Segmental bioelectrical impedance analysis (SBIA) and blood rheology: Reducing the gap between in vivo and in vitro?

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Abstract. Bioelectrical impedancemetry (BIA) has been used to evaluate hematocrit and red cell aggregability *in vitro* but whole body impedance measurements are also correlated to some hemorheologic factors, suggesting a relationship between viscosity factors and electric properties of blood. We repeatedly reported correlations with whole body BIA and hematocrit, whole blood viscosity and plasma viscosity, red cell rigidity and RBC aggregation. The SBIA Inbody 770 modelizes body as 5 cylinders and measures impedance at 1, 5, 50, 250, 500, and 1000 kHz. With the SBIA we found that hematocrit is best correlated to leg reactance at 50 kHz but also to leg impedance at 1 and 5 kHz and trunk reactance. RBC aggregation "M" is best correlated to arm reactance at 5 kHz but also to most measurements of segmental impedance (28 correlations found). RBC aggregation "M1" is best correlated to arm reactance at 5 kHz and to 19 other impedance measurements. A predictive equation for "M" from the mean between the two arm reactances at 5 kHz (maXc5) is found: M = 2.1845maXc5-23.958 ($r = 0.665$, $p < 0.001$) that provides a satisfactory Bland-Altman plot (mean difference: 0.000524 range [–1.6;+1.6]. This study suggests that previously reported correlations between BIA and viscosity factors were not spurious, and that in a narrow cylinder such as the arm the structure of circulating blood (hematocrit, red cell aggregation) may influence the passage of an electric current by increasing reactance.

Keywords: Segmental bioelectrical impedance, hematocrit, myrenne aggregometer, red blood cell aggregation, electrical conductance, light transmission

List of symbols

BIA	Bioelectrical impedancemetry
RBC	red blood cell
SEM	standard error on the mean

1. Introduction

Bioelectrical impedancemetry (BIA) has been investigated with the aim of proposing new *in vitro* techniques for evaluating hematocrit and red cell aggregability *in vitro* [1]. The fair correlations found

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Table 1
Clinical characteristics of the 10 subjects of the study (mean + SEM)

Age (years)	22.00 ± 1.88
Weight (kg)	75.23 ± 10.91
Height (cm)	181.00 ± 5.35
Body mass index (kg/m²)	22.81 ± 2.74
Hematocrit (%)	45.79 ± 3.30

in vitro between BIA and these hemorheologic parameters lead to think that they may influence the electrical properties of the whole body. Accordingly, we repeatedly reported correlations between electrical properties of the whole body (impedance, resistance, reactance) and some hemorheologic factors (hematocrit, whole blood viscosity and plasma viscosity, red cell rigidity and RBC aggregation). However these correlations were difficult to interpret [4, 20] and it was likely that the complex shape of the human body was the mean reason for this. More recently segmental bioimpedance has become largely available and provides a more precise prediction of body composition [3, 5]. The robustness of this approach is largely due to the fact that trunk, arms and legs are separately analyzed as cylinders whose impedance reflect their own biophysical properties.

The SBIA Inbody 770 is one of the most recent devices of segmental BIA and it modelizes body as 5 cylinders, measuring their impedance at 1, 5, 50, 250, 500, and 1000 kHz. From these measurements it provides a set of values of reactance and impedance at various frequencies for the 5 segments of body. We investigated whether such parameters were correlated to hematocrit and red cell aggregation during a protocol where they were measured before and after injection of a single dose of the erythropoietin analogue methoxy polyethylene glycol-epoetin-β (Mircera®).

2. Subjects and methods

2.1. Study subjects

Characteristics of subjects are shown in Table 1. They were included in a study aiming at investigating whether a single injection of the long acting erythropoietin analogue methoxy polyethylene glycol-epoetin-β (Mircera) can be detected by various analytical procedures including metabolomics and proteomics. In this study we also measured red cell aggregability and the results of this aggregability study are shown in a separate paper. In the current paper we only focus on the relationships between impedance data and hemorheologic measurements.

2.2. Bioelectrical impedance measurements

Bioimpedance analysis as performed with the SBIA Inbody 770 impedancemeter kindly provided for this study by InBody FRANCE, 515 Rue Alfred Nobel, 34000 Montpellier (France). This device measures total resistance of the body to an alternative electric current of very low intensity at the following frequencies: 1, 5, 50, 250, 500,1000 kHz.

DSM-BIA was performed using the In-Body (770) body composition analyzer. This equipment has previously been shown to have high test-pretest reliability and accuracy [5]. Unlike conventional BIA equipment which often takes only partial measurements and therefore relies upon formulas to estimate whole body composition, the DSM-BIA technique employs the assumption that the human body is composed of 5 interconnecting cylinders and takes direct impedance measurements from the

various body compartments. A tetrapolar eight-point tactile electrode system is used, which separately measures impedance of the subject's trunk, arms, and legs at six different frequencies (1 kHz, 5 kHz, 50 kHz, 250 kHz, 500 kHz, 1000 kHz) for each of the body segment. The In-Body (770) body composition analyzer has in-built hands and feet electrodes. Subjects wore normal indoor clothing and advised to stand barefooted in upright position with their feet on the feet electrodes on the machine platform and their arms abducted with hands gripping on to the hands electrodes on the handles. Subjects were not require to fast for the test [8, 10, 11, 19] The results that interested us in this study were crude values of electric parameters, *i.e.* impedance, resistance and reactance in all segments analyzed by the BIA.

2.3. Hemorheological in vitro measurements

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). RBC aggregation was assessed with the Myrenne aggregometer [17] which gives two indices of RBC aggregation: "M" (aggregation during stasis after shearing at $600\,s^{-1}$) and "M1" (facilitated aggregation at low shear rate after shearing at $600\,s^{-1}$). Hematocrit was measured together with other hematological markers with the Coulter analysis.

2.4. Statistics

Results are presented as mean $\pm$ the SEM. A value of $p<0.05$ was considered as significant. Comparisons were made with nonparametric tests. Correlations were tested by least square fitting for linear, exponential, logarithmic and power relationships.

3. Results

The main results of this experiment are presented in a separate paper. After injection of Mircera® a decrease in mean RBC corpuscular volume at day 2 ($p<0.01$) and day 10 ($p<0.02$), a rise in reticulocyte count ($p<0.001$) between day 4 and day 17 and a decrease in ferritin a day 5 ($p<0.05$) were evidenced. Hemoglobin decreased at day 4 ($p<0.005$). Hematocrit was unchanged. There was a dramatic (+67%) increase in RBC aggregation index "M" (from 9.49 ± 1.01 to 17.66 ± 1.8 $p<0.01$). A decrease in systolic blood pressure was also observed during the period from day 4 to day 17 (at day 10: -11.90 ± 2.28 mmHg $p<0.001$; at day 17: -15.80 ± 2.83 $p<0.001$. There was also a decrease in diastolic blood pressure, mean and pulse pressure. All these results are commented in a separate study.

Table 2 shows all the significant correlations that were found between the hemorheological parameters studied here (*i.e.*, RBC aggregation and hematocrit) and impedance measurements.

As shown on Fig. 1 hematocrit is best correlated to leg reactance at 50 kHz (left leg $r=0.410$ $p<0.001$). It is also correlated to right leg reactance ($r=0.353$ $p<0.01$), to leg impedance at 1 and 5 kHz, and to trunk reactance (see Table 2).

RBC aggregation "M" is best correlated to arm reactance at 5 kHz (right arm $r=0.659$ $p<0.001$ (Fig. 2); left arm $r=0.617$ $p<0.001$) but also to most measurements of segmental impedance (28 correlations found). RBC aggregation "M1" is best correlated to arm reactance at 5 kHz (right arm $r=0.549$ $p<0.001$; left arm $r=0.511$ $p<0.001$) and to 19 other impedance measurements. Finally a predictive equation for "M" from the mean between the two arm reactances at 5 kHz (maXc5) is found: $M = 2.1845 maXc5 - 23.958$ ($r=0.665$ $p<0.001$) that provides a satisfactory Bland-Altman plot (mean difference: 0.000524 range [–1.6; +1.6] (Fig. 3).

Table 2
Coefficients of correlation between measurements of segmental BIA and the three hemorheolgic parameters measured in the study.

Main predictors of hematocrit	Main predictors of RBC aggregation "M"	Main predictors of RBC aggregation "M1"
50 kHz-LL Xc	5 kHz-RA Xc	5 kHz-RA Xc
$r = 0.410$	$r = 0.660$	$r = 0.549$
50 kHz-LA Xc	5 kHz-LA Xc	5 kHz-LA
$r = 0.353$	$r = 0.617$	$r = 0.511$
5 kHz-LL Xc	5 kHz-RL Z	1 kHz-RL Z
$r = 0.353$	$r = 0.462$	$r = 0.453$
50 kHz-RA Xc	1 kHz-RL Z	5 kHz-RL Z
$r = 0.344$	$r = 0.462$	$r = 0.450$
	50 kHz-RA Xc	50 kHz-RL Xc Z
	$r = 0.454$	$r = 0.439$
	50 kHz-RL Xc	50 kHz-RA Xc
	$r = 0.448$	$r = 0.420$
	1 Mhz-RL Z	50 kHz-RL Z
	$r = 0.446$	$r = 0.418$
	250 kHz-LL Xc	1 Mhz-RL Z
	$r = 0.440$	$r = 0.416$
	50 kHz-RL Z	500 kHz-RL Z
	$r = 0.432$	$r = 0.403$
	500 kHz-RL Z	250 kHz-RL Z
	$r = 0.426$	$r = 0.394$, $p < 0.01$
	50 kHz-LL Xc	50 kHz-LA Xc
	$r = 0.416$	$r = 0.369$, $p < 0.01$
	250 kHz-RL Z	50 kHz-LL Xc
	$r = 0.413$	$r = 0.354$, $p < 0.01$
	1 kHz-LL Z	5 kHz-RL Xc
	$r = 0.410$	$r = 0.335$, $p < 0.02$
	5 kHz-LL Z	1 kHz-LL Z
	$r = 0.405$	$r = 0.331$, $p < 0.02$
	50 kHz-LL Z	5 kHz-LL Z
	$r = 0.389$	$r = 0.319$, $p < 0.05$
	50 kHz-LA Xc	250 kHz-LL Xc
	$r = 0.378$	$r = 0.313$, $p < 0.05$
	250 kHz-LL Z	50 kHz-LL Z
	$r = 0.362$	$r = 0.299$, $p < 0.05$
	500 kHz-LL Z	5 kHz-LL Xc
	$r = 0.355$	$r = 0.296$, $p < 0.05$
	1 Mhz-LL Z	250 kHz-LL Z
	$r = 0.348$	$r = 0.273$, $p < 0.05$
	50 kHz-TR Xc	
	$r = 0.340$	
	5 kHz-TR Z	
	$R = 0.336$	
	5 kHz-RA Phase Angle	
	$r = 0.335$	

Table 2
(*Continued*)

Main predictors of hematocrit	Main predictors of RBC aggregation "M"	Main predictors of RBC aggregation "M1"
	5 kHz-LA Phase $r = 0.334$ 1 kHz-TR Z $r = 0.323$ 5 kHz-RL Xc $r = 0.306$ 50 kHz-TR Z $r = 0.285$ 5 kHz-LL Xc $r = 0.272$	

Abbreviations: Xc = reactance; Z = impedance; LL = left leg; LA = left arm; RA= right arm; LA = left arm; TR = trunk.

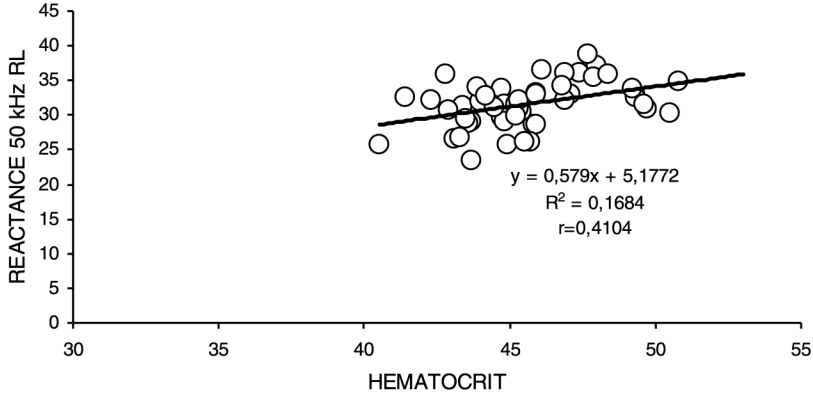

Fig. 1. Positive correlation between systemic hematocrit and leg reactance at 50 kHz.

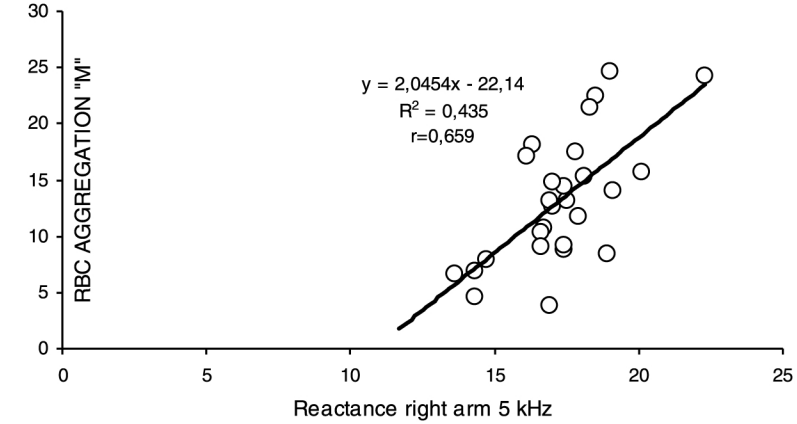

Fig. 2. Positive correlation between red cell aggregation "M" and right arm reactance at 5 kHz.

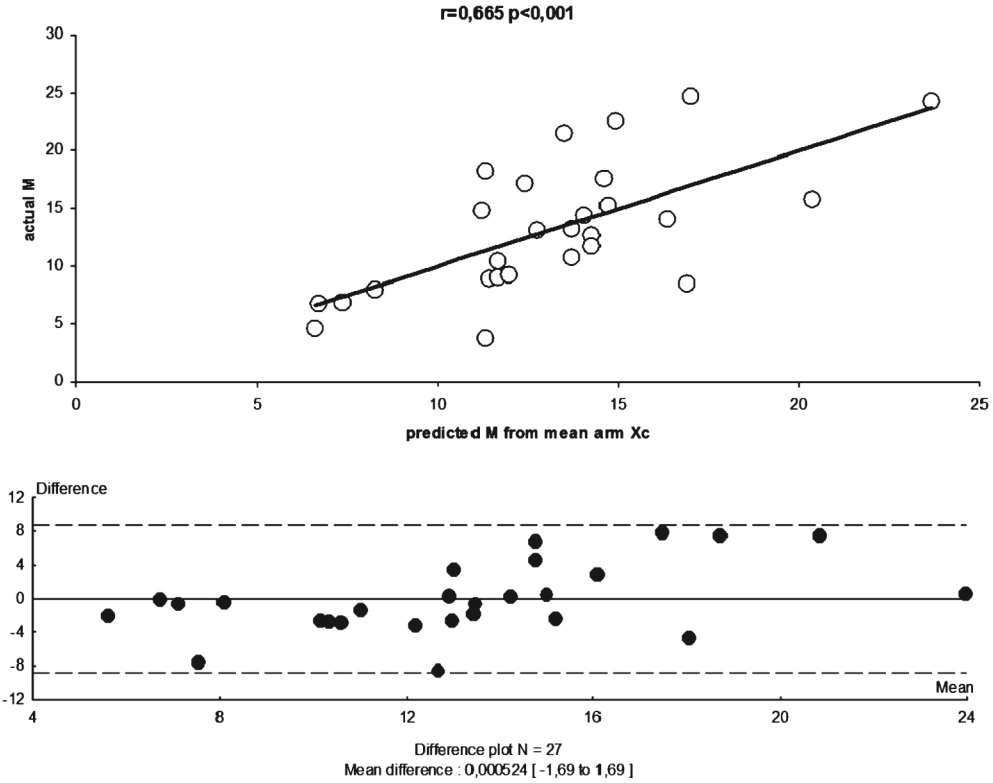

Fig. 3. Agreement of a prediction of RBC aggregation index "M" with its value predicted with the equation: M = 2.1845 maXc5-23.958 with maXc5 being the mean of reactances of the two arms. Upper pannel: correlation coefficient; lower pannel: Bland-Altman plot.

4. Discussion

Bioelectrical impedance analysis is widely used for the routine assessment of fat mass, fat free mass and body fluid volumes [16, 22] but there are other determinants of the electric properties of the body that affect the impedance of living tissues and thus can be reflected to some extent by BIA measurements.

This study evidences a host of correlations between BIA crude measurements and both haematocrit and red cell aggregability. Among them, the most significant are between hematocrit and leg reactance at 50 kHz, and between arm reactance at 5 kHz and RBC aggregation index "M". We show a predictive equation for "M" from the mean between the two arm reactances at 5 kHz that provides a quite satisfactory Bland-Altman plot.

In preceding studies using whole body BIA (see review in [4, 21] we reported several predictions of viscosity factors with r coefficients ranging between $r = 0.441$ and $r = 0.761$ but their meaning was sometimes difficult to understand. All are shown on Table 3.

Although some of the previous correlations shown on Table 3 yielded satisfactory coefficients of correlations and/or Bland-Altman plots, the correlations shown here are probably more accurate than those we previously reported in our preceding papers using whole body BIA. Both hematocrit and red cell aggregation are known to increase the impedance of blood *in vitro* or measured by electrodes set within vessels directly in the blood flow, and this is exactly what is reflected with our *in vivo* measurements.

Table 3
Previously reported equations predicting viscosity factors from whole body BIA measurements.

Formula	Correlation coefficient	Bland-Alman plot: mean difference (range).	References
$Hct = 50.42 \exp(-3.07 \cdot 10^{-4} Z_1)$	$r = -0.485$	–0.187(–0.976–0.602)	Varlet-Marie et al., *Clin Hemorheol Microcirc* **28** (2003) 129–137
$WBV = -513.4069 Z_{100} + 4.1466$	$r = 0.518$	5.9×10^{-5} (–0.127–0.127)	Varlet-Marie et al., *Clin Hemorheol Microcirc* **28** (2003) 129–137
PV = 1.07+0.00568 (W/FFM)–0.000154 Z_{10}	$r = 0.441$	0.023(–0.0242+0.0289)	Varlet-Marie et al., *Clin Hemorheol Microcirc* **28** (2003) 129–137
$Hct = -0.029\ Z_{50} + 54.621$	$r = -0.591$	–0.0335 (–0,686–0,619)	Varlet-Marie et al., *Clin Hemorheol Microcirc* **30** (2004) 471–475
$Hct = -0.0352\ Z_{50} + 58.741$	$r = -0.686$	0(–1.04–1.04)	Varlet-Marie et al., *Clin Hemorheol Microcirc* **30** (2004) 393–398
$WBV = -0.0032\ Z_{50} + 4.8621$	$r = -0.541$	-1.82×10^{-5} (–1.04–1.04)	Varlet-Marie et al., *Clin Hemorheol Microcirc* 30 (2004) 393–398 393
k = 0,005809 Re/ECW + 1,1784	$r = 0{,}487$	0124 (–0,00481–0,00296)	Brun et al., *Series on Biomechanics* 25(1-2) (2010) 100–104
$M = -27{,}4755\ \rho_e + 1121{,}57029$	$r = 0.463$	94 (–0,842–0,842).	Brun et al., *Series on Biomechanics* **25(1-2)** (2010) 100–104
$S_{10} = -59{,}38579\ (\rho_e\text{-}40) + 63{,}083$	$r = 0.761$	0,000722 (–1,77–1,77).	Brun et al., *Series on Biomechanics* **25(1-2)** (2010), 100–104

Abbreviations: Hct = hematocrit; WBV= whole blood viscosity at 1000 s–1; Z1 = whole body impedance at a frequency of 1 kHz; Z10 = whole body impedance at a frequency of 10 kHz; Z50 = whole body impedance at a frequency of 50 kHz; Z100 = whole body impedance at a frequency of 100 kHz; PV = plasma viscosity; W/FFM =water in fat free mass calculated by whole body BIA at 50 kHz; "k" = red blood cell rigidity index calculated from viscometry with Quemada's equation; ECW: extracellular water volume calculated with; Re = extracellular resistance and ?e = extracellular resistivity predicted with Hanai's mixture conductivity theory. according to Jaffrin [7]; M= Myrenne index of red blood cell aggregation (see text); "S10 SEFAM index of red blood cell aggregation.

The measurement of red cell aggregation used in this study is the most classical and widely used one. It has been recently shown to be well correlated with the measurement of the yield stress provided by viscometric measurements, supporting its validity [10]. On the other hand, *in vitro* experiments conducted by the late Oguz Baskurt show that despite some discrepancies electrical conductance and light transmission of aggregating blood at various hematocrits in plasma or in isotonic saline have similar time courses and are related to each other [2]. Concerning hematocrit, all *in vitro* studies [6, 14, 15, 18] show that it increases impedance, and this is also found with electrodes put *in vivo* in the blood stream [12, 13]. Therefore the equation found in this paper is also logic. By contrast it is still difficult to understand why in whole body measurements we repeatedly evidenced negative correlations, that are fully contra-intuitive.

5. Conclusions

On the whole this study evidences a correlation between hematocrit and leg reactance, and a correlation between red cell aggregation and arm reactance. Further testing of this approach is needed. This study suggests that the previously reported correlations between whole body BIA and viscosity factor were probably not spurious, and that in a narrow cylinder such as the arm the structure of circulating blood (hematocrit, red cell aggregation) may influence the passage of an electric current by increasing

reactance. Whether this may provide a reliable window on hemorheology remains of course to be studied.

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