Children with severe acute malnutrition
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Citation for published version (APA):

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Download date: 11 Dec 2018
Chapter 6

The clinical use of longitudinal bio-electrical impedance analysis in children with severe acute malnutrition

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Manuscript in preparation
ABSTRACT

**Background & Aims:** The severity of malnutrition in children is normally assessed using anthropometry: weight for height (W/H), height for age (W/A), and mid upper arm circumference (MUAC) which measure body composition indirectly. Altered body composition (loss of fat-free mass) is linked to poor clinical outcome and can be estimated by bio-electrical impedance analysis (BIA). We aimed to assess how BIA parameters in children with severe acute malnutrition (SAM) with or without edema: 1) change during clinical stabilization; or 2) add a prognostic value to clinical outcome when combined with ‘classic’ anthropometry.

**Methods:** For this prospective, observational study, we enrolled children, aged 6-60 months, that were admitted for complicated SAM at the Queen Elizabeth Central Hospital in Blantyre, Malawi. Height, weight, MUAC and bio-electrical impedance were measured on admission and after clinical stabilization. Resistance (R) was considered to reflect body fluids, reactance (Xc) soft tissue, and phase angle (PA) the arc tangent relation of Xc/R. The R and Xc values were divided by height to correct for body size (R/H and Xc/H).

**Results:** We studied 183 SAM children (edematous 53%; age 23.0±12.0 months; male 54%) and 11 healthy controls (age 14.6±5.3 months; male 73%). On admission, edematous SAM children had lower bio-electrical impedance parameters (R, Xc, PA) than non-edematous children (p<0.0001); and after clinical stabilization BIA increased only in children with edema (p<0.0001). MUAC and W/H z-scores were negatively correlated with R/H (-0.5967, p<0.0001 and -0.6786, p<0.0001) and this relationship was stronger in edematous cases. Children that died had lower W/H z-scores (-2.7 ± 1.8 vs -4.1 ± 1.8, p=0.0002) and lower MUAC (11.6 ± 1.6 vs 10.3 ± 1.8, p < 0.0001). Combining BIA and anthropometric variables did not improve classification error rates or sensitivity and specificity in predicting clinical groups or outcomes compared to using anthropometric variables alone. However, BIA did help better distinguish children with edematous SAM from community children. Overall, the variability in BIA measures was high and their added predictive value low.

**Conclusions:** During clinical stabilization, BIA parameters increase in children with edematous SAM, which likely reflects fluid loss. BIA is correlated with classic anthropometry, and this more so in children with edema. Classic anthropometry is associated with mortality but BIA, as currently implemented, does not add prognostic value in predicting the clinical outcome of children with SAM. Unless the method is improved, our data does not support the clinical use of BIA in low-resource settings for care of children with complicated SAM.
INTRODUCTION

Severe acute malnutrition (SAM) in children remains a major global health problem, hence its inclusion in the Sustainable Development Goals.(1,2) Nearly half (45%) of global under-five mortality is related to poor nutrition and the vast majority of these children live in Sub Saharan Africa and South east Asia.(3) Despite adherence to WHO protocols the case fatality rate in children with SAM remains unacceptably high (up to 35%).(4) Thus, current SAM management and identification of children at risk of clinical deterioration needs to be urgently improved.(1,5–7)

SAM is defined by WHO standards, as any of the following: for non-edematous SAM/marasmus, a weight for height (W/H) below -3 standard deviation (SD), a mid-upper arm circumference (MUAC) of less than 115mm, or, for edematous SAM/kwashiorkor, the presence of bilateral edema.(8) Current management strategies are ‘blanket approaches’ that disregard the different presentations of SAM(4) and their associated clinical risk.

Several recent studies show higher mortality in children with non-edematous SAM.(9–11) In the 1960s, the four-surface electrode technique of bioelectrical impedance analysis (BIA) was introduced and several multi-frequency analyzers have since become available.(12) Over the past two decades, BIA has proven to be non-invasive, inexpensive, and easy to implement. This method of estimating body composition is now widely used for clinical assessment in high resource settings since it is non-invasive, portable and relatively inexpensive.(12–17) Body composition is not quantified by BIA directly but is calculated from body reactance (Xc) and resistance (R) which are measured by changes that occur in a small alternating electrical current when it passes through the body.(18,19) Reactance arises from cell membranes, and resistance from extra- and intracellular fluid and electrolytes, and their combination is termed ‘(body-)impedance’. (12) It can provide a reliable estimate of total body water (TBW) and fat free mass (FFM) in healthy individuals but requires population and disease-specific equations.(20) The phase angle (PA) is the arc tangent relation of Xc/R converted to degrees.(21) It describes the phase shift between the current and voltage that results from the electrochemical membrane.(19) It is considered to be an indicator of membrane integrity and body cell mass. In several diseases a reduced PA is associated with reduced survival.(14) BIA measurements can also be interpreted using bioelectrical impedance vector analysis (BIVA) as developed by Piccoli et al.(22,23) This method plots the raw impedance parameters R and Xc normalized by height as a bivariate vector in the ‘R-Xc graph’ (24) and differences in these R-Xc vectors can be useful (e.g. to monitor hydration status or muscle mass changes).(25)

In addition to estimating body composition or hydration status, components of BIA have been proposed to be useful prognostic markers and shown to correlate with clinical outcome in two high-resource studies in children with renal disease or undergoing hematopoietic stem cell transplantation.(26,27) Girma et al. recently published a cross-
sectional paper on BIA in malnourished children and showed that $X_c$ was associated with serum calcium and chloride while $R$ correlated with serum albumin. The authors suggested that $R$ could be used to monitor nutritional recovery.\(^{(16)}\) However, the study was cross-sectional with a small sample size. Our prospective study aimed to assess whether BIA parameters: 1) change during hospitalization in children with non-edematous or edematous complicated SAM and 2) whether they add a prognostic value to predict clinical outcome compared to using only classic anthropometry.

**MATERIALS AND METHODS**

**Study design and setting**

This prospective, observational study was conducted within the framework of the “F75 trial”, a multicenter, randomized, double-blind intervention trial (ClinicalTrials.gov: NCT02246296). Briefly, the study aimed to determine whether stabilization of malnourished children is improved by reducing carbohydrates and removing lactose in F75, the standard milk formula recommended by the WHO. The trial randomized children with SAM to either receive the standard F75 milk or a modified formulation which was iso-caloric but containing more triglycerides and less carbohydrates. In Malawi, the trial was conducted at the Nutrition Rehabilitation Unit (NRU) in the Pediatric Department of Queen Elizabeth Central Hospital in Blantyre and enrolled 320 patients between December 2014 and December 2015 (manuscript in preparation). Our BIA sub-study started recruiting patients later from February 2015 until the trial stopped. The study was approved by the Malawi College of Medicine Research and Ethics Committee (COMREC nr P.03/14/1540) and conducted according to guidelines of Good Clinical Practice which are based on the principles of the Declaration of Helsinki.\(^{(28)}\)

**Participants**

Children aged 6 – 96 months admitted with *complicated* SAM (i.e. those with signs of severe systemic illness and/or poor appetite)\(^{(4)}\) were screened for eligibility for the F75 trial. SAM was defined according to WHO standards (see above).\(^{(29)}\) Children were excluded if parental consent was not obtained or if the child had known allergies to milk products. For the BIA study, only children between 6-60 months were included and additional exclusion criteria were: 1) the presence of open skin lesions on hands or feet that would impede the positioning of BIA electrode stickers, 2) inability to stretch the limbs due to cerebral palsy (CP), and 3) significant body asymmetry such as amputations, unilateral hemiparesis, and neuromuscular conditions causing localized changes in perfusion or
tissue atrophy.(13) Also, for the BIA study, we enrolled children during weekdays and office hours only.

For comparison, healthy children (n=11) were later recruited for BIA analysis. These children were measured using the same equipment between January and June 2017 within the framework of another observational study conducted by The Childhood Acute Illness and Nutrition (‘CHAIN’) Network (ClinicalTrials.gov Identifier: NCT03208725). These healthy controls were recruited from the community to establish expected norms for demographic and biological factors.

**Inpatient care**

The standard clinical care for children with SAM consists of three distinct phases. In ‘phase 1’ or ‘stabilization phase’, F75, a low protein milk with reduced caloric energy (80-100 kcal/kg/day) is given. Once stabilized, a child is moved to the ‘transition phase’ and receives either ready-to-use therapeutic foods (RUTF) or a milk formula known as F100. Compared to F75, RUTF and F100 have higher energy density and protein content. A child is discharged when clinically stable and able to finish their RUTF feeds (‘rehabilitation phase’/’phase 2’).

BIA measurements were taken at hospital admission and after stabilization (i.e. on the first day of transition). All children admitted to the NRU had a thick blood film examined for parasitemia, hematocrit counts and were offered a rapid Human Immunodeficiency Virus (HIV) antibody test with appropriate pre- and post-counselling. These tests were also performed in the recruited community children.

**Data collection**

Weight was measured using a digital scale (Marsden Portable Digital Baby Scale - Class III MS-4101). The height of children under 24 months was measured in supine position using length boards, whereas stadiometers were used for those aged 24 months and older. Edema was scored based on the WHO grading system.(8)

BIA output variables (Xc, R and PA) were measured using a Bioelectrical Impedance Analyzer (Bodystat QuadScan4000) at 50 kHz. Self-adhesive disposable electrodes were attached at the right hand and foot just proximal to the fingers and toes, injecting leads were connected to both electrodes and the measuring leads to those on the right wrist and right ankle. Measurements were taken in triplicates but tests were repeated up to 5 times if the variance in R or Xc was above 5%.

**Statistical methods**

Data were collected on standardized proforma’s, entered into a database and analyzed with Stata (Release 13) (30) and R (Version 3.4.0). Differences in baseline characteristics of participants were assessed using Fisher exact test, or logistic regression. As children
with non-edematous and edematous SAM display different clinical and biochemical characteristics, we conducted sub-analyses. For BIA, we used logistic regression to analyze group differences at admission and logistic mixed effects models to evaluate changes between admission and transition phase while accounting for repeated measures within subjects. The sample size was based on a convenience sample.

To compare the prognostic value of using anthropometrics alone or anthropometrics complemented by BIA, we conducted Partial Least Squares discriminant analysis (PLS-DA) using the mixOmics package. Values were offset by 10, log transformed, mean centered and scaled. Multilevel PLS-DA was used to compare admission and nutritional stabilization to account for repeated measures. The discriminative power of the PLS-DA models to classify groups was assessed using the balanced error rate (BER) based on centroid distance obtained from leave-one-out cross-validation.

BIVA was performed as described by Piccoli to test for differences in BIA between groups of: 1) children with or without edema on admission; 2) between patients at admission and after clinical stabilization. The height-corrected values of R and Xc were plotted with ellipses indicating the 95% confidence area of the means as previously described. Differences in independent multivariate means were tested using Hotelling’s T2 test (e.g. between edema and non-edema or survival and mortality) whereas paired Hotelling’s T2 test was used to evaluate changes between patients at admission and after stabilization. Hotelling’s T-test is a multivariate extension of the Student-T test and p<0.05 where considered to be significant. Shifts in BIVA were interpreted as previously described and illustrated in Supplemental Figure 1.

Spearman’s correlation was used to relate log-transformed height-corrected BIVA parameters (i.e. R, Xc) and classic measures of anthropometry (i.e. W/H, W/A, MUAC).

RESULTS

Between December 2014 and December 2015, 183 patients were recruited for our BIA sub-study. Anthropometry and BIA measurements were successfully conducted on admission in 175 children (non-edematous n=81, edematous n=94), and in 155 patients after clinical stabilization (non-edematous n=73, edematous n=82); with 147 children (non-edematous n=68, edematous n=79) having both time points (Supplemental Figure 2. Flow chart). Overall mortality in SAM patients was 17% (total deaths, n=31; non-edematous, n=17; edematous, n=14). Of these children, some passed away before clinical stabilization (n=13) while others died after (n=18). Baseline characteristics are detailed in Table 1 and separated by nutritional status and clinical outcome in Supplemental Table 1. Children with edematous SAM were older (p=0.002), and less likely to be HIV positive (16% vs. 38% in the non-edematous group, p=<0.001).
We measured anthropometry and BIA in 11 children recruited from the community. These controls were younger than our patient group with SAM (14.6 ± 5.3 months versus 23.0 ± 12.0 months, p=0.01) but their anthropometric measures tended to be normal for their age; with their lowest measurement being H/A Z-score of -1.1 ± 1.6.

**Table 1 – Characteristics of community children and patients with severe acute malnutrition.**

<table>
<thead>
<tr>
<th></th>
<th>Control Non-edematous</th>
<th>SAM Edematous</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>8 (73)</td>
<td>99 (54)</td>
<td>.4</td>
</tr>
<tr>
<td>HIV reactive*, n (%)</td>
<td>0 (0)</td>
<td>47 (26)</td>
<td>.07</td>
</tr>
<tr>
<td>Age, mon</td>
<td>14.6 ± 5.3</td>
<td>23.0 ± 12.0</td>
<td>.01</td>
</tr>
<tr>
<td>Height-for-age, z-score</td>
<td>-1.1 ± 1.6</td>
<td>-3.4 ± 1.5</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Weight-for-age, z-score</td>
<td>-0.7 ± 1.1</td>
<td>-3.8 ± 1.6</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Weight-for-height, z-score</td>
<td>-0.2 ± 1.3</td>
<td>-3.0 ± 1.8</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>14.0 ± 1.1</td>
<td>11.4±1.7</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Time to stabilization, days</td>
<td>-</td>
<td>3.3 ± 2.0</td>
<td>-</td>
</tr>
<tr>
<td>Duration of admission, days**</td>
<td>-</td>
<td>5.6 ± 3.3</td>
<td>-</td>
</tr>
<tr>
<td>Time to death, days</td>
<td>- 6.5 ± 4.2</td>
<td>7.1 ± 5.1</td>
<td>-</td>
</tr>
<tr>
<td>Death, n (%)</td>
<td>- 31 (17)</td>
<td>17 (16)</td>
<td>-</td>
</tr>
</tbody>
</table>

Data in cell are mean +/- SD or n (%), *3 SAM patients with unknown HIV status; ** Duration of admission calculated in only patients that survived. Significance test performed with either Fisher exact or logistic regression as appropriate. Significance threshold, p < 0.5. MUAC, mid upper arm circumference; SAM, severe acute malnutrition.

**BIA in children with or without edematous SAM on admission and after clinical stabilization**

Bio-electrical impedance values are shown in Table 2 and BIVA plots of height adjusted R and Xc values are presented in Figure 1. SAM children without edema had higher resistance index than controls (1241 ± 250 ohms/m vs. 993 ± 188 ohms/m, p<0.001), whereas children with edema had lower PA (2.3 ± 1.3 vs. 3.2 ± 0.5 degree, p=.04) and lower indices of both resistance and reactance (respectively, 803 ± 272 vs. 993 ± 188, p=.03; 54 ± 9 ohms/m and 33 ± 20 ohms/m, p=.005). Compared to children without edema, those with edematous SAM had lower BIA values (PA, R and Xc indices) both on admission and after stabilization; BIVA vectors of edematous and non-edematous SAM patients differed between the two time points (p<0.001 and p<0.001 respectively, Figure 1. In children with edematous SAM, BIA values increased between admission and stabilization (PA 2.3 ± 1.3 degree vs. 2.9 ± 2.1 degree, p=.006; Resistance index 803 ± 272 ohm/m vs. 939 ±
297 ohm/m, \( p = 0.002 \); Reactance index \( 33 \pm 20 \) ohm/m vs. \( 48 \pm 35 \) ohm/m, \( p = 0.0007 \) and stabilization values of all BIA parameters for children with edematous SAM no longer differed from those of controls. Concordantly, the BIVA vector of children with edematous SAM showed a shift along the major hydration axis after stabilization (\( p = 0.001 \)), which likely reflects loss of edema in these patients (explained in Supplemental Figure 1). In contrast, the BIVA parameters of children with non-edematous SAM did not differ significantly between admission and stabilization, while their resistance index remained different from controls.

**Figure 1** BIVA vector analysis.
Bio-electrical vector analyses (BIVA) of healthy controls, SAM patients with and without edema on admission and after stabilization. CP, community participants \((n=11)\); nE, Non-edematous children with SAM on admission \((n=81)\); nE′, Non-edematous children after stabilization \((n=73)\); E, Edematous children on admission \((n=94)\); E′ Edematous children after stabilization \((n=82)\). Black dots represent the centroids and ellipses indicate the 95% confidence interval of the mean for each group as indicated by corresponding label. The mean shift between admission and stabilization of children with or without edema was tested using paired Hotelling’s T2 test, \( p < 0.05 \) was considered significant.
### Table 2 – Bio-electrical impedance values of community control children and patients with either edematous or non-edematous SAM on admission and stabilization

<table>
<thead>
<tr>
<th></th>
<th>Admission</th>
<th>Stabilization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (^a)</td>
<td>SAM (^b)</td>
</tr>
<tr>
<td>Resistance, Ohm</td>
<td>736 ± 93</td>
<td>775 ± 236</td>
</tr>
<tr>
<td>Reactance, Ohm</td>
<td>41 ± 6</td>
<td>35 ± 18</td>
</tr>
<tr>
<td>Phase angle, degree</td>
<td>3.2 ± 0.5</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td>Resistance index</td>
<td>993 ± 188</td>
<td>341 ± 9</td>
</tr>
<tr>
<td>Reactance index</td>
<td>54 ± 9</td>
<td>47 ± 26</td>
</tr>
</tbody>
</table>

Bio-electrical impedance values were measured at 50 kHz, resistance and reactance index = value/height in m. Data are presented as mean +/- SD and logistic regression was used to assess group differences and mixed effect logistic regression to compare admission and stabilization while accounting for repeated measures within subjects. Significance, p < 0.5.
Association of BIA and classic anthropometry with clinical outcomes

Overall, BIA values negatively correlated with anthropometric measures, but this relationship was mostly driven by children with edematous SAM. Correlations between bio-electrical impedance and anthropometry among children with SAM are shown on admission in Table 3 and after stabilization in Supplemental Table 2 and Supplemental Figure 3. In the edematous group, significant negative correlations were found between both resistance index, reactance index and all anthropometry both on admission and after stabilization. In the non-edematous group, the correlation between BIA and anthropometry was weak both on admission and after clinical stabilization.

We then used PLS-DA models to evaluate the relationships between variables and their collective capacity to distinguish groups of children with edematous SAM, non-edematous SAM, or community participants (Figure 2). The anthropometric variables of W/H, W/A and MUAC were highly correlated as indicated by their parallel directional arrows (Figure 2A). Using only anthropometric measures (W/H, W/A, H/A, MUAC), groups separated mainly along Variate 1, i.e., the first composite variable that summarizes 75% of the variance of the 4 anthropometric measures. Unsurprisingly, the PLS-DA ROC curves indicate that anthropometric measures have predictive value and can classify children into their respective nutritional groups (AUC values: Controls vs. others, 0.94; non-edema vs. others 0.83; edema vs. others, 0.74). However, combining anthropometric measures with BIA values only marginally improved classification of non-edematous and edematous children as evaluated by the distance between group centroids but did improve differentiation of community children from those with SAM (i.e. community children scored higher along composite Variate 2). Also, the Balanced Error Rate (BER) based on centroid distance was 0.27 for the PLS-DA model using only anthropometric variables, and was 0.25 when using both anthropometric measurements and BIA values. The specific class error rates based on centroid distance also did not significantly improve as they were 0.56 vs. 0.48 for edematous SAM, 0.27 vs. 0.26 for non-edematous SAM, and 0 vs. 0 for community patients. Thus, using more complex composite measures that include both anthropometry and BIA does not improve the group classification of children with SAM compared to using only anthropometry. Also, these error rates are likely underestimated as we used a leave one out cross-validation approach due to the small sample size of the sub analyses. Overall, bio-electrical impedance may help to differentiate children with edematous SAM from controls who may have similar anthropometric measures.

The BIVA values of children that died in the edematous group did significantly differ from those that survived (p=0.004) but this subgroup of children was small (n=14) and the difference was driven by 3 outliers which suggests that this relationship may be spurious (Supplemental Figure 4A shows BIVA on admission of children with edematous SAM who survived or died). BIVA on admission of SAM children that died before stabilization did not differ from those that died after stabilization (p=0.8); furthermore, BIVA did not
### Table 3 – Correlations between bio-electrical impedance indices and anthropometry at hospital admission of children with severe acute malnutrition

<table>
<thead>
<tr>
<th>Admission</th>
<th>SAM patients</th>
<th></th>
<th></th>
<th>Non-Edematous</th>
<th></th>
<th></th>
<th></th>
<th>Edematous</th>
<th></th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r (95% CI)</td>
<td>df</td>
<td>R²</td>
<td>df</td>
<td>R²</td>
<td>df</td>
<td>R²</td>
<td>df</td>
<td>R²</td>
<td>df</td>
<td>R²</td>
<td></td>
</tr>
<tr>
<td>Resistance index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Weight-for-height</td>
<td>-0.63 (-0.71, -0.53)</td>
<td>173 .4</td>
<td>&lt;.001</td>
<td>-0.18 (-0.39, 0.04)</td>
<td>79 .03</td>
<td>.1</td>
<td>-0.54 (-0.67, -0.38)</td>
<td>92 .3</td>
<td>&lt;.001</td>
<td>-0.58 (-0.87, 0.03)</td>
<td>9 .3</td>
<td>.06</td>
</tr>
<tr>
<td>Weight-for-age</td>
<td>-0.50 (-0.61, -0.38)</td>
<td>173 .3</td>
<td>&lt;.001</td>
<td>-0.04 (-0.25, 0.18)</td>
<td>79 .0</td>
<td>.7</td>
<td>-0.42 (-0.57, -0.24)</td>
<td>92 .2</td>
<td>&lt;.001</td>
<td>-0.49 (-0.84, 0.16)</td>
<td>9 .2</td>
<td>.1</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>-0.47 (-0.58, -0.35)</td>
<td>173 .2</td>
<td>&lt;.001</td>
<td>0.03 (-0.25, 0.19)</td>
<td>79 .0</td>
<td>.76</td>
<td>-0.39 (-0.55, -0.20)</td>
<td>92 .2</td>
<td>&lt;.001</td>
<td>-0.70 (-0.92, -0.17)</td>
<td>9 .5</td>
<td>.02</td>
</tr>
<tr>
<td>Reactance index</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Weight-for-height</td>
<td>-0.52 (-0.62, -0.40)</td>
<td>173 .3</td>
<td>&lt;.001</td>
<td>0.06 (-0.16, 0.27)</td>
<td>79 .0</td>
<td>.6</td>
<td>-0.39 (-0.55, -0.20)</td>
<td>92 .2</td>
<td>.001</td>
<td>0.01 (-0.59, 0.60)</td>
<td>9 .0</td>
<td>1</td>
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<tr>
<td>Weight-for-age</td>
<td>-0.45 (-0.56, -0.33)</td>
<td>173 .2</td>
<td>&lt;.001</td>
<td>0.07 (-0.15, 0.28)</td>
<td>79 .0</td>
<td>.5</td>
<td>-0.33 (-0.50, -0.14)</td>
<td>92 .1</td>
<td>.001</td>
<td>-0.32 (-0.77, 0.35)</td>
<td>9 .1</td>
<td>.3</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>-0.41 (-0.53, -0.28)</td>
<td>173 .2</td>
<td>&lt;.001</td>
<td>0.13 (-0.10, 0.34)</td>
<td>79 .0</td>
<td>.3</td>
<td>-0.29 (-0.46, -0.09)</td>
<td>92 .08</td>
<td>.005</td>
<td>0.05 (-0.56, 0.63)</td>
<td>9 .0</td>
<td>.9</td>
</tr>
<tr>
<td>Phase angle</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-for-height</td>
<td>-0.23 (-0.37, -0.09)</td>
<td>173 .05</td>
<td>.002</td>
<td>0.20 (-0.02, 0.40)</td>
<td>79 .04</td>
<td>.08</td>
<td>-0.12 (-0.31, 0.09)</td>
<td>92 0</td>
<td>.26</td>
<td>0.32 (-0.35, 0.77)</td>
<td>9 .1</td>
<td>.3</td>
</tr>
<tr>
<td>Weight-for-age</td>
<td>-0.25 (-0.38, -0.10)</td>
<td>173 .06</td>
<td>.001</td>
<td>0.1 (-0.12, 0.31)</td>
<td>79 .0</td>
<td>.4</td>
<td>-0.15 (-0.35, 0.05)</td>
<td>92 0</td>
<td>.1</td>
<td>-0.04 (-0.63, 0.57)</td>
<td>9 .0</td>
<td>.9</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>-0.21 (-0.35, -0.07)</td>
<td>173 .04</td>
<td>.005</td>
<td>0.15 (-0.07, 0.35)</td>
<td>79 .0</td>
<td>.2</td>
<td>-0.11 (-0.31, 0.09)</td>
<td>92 0</td>
<td>.3</td>
<td>-0.45 (-0.20, 0.83)</td>
<td>9 .2</td>
<td>.2</td>
</tr>
</tbody>
</table>

Bio-electrical impedance values were measured at 50 kHz, resistance and reactance index = value/height in m. Correlation (r) was assessed with Pearson product-moment correlation; for this resistance, reactance and phase values were log transformed. CI is 95% confidence interval; df is degrees of freedom (n-2); R² is the coefficient of determination. p-values less than .05 were considered significant.
Comparisons

- Component 1
- Component 2
- Variate 1 (75% explained variance)
- Variate 2 (19% explained variance)
- Component 1
- Component 2
- Variate 1 (62% explained variance)
- Variate 2 (20% explained variance)

A) B) C) D)

non-edema vs. others: 0.83
edema vs. others: 0.74
CP vs. others: 0.94
non-edema vs. others: 0.84
edema vs. others: 0.76
CP vs. others: 0.95
non-edema vs. others: 0.89
edema vs. others: 0.82
CP vs. others: 0.79

Figure 2 Partial Least Squares Discriminant Analysis and ROC curves.

Partial Least Squares Discriminant Analysis (PLS-DA models based on distance matrix as a function of nutritional group. A) Correlation plots of anthropometric variables, and C) of anthropometric variables together with bio-electrical impedance variables. B) Individual score plot based only on anthropometric values or on D) both anthropometric and bio-electrical impedance variables; colors indicate the nutritional group of each individual: non-edematous SAM (grey), edematous SAM (salmon), and community children (blue); group centroids are indicated by stars. H/A, height-for-age z-score; W/A, weight-for-age z-score; MUAC, mid upper arm circumference; W/H, weight-for-length z-score; R, resistance; Xc, reactance.

ROC curves are generated from leave-one-out cross-validation of predicted classes and associated average AUC value for the multivariate analysis on A) component 1 and B) component 2 of the PLS-DA models including only anthropometric measures; and on C) component 1 and D) component 2 of the PLS-DA model including both anthropometric and bio-electrical impedance variables.
differ between SAM children that survived until discharge versus of those that died after stabilization (p=0.3). We also ran PLS-DA models to assess whether admission BIA values can be combined to classical anthropometric measurements to help identify children with diarrhea or those at high risk of mortality. For diarrhea, the classification error rates were high, and AUC values was 0.55, i.e. very low and not better than random chance, suggesting that BIA would not be helpful in identifying children with or without diarrhea or grading its’ severity. For mortality, adding the BIA values to anthropometric measures did not improve classification of children with edematous SAM that died versus survived (BER, 0.31 with only anthropometry vs. 0.31 with both anthropometry and BIA); and this was also the case for children with non-edematous SAM that died or survived (BER, 0.31 with only anthropometry vs. 0.31 with both anthropometry and BIA). This suggests that BIA as performed here would not improve the identification of children at high risk of mortality beyond the information captured using classic anthropometry.

DISCUSSION

This study is the first to show how BIA parameters change together with ‘classic’ anthropometry throughout nutritional recovery of children with complicated SAM. As expected, BIA was correlated with measures of anthropometry, however BIA, as currently implemented, did not add significant clinical prognostic value in predicting the clinical outcome of children with complicated malnutrition. BIA and its association with anthropometric and biochemical markers has been recently described in children with malnutrition by Girma et al.(16) Our results corroborate their findings in a larger sample size but also show how BIA parameters change with nutritional and clinical rehabilitation in children with edematous or non-edematous SAM. Similarly, to Girma et al, we found lower BIA parameters (PA, R and Xc) in children with edematous SAM compared to those without edema.(20) Interestingly, control children had intermediate BIA parameters having higher values than children with edematous SAM but lower values than those with non-edematous SAM. This likely reflects differences in hydration status between the two phenotypes, but it is unclear if, upon full loss of edema, the BIA parameters of edematous children will stabilize to control levels or continue to increase and resemble those of SAM children without edema.

Overall, the resistance and reactance indices of children with SAM negatively correlated with anthropometric measures but this relationship was mainly driven by children with edematous SAM. In these children, MUAC and weight reflect not only lean and fat mass but also edema; whereas BIA parameters may better reflect the separate components of fluid, fat and lean body mass; thus, better able to distinguish SAM patients from controls when compared to using anthropometry alone.
In children with non-edematous SAM, anthropometric measures did not correlate strongly with BIA parameters; thus, it becomes unclear if BIA is still able to reflect body composition in severely wasted children that are ill.

We conducted different analyses to draw firm conclusions on whether BIA could have a role in the diagnosis and management of children with complicated SAM. First, we used Piccolis’ BIVA method(32) and found that vectors from children with edematous SAM at admission were shifted after stabilization along the major axis representing changes in tissue hydration (Supplemental Figure 1). This likely reflects the general fluid loss that occurs in children with edematous SAM following nutritional recovery. This shift in multivariate mean was significant, but the variability of BIA parameters was high which reduces the usefulness of BIA in estimating the clinical risk of individual children as evaluated using PLS-DA models.

We compared the classification performance of PLS-DA models that combined all anthropometric variables with or without additional BIA parameters. This showed that as expected anthropometry alone had predictive value to classify children into nutritional groups but that adding BIA only marginally improved model performance. Also, the improvements were mostly in separating controls from children with edematous SAM. Therefore, in theory, BIA could help identify children with nutritional edema that present with subclinical symptoms; however, the robustness of BIA for this task would require further evaluation.

Finally, in our study, BIA did not show clear prognostic value with regards to other clinical outcomes (i.e. diarrhea or mortality).

Although BIA has been in use for over 4 decades, the required population and disease-specific equations to estimate fat mass and lean body mass have not yet been developed (20). Currently, the available equations for young children are based on populations living in developed countries; and appropriate equations to estimate fat/muscle mass from BIA parameters are lacking for children with SAM. Also, the interpretation of BIA parameters and their changes differ between children with edematous or non-edematous SAM. Therefore, pediatric BIA data over a spectrum of nutritional status would be required to facilitate the clinical interpretation of BIA in children with SAM living in low resource settings.

Recently, an analytical variant of BIVA has been proposed: *specific* BIVA.(33) This method has been shown to be more accurate in estimating the relative proportion of fat mass in adults.(33,34) Based on the assumption of Ohm’s law that body impedance is affected by cross-sectional area, *specific* BIVA is thought to improve the normalization of BIA parameters for differences in body size by using not only height but also cross-sectional measures of the arm, waist, and calf. (34) This approach may be particularly relevant to use in children with SAM since both wasting (narrow limbs) and stunting (altered body fat distribution) are thought to contribute disproportionally to body impedance.(35,36)
The need to normalize BIA values by height likely adds significant variability to BIVA since in young children height is challenging to measure accurately. Using specific BIVA together with a robust approach to measuring pediatric height could improve the clinical prognostic value of BIA in children with SAM.

Strengths and limitations
This is the first large prospective longitudinal study reporting BIA changes in children hospitalized for complicated SAM. BIA has inherent limitations that become more problematic when being applied to young, ill, malnourished and stunted children. BIA is also influenced by movement of the child during measurements, feeding status, hydration status and/or urine volume in the bladder. These external factors can be, at least in part, mitigated by performing BIA at a particular time to standardize measurement conditions but this was very challenging to achieve in our low-resource setting. Also, our control participants were obtained from a study that recruited children of a younger age range, however, we found that including their data added value to the interpretation of the BIA values obtained from children with SAM. Finally, it would have be useful to follow changes in BIA parameters throughout the duration of hospitalization to assess the point of stabilization of BIA in children with edematous SAM. If the clinical use of BIA is to be pursued, height measures and robust body size correction methods are to be considered to better establish the specific range of BIA values for each patient groups.

Conclusion
In conclusion BIA parameters correlated with anthropometry but this mainly in children with edematous SAM. Also, BIA values differed significantly between children with edematous or non-edematous SAM. In those with edema, BIA values shifted towards control values after clinical stabilization and this may reflect receding edema. However, this process can be followed with physical examination, and BIA, as currently implemented, is likely too variable to reliably quantify fluid loss, therefore, clinical scoring continues to be more practical and likely just as informative. Based on our results, the current implementation of BIA would not justify its clinical use in the care of ill hospitalized children with SAM and does not add prognostic value beyond what is achieved by clinical examination together with classic anthropometry.

ACKNOWLEDGEMENTS
We would like to thank all study participants and their guardians for taking part in this study and the staff of the nutritional rehabilitation unit “Moyo” of the Queen Elizabeth Central Hospital in Blantyre, for their very hard work.
REFERENCES


Bio-electrical impedance analysis in children with severe acute malnutrition

32. Piccoli A, Pastori G. BIVA SOFTWARE. Padova, Italy: Department of Medical and Surgical Sciences, University of Padova; 2002.

**Supplemental Table 1**—Patient characteristics at admission of children with edematous or non-edematous SAM that survived or died

<table>
<thead>
<tr>
<th></th>
<th>SAM</th>
<th>Non-edematous</th>
<th>Edematous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SAM Survived</td>
<td>SAM Died</td>
</tr>
<tr>
<td></td>
<td>n=11</td>
<td>n=152</td>
<td>n=31</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>8 (73)</td>
<td>84 (55)</td>
<td>15 (48)</td>
</tr>
<tr>
<td>HIV reactive*, n (%)</td>
<td>0 (0)</td>
<td>36 (24)</td>
<td>11 (38)</td>
</tr>
<tr>
<td>Edema, yes</td>
<td>-</td>
<td>83 (55)</td>
<td>14 (45)</td>
</tr>
<tr>
<td>Age, mon</td>
<td>14.6 ± 5.3</td>
<td>23.3 ± 11.7</td>
<td>21.2 ± 13.4</td>
</tr>
<tr>
<td>Height-for-age, z-score</td>
<td>-1.1 ± 1.6</td>
<td>-3.4 ± 1.5</td>
<td>-3.4 ± 1.7</td>
</tr>
<tr>
<td>Weight-for-age, z-score</td>
<td>-0.7 ± 1.1</td>
<td>-3.7 ± 1.6</td>
<td>-4.5 ± 1.6</td>
</tr>
<tr>
<td>Weight-for-height, z-score</td>
<td>-0.2 ± 1.3</td>
<td>-2.7 ± 1.8</td>
<td>-4.1 ± 1.8</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>14.0 ± 1.1</td>
<td>11.6 ± 1.6</td>
<td>10.3 ± 1.8</td>
</tr>
<tr>
<td>Time to stabilization, days</td>
<td>-</td>
<td>3.2 ± 2.0</td>
<td>4.6 ± 2.3</td>
</tr>
<tr>
<td>Duration of admission, days</td>
<td>-</td>
<td>5.6 ± 3.3</td>
<td>-</td>
</tr>
<tr>
<td>Time to death, days</td>
<td>-</td>
<td>-</td>
<td>6.5 ± 4.2</td>
</tr>
</tbody>
</table>

Data in cell are mean +/- SD or n (%). *3 SAM patients with unknown HIV status; missing HIV status died, n=2. Significance test performed with either Fisher exact or logistic regression as appropriate. Significance threshold, p < 0.5. MUAC, mid upper arm circumference; SAM, severe acute malnutrition.
### Supplemental Table 2 – Correlations between bio-electrical impedance indices and anthropometry in children with severe acute malnutrition after stabilization

<table>
<thead>
<tr>
<th>After stabilization</th>
<th>SAM patients</th>
<th>Non-Edematous</th>
<th>Edematous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r (95% CI)</td>
<td>df</td>
<td>$R^2$</td>
</tr>
<tr>
<td><strong>Resistance index</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-for-height</td>
<td>-0.48 (-0.59, -0.34)</td>
<td>150</td>
<td>.2</td>
</tr>
<tr>
<td>Weight-for-age</td>
<td>-0.42 (-0.54, -0.28)</td>
<td>150</td>
<td>.2</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>-0.33 (-0.46, -0.18)</td>
<td>149</td>
<td>.1</td>
</tr>
<tr>
<td><strong>Reactance index</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-for-height</td>
<td>-0.33 (-0.47,-0.18)</td>
<td>150</td>
<td>.1</td>
</tr>
<tr>
<td>Weight-for-age</td>
<td>-0.34 (-0.47,-0.19)</td>
<td>150</td>
<td>.1</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>-0.27 (-0.41,-0.11)</td>
<td>149</td>
<td>.07</td>
</tr>
<tr>
<td><strong>Phase angle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-for-height</td>
<td>-0.12 (-0.27, 0.04)</td>
<td>150</td>
<td>.01</td>
</tr>
<tr>
<td>Weight-for-age</td>
<td>-0.16 (-0.31, 0.0)</td>
<td>150</td>
<td>.03</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>-0.16 (-0.31, 0.0)</td>
<td>149</td>
<td>.03</td>
</tr>
</tbody>
</table>

Bio-electrical impedance values were measured at 50 kHz, resistance and reactance index = value/height in m. Correlation ($r$) was assessed with Pearson product-moment correlation; for this resistance, reactance and phase values were log transformed. CI is 95% confidence interval; df is degrees of freedom ($n$-2); $R^2$ is the coefficient of determination. $p$-values less than .05 were considered significant.
Supplemental Figure 1 Interpretation of individual vector position on the RXc graph.

Vector BIA with the RXc graph method, allows an evaluation of soft tissues through patterns based on percentiles of their electrical properties without prior knowledge of body weight. From clinical validation studies in adults, vectors falling out of the 75% tolerance ellipse indicate an abnormal tissue impedance, which is interpreted and ranked following the two directions of major and minor axis of tolerance ellipses:

1. Vector displacements parallel to the major axis of tolerance ellipses indicate progressive changes in tissue hydration (dehydration with long vectors, out of the upper pole, and hyperhydration with apparent edema with short vectors, out of the lower pole);
2. Vectors falling (steady state) or migrating (dynamic state) parallel to the minor axis, above (left) or below (right) the major axis of tolerance ellipses indicate more or less cell mass, respectively, contained in soft tissues (i.e. vectors with a comparable R value and a higher or lower Xc value, respectively).
3. Different trajectories indicate combined changes in both hydration and tissue mass.

Piccoli A, Pastori G: BIVA software. Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, 2002 (available at E-mail: apiccoli@unipd.it)
Chapter 6

Recruited into F75 trial
n=320

Excluded, n=137
Out of office hours
Unable to stretch limbs
Open skin lesions
Too sick
Withdrew from F75 study
Both Limbs taped
Died before Test 1

Enrolled for BIA Study
SAM, n=183
Community Participants, n=11

Controls
n=11

Test 1 - BIA on admission
n=175

Died before stabilization, n=13
BIA after stabilization failed, n=15

n=8*

Test 2 - BIA after stabilization
n=155

**Supplemental Figure 2** Flowchart

**Supplemental Figure 4** BIVA of children with different grades of edema who died and survived.
A: BIVA on admission of children with edematous SAM who survived (n=83) or died (n=14). Black dots represent the centroids and ellipses indicate the 95% confidence interval of the mean for each group as indicated by corresponding label. B: BIVA on admission of children with edematous SAM, separated by degree of edema. 1 = +, 2 = ++, 3 = +++.
**Bio-electrical impedance analysis in children with severe acute malnutrition**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Edematous SAM, survived</th>
<th>Edematous SAM, died</th>
<th>Non-edematous SAM, survived</th>
<th>Non-edematous SAM, died</th>
</tr>
</thead>
</table>

**Supplemental Figure 3** Individual correlations between bio-electrical impedance and anthropometry on admission and after stabilization.

<table>
<thead>
<tr>
<th></th>
<th>Admission</th>
<th>After-stabilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><img src="#" alt="Graph A" /></td>
<td><img src="#" alt="Graph B" /></td>
</tr>
</tbody>
</table>
|   | $\rho = -0.58$ (95%CI: -0.47, -0.67)  
$R^2 = 0.3; p<0.0001$ | $\rho = -0.45$ (95%CI: -0.31, -0.56)  
$R^2 = 0.2; p<0.0001$ |
| B | ![Graph C](#) | ![Graph D](#) |
|   | $\rho = -0.43$ (95%CI: -0.31, -0.54)  
$R^2 = 0.19; p<0.0001$ | $\rho = -0.28$ (95%CI: -0.13, -0.41)  
$R^2 = 0.08; p<0.0001$ |
| C | ![Graph E](#) | ![Graph F](#) |
|   | $\rho = -0.15$ (95%CI: -0.001, -0.28)  
$R^2 = 0.02; p<0.05$ | $\rho = -0.06$ (95%CI: 0.09, -0.21)  
$R^2 = 0.004; p<0.4$ |