fat mass; ICW: intracellular water; kg, kilograms; L, liters; MRC-ss, Medical Research Council sum-score; n, number; PFIT-s, Physical Function in Intensive care Test-scored; TBW, total body water; %, percentage.

Table 4: Associations between BIS derived body masses and volumes at awakening with muscle strength and physical function

		Rho P value							
Measure		MRC-ss			Handgrip	MRC-ss	PFIT-s		
Timepoint	Awakening	**Awakening**			**Awakening**	**ICU discharge**	**ICU discharge**		
Site		Site-A	Site-B	All available	Site-B	Site-A	Site-A	Site-B	All available
n		n=46	n=19	n=65	n=32	n=44	n=44	n=15	n=59
TBW	(L)	-0.05 P=0.75	0.53 P=0.020	0.00 P=0.97	0.50 P=0.003	-0.02 P=0.90	-0.14 P=0.36	0.04 P=0.87	0.09 P=0.52
	(%)	-0.15 P=0.34	0.46 P=0.049	-0.09 P=0.46	0.50 P=0.003	0.010 P=0.95	-0.02 P=0.88	-0.13 P=0.63	0.25 P=0.06
ICW	(L)	-0.08 P=0.60	0.57 P=0.011	-0.04 P=0.76	*0.61* *P≤0.001*	-0.05 P=0.76	-0.17 P=0.28	0.10 P=0.73	0.12 P=0.39
	(%)	-0.06 P=0.71	0.27 P=0.27	-0.03 P=0.79	0.28 P=0.11	-0.02 P=0.88	-0.08 P=0.62	0.26 P=0.34	0.19 P=0.15
ECW	(L)	-0.05 P=0.74	0.32 P=0.18	0.03 P=0.81	0.33 P=0.07	-0.02 P=0.89	-0.14 P=0.37	-0.10 P=0.72	0.05 P=0.69
	(%)	0.06 P=0.71	-0.29 P=0.22	0.03 P=0.82	-0.30 P=0.10	0.02 P=0.88	0.08 P=0.62	-0.30 P=0.28	-0.20 P=0.14

ECW:ICW		0.05 P=0.74	-0.34 P=0.15	0.02 P=0.90	-0.32 P=0.08	0.02 P=0.89	0.08 P=0.61	-0.34 P=0.19	-0.21 P=0.11
FFM	(kg)	-0.05 P=0.75	0.53 P=0.019	0.00 P=0.97	0.50 P=0.003	-0.02 P=0.90	-0.14 P=0.36	0.02 P=0.94	0.08 P=0.53
	(%)	-0.15 P=0.34	0.46 P=0.049	-0.09 P=0.46	0.50 P=0.003	0.10 P=0.95	-0.02 P=0.88	-0.13 P=0.63	0.24 P=0.064
FM	(kg)	0.14 P=0.37	-0.25 P=0.31	0.13 P=0.30	-0.19 P=0.30	-0.03 P=0.87	-0.03 P=0.86	0.09 P=0.74	-0.24 P=0.074
	(%)	0.15 P=0.34	-0.55 P=0.049	0.09 P=0.46	-0.50 P=0.003	-0.01 P=0.95	0.02 P=0.88	0.13 P=0.63	-0.24 P=0.064

Italic font rho an p values represent associations interpreted to be statistically significant

BIS, bioimpedance spectroscopy; ECW, extracellular water; FFM, fat free mass; FM, fat mass; ICW: intracellular water, kg, kilograms; L, liters; MRC-ss, Medical Research Council sum-score; n, number; PFIT-s, Physical Function in Intensive care Test-scored; TBW, total body water; %, percentage.

Table 5: Associations between raw impedance variables from site-A and site-B at awakening with muscle strength and physical function

			Rho P value								
			Awakening			**ICU discharge**				**Hospital discharge**	
			MRC-ss	MRC-ss	Handgrip[a]	MRC-ss	PFIT-s	PFIT-s	DEMMI[b]	PFIT-s	DEMMI[b]
n	Site-A, n=48 Median [IQR]	Site-B, n=32 Median [IQR]	Site-A n=46	Site-B n=16	Site-B n=29	Site-A n=44	Site-A n=44	Site-B n=12	Site-B n=15	Site-B n=14	Site-B n=17
Impedance variables at awakening											
Phase angle, 50 kHz (PA °) [c]	4.1 [3.6-4.8]	3.8 [2.9-4.8]	0.23 P=0.13	*0.70* *P=0.001*	*0.64* *P≤0.001*	0.18 P=0.26	0.05 P=0.77	0.44 P=0.10	0.64 P=0.011	0.44 P=0.12	0.60 P=0.01
Impedance ratio, (IR)	0.86 [0.83-0.87]	0.87 [0.82-.0.90]	-0.19 P=0.20	*-0.79* *P≤0.001*	*-0.66* *P≤0.001*	-0.15 P=0.32	-0.04 P=0.82	-0.40 P=0.19	-0.57 P=0.05	-0.51 P=0.10	-0.61 P=0.02
$R_\infty:R_0$	0.76	0.78	0.0	*-0.80*	*-0.64*	-0.02	0.08	-0.39	-0.48	-0.42	-0.56

	[0.71-0.80]	[0.75-0.78] [d]	2 P=0.89	*P≤0.001*	*P≤0.001*	P=0.88	P=0.61	P=0.21	P=0.11	P=0.19	P=0.03
Characteristic frequency (*f*c, kHz)	48.5 [37.9-58.7]	48.2 [40.2-54.2]	0.07 P=0.65	*-0.90* *P≤0.001*	*-0.61* *P≤0.001*	0.02 P=0.92	0.08 P=0.63	-0.56 P=0.06	-0.62 P=0.031	-0.46 P=0.15	-0.48 P=0.07
Cell membrane capacitance (*C*m, nF)	1.77 [1.01-2.64]	1.09 [0.63-1.66] [d]	-0.16 P=0.30	*0.80* *P≤0.001*	*0.68* *P≤0.001*	-0.04 P=0.78	-0.13 P=0.39	0.23 P=0.48	0.20 P=0.54	0.53 P=0.10	0.63 P=0.01

Italic font rho an p values represent associations interpreted to be statistically significant

DEMMI, deMorton mobility index; IQR, inter-quartile range; MRC-ss, Medical Research Council sum-score; n, number; PFIT-s, Physical Function in Intensive care Test-scored

[a] median [IQR] handgrip strength at awakening was 15.0 [5.5-22.5] kg

[b] median [IQR] DEMMI score of participants at ICU discharge was 20 [8-24], and at hospital discharge was 48 [36-71]

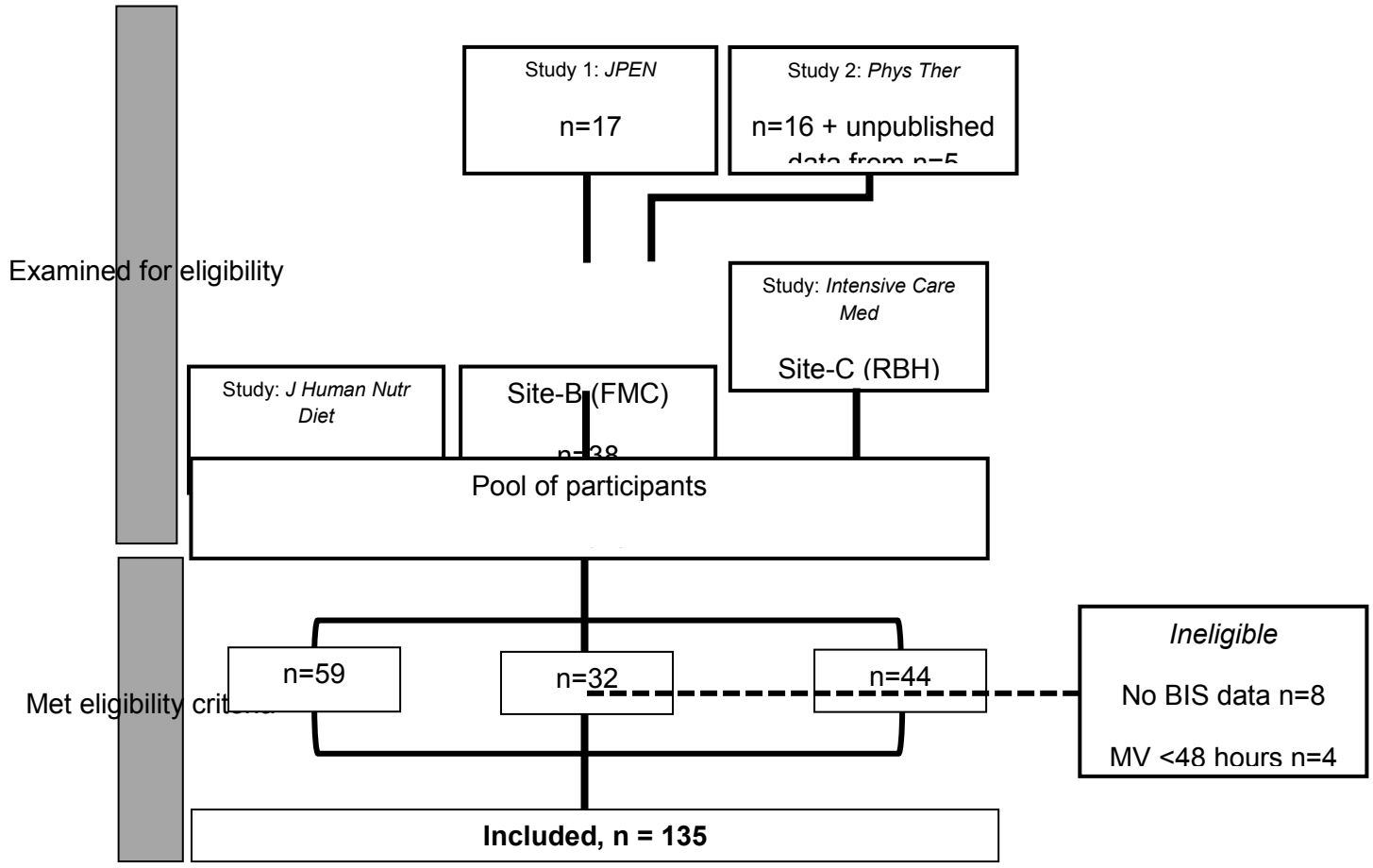
[c] n=3 more participants for phase angle analysis at site-B for each outcome

[d] significant difference between site-A and site-B at awakening

FIGURE CAPTIONS

Figure 1: Flow of participants through study and data summary

BIS, bioimpedance spectroscopy, FMC, Flinders Medical Centre; ICU, intensive care unit; MRC-ss, medical research council sum-score; MV, mechanical ventilation, PFIT-s, physical function in intensive care unit test-scored; RBH, The Royal Brisbane and Women's Hospital; RMH, The Royal Melbourne Hospital;



Available data summary

Site-A	Site-B	Site-C	Measure and timepoint
✓	x	✓	BIS at enrollment
✓	✓	x	BIS at first awakening
✓	✓	x	MRC-ss at first awakening
✓	x	✓	MRC-ss at ICU discharge
✓	✓	✓	PFIT-s at ICU discharge
x	✓	x	PFIT-s at Hospital discharge

Supplementary material

Table S1: Cohort characteristics, inclusion and exclusion criteria from original studies

Table S2: Body composition analysis methods at each site with the SFB7

Table S3: Associations between raw impedance variables from site-A at enrollment with strength, function and other variables

Table S4 Associations between BIS derived body volumes and masses at with other outcome variables

Table S5 Associations between raw impedance variables from site-A and site-B awakening with other outcome variables

Figure S1: Bioimpedance vector analysis (BIVA) of critically ill site-A (dashed line, n=48), critically ill site-B (spotted line, n=32) and healthy control data (solid line, n=