Bioimpedance spectroscopy as technique of hematological and biochemical analysis of blood

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Abstract. Bioimpedance spectroscopy may become a useful method for the express analysis and monitoring of blood parameters. The aim of this study was to identify biochemical and hematological parameters of blood that can be accurately predicted by means of bioimpedance technique. Hematological (red blood cell and white blood cell parameters) and biochemical (total proteins, albumins, fibrinogen, sodium, potassium, chloride ion concentrations in plasma) parameters were measured with a hematological analyzer and routine methods. Bioimpedance spectroscopy of the whole blood (1.5 ml) in frequency range 5–500 kHz (31 frequencies) was performed using BIA analyzer ABC-01 “Medass”. Frequency relationships of resistance and reactance of the whole blood and the parameters of the Cole model were investigated. Close simple and multiple correlations of bioimpedance indices were observed only with erythrocyte parameters (Ht, Hb, RBC). Thus bioimpedance analysis of the whole blood can accurately predict red cell parameters but it is less effective for estimation of plasma biochemical and white cell parameters.

1. Introduction
Investigation of biochemical and hematological blood parameters is important for physiology and medicine. It aims at searching for more accessible, inexpensive and exact methods of estimation of composition of blood. For this, manual direct microscopy of blood cells, automated blood cell counters [1] and routine biochemical techniques [2] are used. Many of them are labour-consuming and expensive. Bioimpedance spectroscopy of blood may be useful and convenient methods for estimation of blood parameters. It was shown that BIA parameters of blood are related to various morphological and biochemical indices of blood [3, 4], as well as to biochemical modification of blood during storage [5]. The purpose of our work was to identify biochemical and hematological parameters of blood that can be accurately predicted by means of bioimpedance technique.

2. Materials and methods

2.1. Blood samples
Blood samples were collected from 46 donors and placed into 4 plastic tubes (Improvacuter). For bioimpedance analysis 3 ml blood sample was drawn into tubes containing heparin. For biochemical serum analysis 4 ml blood sample was drawn into empty tubes. For fibrinogen measurement 2 ml blood sample was drawn into tubes containing sodium citrate, and for hematological analysis 2.5 ml of blood was drawn into tubes with EDTA.
2.2. BIA measurements of blood

The electrical measurements of blood (1.5 ml) were done in a conductivity cell (Fig. 1). The conductivity cell is a uniform cylindrical plastic tube 8 mm in diameter (d) with four gilded copper wire electrodes and inner interelectrode height (h) 9 mm. Current (CE) and potential electrodes (PE) of 3 mm and 0.5 mm in diameter were applied by piercing the plastic tube. The interelectrode distance between CE and PE was 2.5 mm, and 3 mm between PE and PE. The electrodes were connected to BIA analyzer ABC-01 “Medass” (SRC Medass, Russia). Measurements of impedance (Z), resistance (R), reactance (Xc), and phase angle (Phi) of blood samples were carried out at room temperature (21±1°C) in the frequency range from 5 kHz to 500 kHz (31 frequencies). For statistical analysis, the values of phase angle at 10, 50 and 500 kHz (Phi10, Phi50 and Phi500 respectively) were used. The Cole model parameters of extracellular (Re) and intracellular resistance (Ri), as well as parameter Alpha and characteristic frequency (Fch) were calculated by the analyzer software. Example of the impedance plot (the frequency dependence of reactance from resistance) for the whole blood sample with a hematocrit of 47% is shown in Fig. 2. Coefficient of variation for all parameters obtained by this method was less than 1.0%.

As BIA measurement was done in the vertical conductivity cell, the parameters Re, Phi, Alpha have changed in time due to sedimentation of red blood cells between potential electrodes. We estimated the rate of change of Re (ΔRe) and Alpha (ΔAlpha) for 1 minute in percents as follows, \( \Delta Re(\%) = \frac{(Re4-Re64) \times 100}{Re64} \), where Re4 and Re64 are Re at 4 and 64 seconds of BIA monitoring, respectively. The similar formula was used for calculating ΔAlpha. Descriptive statistics for ΔRe and ΔAlpha are presented in Table 1.

![Image of conductivity cell](image)

![Image of impedance plot](image)

2.3. Biochemical and hematological measurements of blood

Biochemical parameters, total protein concentration (TP), albumin (Alb) and globulin (Glb) concentrations, sodium (Na), potassium (K), and chloride (Cl) ion concentrations were measured according to conventional methods using the Sapphire 400 chemical analyzer. Plasma fibrinogen level (Fib) was determined by the method of A. Clauss.

Hematological parameters, hematocrit (Ht), mean corpuscular volume (MCV), red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), mean content of hemoglobin (MCH), leucocytes (WBC), neutrophils (Neu), lymphocytes (Lym), monocytes (Mon), platelets (Plt) in blood were analyzed using the MEK-8222I/K hematological analyzer. Reticulocytes (Ret) and erythrocytes sedimentation rates (ESR) for 1 hour at room temperature were determined by routine methods.

Correlations were tested by linear and multiple regression analysis. As the distribution of ESR was very skew, correlation analysis was performed after logarithmic transformation of ESR.

| Table 1. Descriptive statistics for the rate of change of blood bioimpedance parameters Re (ΔRe) and Alpha (ΔAlpha) for 1 minute (n=17) |
|Mean| Std. Dev.| p| Mean| Std. Dev.| p|
|---|---|---|---|---|---|
|Re4, Ohm| 93,9| ±10,5| Alpha4| 0,937| ±0,015|
|Re64, Ohm| 95,8| ±11,4| Alpha64| 0,922| ±0,014|
|ΔRe, Ohm| 1,9| ±1,6| ΔAlpha| -0,015| ±0,016| 0,0008|
3. Results

The results of correlation analysis between hematological and bioimpedance blood parameters are represented in Table 2. As is shown, hematological parameters RBC, Hb, Ht, MCV, MCH, MCHC were significantly related to Re, Ri, Phi10, Phi50, Phi500, Fch and Alpha. All correlations of the indices with Re, Phi10, Phi50, Phi500 were positive, but with Ri, Fch and Alpha were negative. Concentration of Ret was weakly correlated to Re only and WBC was weakly related to Re and to Phi500 (all p<0.05). Neu, Lym, Mon and Plt were not related to bioimpedance blood parameters.

Table 2. Correlations (r) between hematological and bioimpedance blood parameters (n=46)

|       | RBC   | Hb    | Ht    | MCV  | MCH  | MCHC | WBC  | Ret   |
|-------|-------|-------|-------|------|------|------|------|-------|
| Re    | 0.64***| 0.78***| 0.80***| 0.51***| 0.50***| 0.32* | 0.34*| 0.46**|
| Ri    | -0.54***| -0.75***| -0.75***| -0.59***| -0.59***| -0.39*| -0.11| -0.11|
| Phi10 | 0.33*  | 0.46** | 0.43** | 0.29*  | 0.35*  | 0.32* | -0.09| 0.08  |
| Phi50 | 0.56***| 0.73***| 0.72***| 0.50***| 0.52***| 0.40**| 0.26 | 0.26  |
| Phi500| 0.68***| 0.82***| 0.83***| 0.51***| 0.53***| 0.39**| 0.32*| 0.33  |
| Fch   | -0.44**| -0.56***| -0.54***| -0.36* | -0.40**| -0.37*| -0.28| -0.19 |
| Alpha | -0.16  | -0.47***| -0.47***| -0.59***| -0.55***| -0.28| -0.04| -0.12 |

(*, **, *** - p <0.05, <0.01, <0.001 respectively)

Multiple regression analysis showed that a sum of bioimpedance parameters (Ri, Fch, Phi500, Re in relation to RBC and to MCHC; Ri, Fch, Phi500, Re, Alpha in relation to Ht, Hb, MCV, MCH) explained 82% of Ht dispersion (R^2=0.82, p<0.001), 78% of Hb dispersion (R^2=0.78, p<0.001), 61% of RBC dispersion (R^2=0.61, p<0.001), 47% of MCV dispersion (R^2=0.47, p<0.001), 45% of MCH dispersion (R^2=0.45, p<0.001), and 25% of MCHC dispersion (R^2=0.25, p=0.018). Thus the best multiple correlation was obtained between a sum of bioimpedance blood parameters (Ri, Fch, Phi500, Re, Alpha) and hematocrit. Correlation between calculated Ht and Ht measured by hematological analyzer was r=0.91 (p<0.001, Fig. 3).

The results of bivariate correlation analysis between biochemical and bioimpedance blood parameters are shown in Table 3. Of biochemical parameters, only Glb, Fib, TP, Ki and ESR weakly correlated with the bioimpedance parameters. Higher correlations of Glb were with Phi50 (r=0.42, p=0.004) and Fch (r=-0.44, p=0.002). Similar result was obtained for Fbr (r=0.44, p=0.003 and r=-0.54, p<0.001 with Phi50 and Fch respectively) and TP (r=0.40, p=0.006 and r=-0.40, p<0.006 with Phi50 and Fch respectively).

Table 3. Correlations (r) between biochemical and bioimpedance blood parameters (n=46)

|       | Alb   | Glb   | Fib   | TP    | Ki    | Nai   | Cli   | ESR   | ESR/Ht |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| Re    | 0.13  | 0.16  | 0.27^ | 0.20  | 0.01  | 0.18  | 0.09  | -0.20 | -0.37**|
| Ri    | -0.03 | -0.23 | 0.06  | -0.23 | -0.35*| -0.10 | -0.24 | 0.32* | 0.49***|
| Phi10 | -0.16 | 0.40**| 0.21  | 0.32* | 0.07  | 0.03  | 0.04  | 0.08  | -0.05  |
| Phi50 | 0.03  | 0.42**| 0.44**| 0.40**| 0.00  | 0.09  | 0.05  | -0.10 | -0.28  |
| Phi500| 0.06  | 0.30* | 0.31  | 0.31* | 0.04  | 0.17  | 0.10  | -0.23 | -0.40**|
| Fch   | 0.02  | -0.44**| -0.54*| -0.40**| 0.14 | -0.06 | 0.07  | 0.00  | 0.15  |
| Alpha | -0.14 | -0.08 | -0.07 | -0.12 | -0.25 | 0.03  | -0.12 | 0.01  | 0.17  |

(^, *, **, *** - p <0.1, <0.05, <0.01, <0.001 respectively)

Multiple regression analysis showed that both of the parameters Phi50 and Fch explained 19% Glb dispersion (R^2=0.19, p=0.010), 29% of Fbr dispersion (R^2=0.29, p=0.001) and 17% TP dispersion.
(R²=0.17, p=0.018). Ions Nai, Cli were not associated with the bioimpedance parameters. Only serum Ki was significantly associated with Ri (r=-0.35, p=0.017).

Correlations of the parameters ∆Re and ∆Alpha with biochemical and hematological parameters are presented in Table 4. As is shown, TP (p=0.009), Glb (p=0.013), Fbr (p=0.012) and ESR (p=0.046) were all positively related to the rate of change of blood Re for 1 minute (∆Re). On the contrary, TP (p<0.1) and Glb (p=0.046) were negatively related to the rate of change of Alpha for 1 minute (∆Alpha).

Table 4. Correlations (r) of the change of Re (∆Re) and Alpha (∆Alpha) with biochemical and hematological parameters (n=17)

|        | RBC | Ht  | MCV | ESR | Alb | TP  | Ki  | Nai | Fbr | Glb |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ∆Re    | 0.35| 0.50*| 0.19| 0.49*| 0.24| 0.61**| -0.10| -0.19| 0.59*| 0.59*|
| ∆Alpha | -0.33| -0.40| -0.16| -0.22| 0.05| -0.46^| -0.40| -0.19| -0.09| -0.49*|

(^, *, ** - p <0.1, <0.05, <0.01 respectively)

4. Discussion and conclusions

Our data show that the bioimpedance parameters of the whole blood correlate closely with the red blood indices: Ht, Hb, RBC. Similar correlations with Ht were obtained elsewhere [3-5]. All BIA parameters of blood were moderately associated with characteristics of single erythrocytes: MCV, MCH and especially MCHC. These low correlations were obtained because the impedance parameters of our BIA method reflect integral characteristics of whole blood: the total red cell count and plasma volume in a zone of measurement, instead of characteristics of single blood cells. Possibly, reduction of the sizes of conductivity cell till the sizes of one erythrocyte allows estimating characteristics of a single blood cell. So the BIA technique needs further development for estimating these blood indices. Hence, the bioimpedance analysis of whole blood can be used for fast and accurate determination of total red blood cell parameters (Ht, RBC, Hb) only. Electrical parameters (Re, Ri, Alpha, Phi) of the whole blood poorly reflected the concentration of white blood cells. This is possibly due to a comparatively low concentration of leukocytes in blood. Our results show that the extracellular fluid resistance (Re) of the whole blood is not related to the main extracellular plasma ions: sodium and chloride ion concentrations. This could be ascribed to a stronger influence of plasma proteins on the impedance parameters. Phase angle of blood at low frequencies (Phi50), characteristic frequency (Fch), as well as the rate of changes of Re and Alpha (∆Re, ∆Alpha) in vertical measuring cells for 1-2 minute, can roughly estimate the concentration of macromolecular plasma globulins (gamma-globulins, alpha2-globulins and fibrinogen) and ESR. The observed changes in extracellular resistance and parameter Alpha possibly reflect erythrocyte aggregation process in blood caused by large protein molecules. This assumption requires additional tests of the blood bioimpedance characteristics along with measurements of aggregation parameters of red blood cells.

References

[1] Pohland D 1989 J. Clin. Chem. Clin. Biochem. 27, 41-47
[2] Faulkner W.R., Meites S.1982 Selected methods of clinical chemistry (Washington DC)
[3] Zhao T X 1996 J. Med. Eng. Technol. 20 115-120
[4] Zhao T X 1993 Physiol. Meas. 14 299-307
[5] Ulgen Y, Sezdi M 2007 Med. Biol. Eng. Comput. 45 653-660