Abstract

Objective: To observe the behavior of the plotted vectors on the RXc (R = resistance – and Xc – reactance corrected for body height/length) graph through bioelectrical impedance analysis (BIVA) and phase angle (PA) values in stable premature infants, considering the hypothesis that preterm infants present vector behavior on BIVA suggestive of less total body water and soft tissues, compared to reference data for term infants.

Methods: Cross-sectional study, including preterm neonates of both genders, in-patients admitted to an intermediate care unit at a tertiary care hospital. Data on delivery, diet and bioelectrical impedance (800 mA, 50 kHz) were collected. The graphs and vector analysis were performed with the BIVA software.

Results: A total of 108 preterm infants were studied, separated according to age (< 7 days and ≥ 7 days). Most of the premature babies were without the normal range (above the 95% tolerance intervals) existing in literature for term newborn infants and there was a tendency to dispersion of the points in the upper right quadrant, RXc plan. The PA was 4.92° (±2.18) for newborns < 7 days and 4.34° (±2.37) for newborns ≥ 7 days.

Conclusion: Premature infants behave similarly in terms of BIVA and most of them have less absolute body water, presenting less fat free mass and fat mass in absolute values, compared to term newborn infants.

Introduction

Bioelectrical impedance analysis (BIA) is a method used for body composition analysis based on the measurement of electrical ionic conduction of soft tissue (body fluids), assuming that fatty and bone tissues are poor electric conductors. BIA presents two bioelectrical parameters: body resistance (R) and reactance (Xc), which, alone, do not allow to directly assess total body water (TBW) and extracellular water (ECW). In such cases, conventional BIA equations are used, which presuppose constant density and hydration of fat-free mass (FFM) and are applicable only in specific populations for which they were developed. Changes in newborn (NB) body weight are difficult to interpret, because they may represent abnormalities both in adipose tissue or in its hydration. Thus, one cannot consider the constant hydration of FFM, and the conventional method of BIA is deemed inappropriate. As in the first 28 days, body water turnover in infants is very large, they become susceptible to hydro-electrolytic...
imbalance. In this sense, knowing the detailed body composition during the late postnatal period may be important for nutritional care and treatment.⁶,⁷

Considering the need to overcome the limits imposed by conventional BIA and to use the gold standards for the development of predictive equations, one can make use of bioelectrical impedance vector analysis. The graphic method of resistance and reactance corrected for height (R/Xc) is based on the analysis of the bivariate distribution of the impedance vector in a healthy population with specific characteristics, with the use of impedance measures standardized by height (H), which are plotted as bivariate vectors.⁸ It is possible to make three types of BIVA assessment: a single vector measured in an individual for the first time; the follow-up of a single patient (repeated/serial measures); and of subject groups. In BIVA, R and Xc, corrected for H, are plotted as vectors on the R/Xc² plan.

BIVA allows evaluating the patient by direct measurements of impedance vector and is independent of equations or models, being affected only by errors in impedance measurements and by biological variables of individuals.⁹

Phase angle (PA) is important in the assessment of severity and prognosis, once it reflects different electrical properties of tissues that are affected by diseases, nutritional status, and hydration.⁹ It is derived mathematically from an arc tangent ratio between Xc and R (Xc/R)².¹⁰ PA reflects changes in electric conductivity of the body, indicating changes in cell membrane integrity as well as in intercellular space.¹⁰ Studies suggest that the PA values, obtained by BIA, are related to the prognosis (morbidity and mortality) of the patient.¹¹ The evaluation of PA may be superior to other nutritional, anthropometric and serum indicators.¹²

We hypothesize that preterm NBs present vector behavior in BIVA suggestive of less water and soft tissues compared to the reference for term NBs. Therefore, the aim of this study was to observe the behavior of the vectors of preterm NBs plotted on the R/Xc (R/H and Xc/H) graphs related to the neonatal period⁴,¹³ and the values of PA.

**Methods**

It is a cross-sectional study performed in the intermediate care unit of the Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (HCFMRP-USP), between February and October 2009.

The study included 108 clinically stable preterm NBs of both sexes. The study excluded: term and post-term NBs, infants with some disease that could interfere, directly or indirectly, in the body composition by changes in intra or extracellular volume and NBs with congenital anomalies and/or genetic problems that would prevent the proper assessment of weight, length and BIA.

Data such as sex, gestational age at birth (GA), weight and length (at birth and present), BIA (R and Xc) and diet (breast milk or infant formula) were collected. The GA adequacy of the neonates at birth was evaluated through the curve of Alexander et al.¹⁴ Data regarding the diet of the NBs were obtained from medical records, and were classified according to the breastfeeding recommendations of the World Health Organization.¹⁵

Anthropometric measurements were obtained parallel to BIA, following the recommendation for the clinical application of Kyle et al.,⁹ with the limitations for this age group. We used the RJL System® Model Quantum II (800 mA e 50 kHz) for the measurement of BIA. Adhesive electrodes were placed at previously standardized points on the hand and foot of the newborn in supine position. BIA data were acquired when they remained quiet, preferably in quiet sleep, avoiding contact between the upper limbs and trunk, and between the legs.

R and Xc values were measured in triplicate in all NBs and the mean value of these measures was calculated. The apparatus was calibrated according to instructions of the manufacturers (after 20 assessments) through a 500Ω resistor provided by the manufacturer.

To interpret the results, BIVA was used, a method based on the analysis of the bivariate impedance vector distribution in a healthy population, which uses the plot of the direct measurement of the R and Xc vector components of the NB. The impedance measurements standardized by the length of preterm newborns (expressed both R/H and Xc/H in Ω/m) are plotted as bivariate vectors with its tolerance intervals. Two reference populations were used (term NBs) which are ellipses in the R/Xc plan.⁸ The preterm NBs can be routinely monitored in relation to the variation of body fluid and nutritional status, without making any assumptions about body composition values.

The BIVA Software 2002¹⁶ was used to plot the data of the NBs on the tolerance ellipses of the RXc plan and for the comparisons among groups of NBs through confidence intervals. Two reference populations were used (term NBs) to plot the data on the R/Xc plan: healthy NBs younger than 7 days (< 7d)⁴ and NBs older than or equal to 7 days of life (≥ 7d).¹³

PA was obtained through the arc tangent ratio Xc/R. To transform the result from radians to degrees (°), the result obtained was multiplied by 180°/π.¹⁷

The variables studied were presented as mean and standard deviation. The r (Pearson) linear correlation coefficient between the R/H and Xc/H variables was calculated. The vectors were analyzed by the T-Square Hotelling tests and univariate analysis (F test). Tolerance interval of 95% and significance level of 5% were used for all the analysis.
The study was conducted in accordance with Resolution 196/96 of the National Health Council, and all procedures involving humans/patients were approved by the Ethics Committee in Research with Humans within the HCFMRP-USP. All mothers or guardians of the NBs signed written consent to participate in the study.

Results

The study included 108 preterm NBs, grouped according to age at the time of the study, in order to further comparative analysis with populations described in literature.

The general characteristics of NBs at birth, such as anthropometric data, GA and GA adequacy; as well as data concerning their diet at the time of the study, are presented in Table 1. There are differences in data on weight and length because the groups present different clinical situations and gestational lengths. Data on diet show that there is prevalence of exclusive breastfeeding in the group < 7d, while in the ≥ 7d group continued breastfeeding predominates.

Table 2 presents data obtained at the time of BIA measurement, such as weight, length, Body Mass Index (BMI), R, Xc, R/H and Xc/H and PA, in the present study (< 7d and ≥ 7d) compared to the studies of Piccoli et al. and of Margutti et al. At the moment of BIA evaluation, the NBs < 7d presented mean age of 2.56 ± 1.35 days; mean weight 2,228.32 ± 464.68 grams; and mean length 0.45 ± 0.024 meters. The NBs ≥ 7d presented mean of 14.70 ± 7.22 days, 1,775.32 ± 370.35 grams and 0.42 ± 0.028 meters, respectively for age, weight and length. Mean values of R and Xc corrected for length of preterm NBs < 7d or ≥ 7d are apparently higher in the present study, compared to the studies of Piccoli et al. and of Margutti et al.

Data on PA in NBs < 7d are similar to those found by Piccoli et al. Although one cannot say the same in relation to the values of Margutti et al., there is a great variability in the values of premature infants ≥ 7d (Table 2).

Figure 1 illustrates the distribution of the NBs studied on the RXc plan, separated by age from both sexes. The point result of z score of R and Xc, for all NBs studied, regardless of age, pointed to a scattering vector in the upper right quadrant (URQ), consistent with state of lower absolute amount of body water and soft tissues, when compared to the reference populations.

Figure 2 shows the graphical comparison between the vectors of the groups of preterm newborns < 7d and ≥ 7d with their respective literature references for confidence intervals, showing statistically significant difference (p < 0.05).

Discussion

The present study included clinically stable preterm NBs of both genders. BIVA was used in order to verify the state of hydration and nutritional status, without making any assumptions about body composition values.

Mean values of R/H and Xc/H (Ω/m) were higher, in absolute terms, when compared to the studies of Piccoli et al. and of Margutti et al.

Studies of body composition in NBs led by Pludowsky et al. and Ahmad et al. demonstrated that preterm NBs

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**Table 1** - General characteristics of newborns (younger than 7 days and older or equal to 7 days of life) at birth and during the study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Younger than 7 days</th>
<th>Older or equal to 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborns (n)</td>
<td>68</td>
<td>40</td>
</tr>
<tr>
<td>At birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>44.8 ± 2.4</td>
<td>41.6 ± 2.9</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2,340.0 ± 530.7</td>
<td>1,709.0 ± 383.7</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>35.0 ± 1.6</td>
<td>32.2 ± 2.0</td>
</tr>
<tr>
<td>AGA, n (%)</td>
<td>59 (86.7%)</td>
<td>37 (92.5%)</td>
</tr>
<tr>
<td>SGA, n (%)</td>
<td>7 (10.3%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>BGA, n (%)</td>
<td>2 (2.9%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Moment of the study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusive breastfeeding, n (%)</td>
<td>35 (51.5%)</td>
<td>8 (20.0%)</td>
</tr>
<tr>
<td>Predominant breastfeeding, n (%)</td>
<td>9 (13.2%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Continued breastfeeding, n (%)</td>
<td>22 (32.2%)</td>
<td>28 (70.0%)</td>
</tr>
<tr>
<td>Artificial feeding, n (%)</td>
<td>2 (2.9%)</td>
<td>4 (10.0%)</td>
</tr>
</tbody>
</table>

AGA = appropriate for gestational age; BGA = big for gestational age; GA = gestational age; SGA = small for gestational age.
Table 2 - Characteristics of infants younger than 7 days in the present study and comparison with data obtained in the literature for the same age group (Piccoli et al.4) and infants older or equal to 7 days in the present study and comparison to the data obtained in literature for the same age group (Margutti et al.13); data are expressed as mean ± standard deviation

<table>
<thead>
<tr>
<th>Newborns younger than 7 days</th>
<th>Newborns older than or equal to 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present study</td>
</tr>
<tr>
<td>Neonates (n)</td>
<td>68</td>
</tr>
<tr>
<td>Age (days)</td>
<td>2.56 ± 1.35</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>44.90 ± 2.43</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2,228.32 ± 464.68</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>10.96 ± 1.49</td>
</tr>
<tr>
<td>R (Ω)</td>
<td>794.67 ± 124.34</td>
</tr>
<tr>
<td>R/H (Ω/m)</td>
<td>1,780.67 ± 324.75</td>
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<tr>
<td>Xc (Ω)</td>
<td>67.92 ± 31.93</td>
</tr>
<tr>
<td>Xc/H (Ω/m)</td>
<td>151.54 ± 70.54</td>
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<tr>
<td>Phase Angle</td>
<td>4.92 ± 2.18</td>
</tr>
<tr>
<td>r</td>
<td>0.31</td>
</tr>
</tbody>
</table>

BMI = body mass index; r = coefficient of linear correction between R/H and Xc/H; R = resistance; R/H = resistance/height; Xc = reactance; Xc/H = reactance/height.

presented an absolute amount of FFM and fat mass (FM) significantly lower than term NBs. However, it is necessary to be careful while interpreting this statement, since a study by Camelo Jr. et al. (unpublished data), conducted in this service, assessed body composition by the method of stable isotope dilution, using deuterium, and concluded that term infants with intrauterine growth restriction (IUGR), have a lower percentage of fat and higher percentage of lean body mass when compared to children who are adequate for gestational age (AGAs) at the expense of larger amounts of TBW. This is explained by the fact that the infants who were small for gestational age (SGAs) presented higher percentage of FFM. However, similar percentage of dry lean mass, i.e., the highest percentage of FFM, is due to a higher amount of water. Thus, although IUGR and prematurity are distinct conditions, it is believed that the same is true when comparing preterm with term NBs.

It is expected that preterm NBs present higher relative amount of water when compared to term NBs, due to an increased hydration of the FFM. However, in absolute terms, the amount of TBW in preterm NBs is lower compared to term NBs, as demonstrated in the present study, because of the smaller amount of total body mass. Our data demonstrated higher values on the RXc plan, which corresponds to the URQ, equivalent to a lower amount of soft tissues and water.

Despite the NBs presented the same chronological age of term NBs, the vector behavior is significantly different, suggesting lower absolute amount of TBW and FFM, although from the percentage, relative point of view, the opposite is expected.

Comparing the < 7d group of the present study with the population of Piccoli et al.4 it is observed that the PA found is similar. However, this similarity is not observed between the ≥ 7d group and the study of Margutti et al.,13 and this discrepancy in values may be partially explained by the large variability in this group and for the low correlation between R/H and Xc/H. Apparently, preterm NBs have worse prognosis when compared to term NBs during the most severe phase of diseases. Thus, one should expect that the PA value found in preterm infants would be lower than the term NBs. However, values of this study were apparently higher for ≥ 7d, which may indicate a phase of improvement and clinical stability.

Nagano et al.,20 in a study in Japan, demonstrated the usefulness of PA in the nutritional assessment of infants. However, we cannot discard limitations of PA in the evaluation of severity and prognosis in preterm infants. Clinical studies correlate low PA with morbidity and mortality in severe patients,12 but studies about PA in NBs are rare in the literature.

By analyzing the results, it was possible to conclude that preterm NBs behaved similarly to each other and most of them had a lower absolute amount of TBW and presented lower quantities of FFM and FM, when compared to the term NBs. We speculate that the serial evaluation until the corrected for the term post-conceptional age,
may demonstrate the normalization of the vectors; this should be analyzed in future studies.

It is important to highlight that the majority of preterm NBs is above the tolerance intervals of 95% existing in the literature for term NBs.

Given the above, specific curves can be created for the population of preterm NBs, since they behave differently from term NBs. Notwithstanding, it should also be stressed that prematurity is a situation of abnormality.

As a limitation of the study, it should be emphasized that the infants studied presented a great difference in GA at birth when compared to the term infants from the reference studies (Piccoli et al.4 and Margutti et al.13). Thus, it is probably correct to say that there is a great difference in the amount of water between the infants in the reference studies and the infants in this study.

Acknowledgements
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