Correlation of bioelectrical impedance analysis phase angle with changes in oxidative stress on end-stage renal disease patients, before, during, and after dialysis

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ABSTRACT
Chronic kidney disease is a condition that promotes oxidative stress. There are conflicting evidence about the role of hemodialysis on oxidative stress, that are mostly related with the various types of membrane materials used, the quality and type of dialysate, the method used, etc. The phase angle (PhA), which is determined with bioelectrical impedance analysis (BIA), measures the functionality of cell membranes. In this study, the correlation of the PhA with parameters of oxidative stress is attempted for the first time. We evaluated parameters of oxidative status as total antioxidant capacity (TAC) in erythrocytes (RBCs) and plasma of patients with ESRD undergoing hemodialysis with low flux synthetic polysulfone membranes. Measurements were recorded from 30 patients (16 men and 14 women) aged 64 ± 14 years before, during, and after dialysis, and in 15 healthy volunteers aged 56 ± 12 years. The PhA was obtained by BIA. The plasma TAC increased significantly (41%, \(p < 0.05\)). Intracellular TAC noted a non-significant increase. Total antioxidant capacity of the patients before and after hemodialysis was significantly lower from the healthy volunteers \((p < 0.05)\) showing that ESRD patients are at the state of increased oxidative stress. The PhA increased in significantly positive correlation with plasma TAC at the end of hemodialysis. The process of hemodialysis with biocompatible synthetic membranes and bicarbonate dialysate improved plasma TAC. The positive correlation of PhA with extracellular TAC could evolve to a method of oxidative stress estimation by BIA but further research is needed.

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Introduction
Hemodialysis is believed to increase oxidative stress of the patients.1 Exposure of blood to the membranes results in a numerous of interactions including the activation of neutrophils (the extent of the activation depends on the biocompatibility of the membranes).2,3 Activation of neutrophils can be aggravated by exposure to light (in normal conditions blood components are not exposed to light).4 Various mediators of inflammation such as cytokines and metabolites of arachidonic acid activate the phagocytic responses. Upon activation, a respiratory burst is induced with the release of oxygen free radicals such superoxide radical and hydrogen peroxide. This leads to tissue damage (e.g. oxidation of lipid membranes).5,6 Free radicals are also produced from erythrocyte iron released as a consequence of hemolysis.7 The hemodialysis solution has to be ultra-pure and not transfected, as the endotoxins of the microbes enter from the solution into the blood through the filter (back filtration), resulting in endotoxemia and oxidative stress.

The most common and possibly the most practical for use method of body composition analysis is bioelectrical impedance analysis (BIA). Application of BIA is also used to measure the resistance \((R)\) and reactance of the body \((Xc)\). The ohmic resistance \((Rz)\) expresses the aqueous condition of the tissues and the reactance \((Xc)\) expresses the amount of energy that can be accumulated in the tissues (tissue cells behave as capacitors).8 These two parameters are used to estimate the phase angle (PhA), an index used to estimate the functionality of the cell membranes. PhA has a prognostic value for...
the survival of renal patients. The greater the PhA, the more intact the cell membranes appear to be and the expectancy and quality of patient’s life is better. The same assessment is seen in other chronic diseases such as cancer, HIV, and malnutrition.

The aim of this study was to examine the correlation of the PhA with parameters of oxidative stress such as the total antioxidant capacity (TAC). To our knowledge, this is the first study attempting to establish a relationship between the PhA and the antioxidant capacity in patients with renal disease, subjected to hemodialysis.

### Subjects and methods

#### Patient population

The study subject population consisted of 30 patients with end-stage chronic kidney disease (CKD) (experimental group – EG), under hemodialysis. The patients were either males (n = 16) or females (n = 14), aged 64.0 ± 14 years. Study population characteristics are summarized in Table 1. Also, 15 healthy volunteers (six males and nine females) aged 56 ± 12 years were recruited as the control group (CG). Patients of EG were treated with low-flux synthetic dialysis membrane, made of polysulfone, whereas bicarbonate dialysis solution was used, throughout the study.

The clinical trial was conducted according to the Guidelines of the Declaration of Helsinki. Patients and healthy volunteers were informed in detail and a written consent was provided before their participation to the study.

### Blood sampling

Blood samples were collected in standard sterile vacuum tubes from patients (EG) at three different time points: pre-, in the middle, and immediately post-hemodialysis. Samples were immediately centrifuged at 3000 rpm for 15 min. Plasma and erythrocytes were separated and kept at −80 °C until analysis. Blood samples were also collected from healthy subjects (before meal), to be compared with those of patients on pre-hemodialysis state.

### Total antioxidant capacity

The estimation of TAC in plasma and RBC was performed by blue CrO5 assay, according to Charalampidis et al. (2009).

### Bioelectric impedance analysis (BIA)

Bioelectric impedance analysis is a very common method, used to estimate the body composition, particularly the body fat in relation to lean body mass. It determines the electrical impedance, upon flow of a low-intensity alternating electric current, through body tissues. Based on this, the total body water can be estimated, which can be directly correlated with the healthy status. The normal values range from 4.8 to 8 (PhA), depending on the gender and the age. Women and elderly present lower PhA.

In the present study, the Bodygram Akern BIA 101 was used to calculate the BIA in patients and healthy subjects. Briefly, four electrodes, two on the right hand and two on the right foot, were placed and an alternating electrical current of 800 μA intensity at a frequency of 50 kHz was sent through the body. Single-frequency bioelectrical impedance analyzers are widely used in clinical practice to examine the prognostic impact of PhA. Bioelectric impedance analysis measurements were taken before, in the middle, and after hemodialysis in all subjects.

### Statistical analysis

Data are expressed as mean ± SD. The analysis showed that there was a normal distribution between data and thus the statistical significance between data means at different time points was determined by Student’s t-test and two-way analysis of variance (ANOVA). Also, for data correlation, the bivariate Pearson correlation was used (SPSS version 17.0, SPSS Inc. Chicago, IL). p Values <0.05 were considered as significant.

### Results

#### Antioxidant capacity

The TAC of the plasma was significantly increased at the end of the hemodialysis session. Specifically, TAC of the plasma presented a 41% increase at the end of the session (p < 0.05) while a 28% increase was already noted.

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Table 1. Study population characteristics.

| 1 | Primary disease: no criteria |
| 2 | Duration of hemodialysis >6 months |
| 3 | Continuous hemodialysis for >2 months after study recruitment. |
| 4 | Not concurrent treatment with antioxidant factors |
| 5 | Constant treatment with iron and erythropoietin regimens during CKD. Plasma measurements were performed 3 weeks after the last i.v. infusion of iron |
| 6 | Physiological body temperature (37 °C) and CRP levels <3-fold increase compared with normal levels |

*Kt/V, a number used to quantify hemodialysis treatment adequacy.*
from the middle of the session (2 h from the beginning) (Figure 1).

Hemodialysis leads to a significant increase of plasma TAC. At the end of the session, on one hand, TAC was raised almost to the levels of the normal values (values from the healthy volunteers). On the other hand, TAC of RBC remained stable and at

**Table 2.** Baseline characteristics and bioelectrical impedance measurements of the healthy volunteers group.

| Men       | Women  |
|-----------|--------|
| Age (years) | 56.2 ± 11.5 |
| Height (m)   | 1.7 ± 0.1 |
| Weight (kg)  | 75.8 ± 19.4 |
| Na/K exchange | 1.1 ± 0.2 |
| Lean body mass (kg) | 30.0 ± 8.7 |
| Fat mass (kg)  | 19.8 ± 12.9 |
| Basal metabolic rate (kcal) | 1615 ± 255 |
| BMI (kg/m²)   | 26.4 ± 6.8 |
| Cell body mass (kg) | 10.3 ± 2.4 |
| Total body water (L) | 41.4 ± 9.5 |
| Total body water (extra-cellular) (L) | 19.5 ± 3.7 |
| Total body water (intra-cellular) (L) | 21.8 ± 5.4 |
| Phase angle (deg) | 5.9 ± 0.9 |
| Resistance, R | 500.5 ± 102.9 |
| Reactance, Xc | 50.8 ± 9.9 |

**Figure 1.** Effects of hemodialysis on TAC of the plasma. *Significantly different from the beginning of the session, p < 0.05. Values are presented as mean ± SD.

**Figure 2.** Comparison of TAC of the plasma and RBC in healthy volunteers and in patients on hemodialysis. aSignificantly different from the healthy volunteers levels, p < 0.05. bSignificantly different from the pre-dialysis levels, p < 0.05. Values are presented as mean ± SD.

**Table 3.** Bioelectrical impedance measurements during the hemodialysis session.

|                          | Pre-dialysis | During dialysis | Post-dialysis |
|--------------------------|--------------|-----------------|---------------|
| Weight (kg)              | 70.5 ± 18.7  | 69.3 ± 18.6     | 68.0 ± 18.3   |
| Phase angle (deg)        | 4.5 ± 0.9    | 4.6 ± 1.0       | 5.0 ± 1.0     |
| Resistance, R            | 482 ± 90     | 512 ± 97        | 548 ± 105     |
| Reactance, Xc            | 38 ± 10      | 41 ± 10         | 48 ± 13*      |
| Total body water (L)     | 39 ± 9       | 38 ± 8          | 36 ± 8        |
| Total body water (extra-cellular) (L) | 21 ± 4 | 19 ± 5 | 18 ± 4* |
| Lean body mass (kg)      | 51 ± 12      | 49 ± 11         | 47 ± 11       |
| Cell body mass (kg)      | 24 ± 8       | 22 ± 8          | 23 ± 7        |
| Fat mass (kg)            | 21 ± 11      | 22 ± 11         | 23 ± 11       |

*Statistically significant difference from the beginning of the session, p < 0.05. Values are presented as mean ± SD.

significant lower levels than of TAC of the healthy volunteers (Figure 2).

**Bioelectrical impedance**

The characteristics of the CG (healthy volunteers) as well as bioelectrical impedance measurements are presented in Table 2. The mean age of the hemodialysis patients was 68 ± 14 years, their body weight was 71 ± 19 kg, and their height was 1.6 ± 0.1 m. During the hemodialysis session, the reactance of the patients increased significantly (p < 0.05), while the extra-cellular water was reduced (p < 0.05). All the others indices measured remained stable. The PhA showed a trend to increase but it did not research statistical significant levels at the end of the session. Results are presented in Table 3.

**Correlation of TAC of the plasma and PhA**

The TAC of the plasma of the patients presented a moderately positive correlation (r = 0.606, p < 0.001) with the PhA. Regression analysis showed a weak positive relationship (F(1,30) = 17.451, p < 0.001) (Figure 3).
According to our knowledge, this is the first study in hemodialysis patients demonstrating a moderately positive relationship between PhA and plasma TAC. Dialysis patients have lower initial TAC plasma compared with healthy subjects. The increase in TAC during the hemodialysis session improves the PhA and, therefore, the function of membranes and cells.

A relatively limitation of this study was the number of patients who participated. Increasing the number of patients would possibly strengthen our observations.

The measurement of bioelectrical impedance is an important clinical-diagnostic tool for each physician to assess the nutrition and hydration of the patients. It represents an affordable and non-invasive method that provides useful information on changes in body composition. Bioelectrical impedance analysis estimates the electric impedance of an electric current passing through the body \(^{17}\) allowing the determination of various BIA parameters. Phase angle is a parameter derived from BIA and it is used as an indicator of cell membrane function as well as a marker of nutritional status in different populations. The PhA changes also depend on gender and age. As mentioned previously, greater values of the PhA are correlated with a better quality of life. The reduction in PhA has been associated with conditions of chronic or acute illness. Some diseases associated with decreased PhA are the acquired immunodeficiency syndrome (AIDS), tuberculosis, renal failure, etc. \(^{16}\) The PhA is also used as a predictor in patients with colorectal cancer, \(^{18}\) advanced pancreatic cancer, \(^{19}\) and breast cancer. \(^{11}\) It can also be a diagnostic tool of nutritional status in patients with chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. \(^{20}\)

According to Stobaus et al., \(^{21}\) the two main factors that determine the PhA is malnutrition (85.4%; \(p < 0.0001\)) and infection (9.6%; \(p < 0.0001\)). The studies have been conducted in individuals with sickle cell disease and burns, confirming its ability as an indicator for evaluating the functioning of the cell membrane. \(^{22}\)

Patients undergoing hemodialysis or peritoneal dialysis and have extracellular cell mass/body mass (ECM/BCM) ratio \(>1.2\) and PhA \(<6\) present poor cardiovascular prognosis. \(^{23}\) The PhA has been found reduced in male patients (but not in females) with Stage 5 chronic kidney disease (CKD) and without diabetes. \(^{24}\) The PhA remained stable over a period of 9 months in patients with CKD following conservative treatment. \(^{25}\) Bellizzi et al. \(^{26}\) observed that CKD patients have significantly lower PhA (\(-22\%\)) than healthy subjects even after adjustments made according to the \(R\) ratio \((R = \text{height}^2/\text{resistance in cm}^2/\text{ohm})\) or the total body water. In an early study of 3009 dialysis patients, it was shown that in 50\% of the men and 75\% of the women, PhA is less than 5.16. \(^{27}\)

Bioelectrical impedance is a useful tool to monitor changes in body composition, so that they can be corrected according to the dry weight and modifications made to the program of treatment. \(^{28}\) Bioelectrical impedance measurements vary in a great extent in patients undergoing dialysis (with the highest values immediately after dialysis), but remain stable and highly reproducible for more than 2 h after the end of dialysis, in a state of dry weight. Thus, the timing of measurement of the bioelectrical impedance is crucial for the proper assessment of body composition, and BIA can be measured at any time after the end of hemodialysis session, provided that no changes in hydration have occurred due to consumption of food or beverages. \(^{29}\)

A statistically significant reduction in extracellular body water was observed that was accompanied with a similar decrease (3 L) in the total body water, indicating that the intracellular body water (about 18 L average) remaining stable. The body cell mass also remained stable. Therefore, the post-dialysis increase of the PhA was not due to the hydration of the cells (it remained stable). Moreover, failure to find a difference between the intracellular water volume of the healthy volunteers and patients suggested that no intracellular dehydration had occurred during the session. Therefore, the increase of the PhA is related directly to the improvement of the cell membrane functionality, since the total volume of the fluids removed, which was ultimately only from the extracellular space.

Oxidative stress plays an important role in the development of morbidity as well as mortality in patients with CKD that are either following a supportive

Figure 3. Correlation of plasma’s TAC with the phase angle.
treatment or a dialysis treatment. Oxidative stress increases cardiovascular risk and is implicated in the complications of CKD; it also reduces the durability of the dialysis method (e.g. exacerbating the damage or fibrosis of the peritoneal membrane), and is potentially involved in the progression of renal disease itself. Hemodialysis is a procedure that is correlated with an increased production of oxidation products that play an important role in endothelium dysfunction and atherogenesis, malnutrition, amyloidosis, etc. Uremia per se could be prime phagocyte oxidative burst. Moreover, patients with CKD present increased levels of plasma F2-isoprostanes. Iron’s supplementation side effects include induction of oxidative stress and increase of lipid peroxidation in the plasma. The above could affect TAC in this group of patients.

In conclusion, PhA could be used as a biomarker of the integrity of the cell membranes in hemodialysis patients and thus as a non-invasive marker to measure oxidative stress.

Disclosure statement
The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References
1. Hashimoto H, Mio T, Sumino K. Lipid abnormalities of erythrocyte membranes in hemodialysis patients with chronic renal failure. Clin Chim Acta. 1996;252:137–145.
2. Nguyen AT, Leithias C, Zingriff J, Herbelin A, Naret C, Descamps-Latscha B. Hemodialysis membrane-induced activation of phagocyte oxidative metabolism detected in vivo and in vitro within micro amounts of whole blood. Kidney Int. 1985;28:158–167.
3. Taylor JE, Scott N, Bridges A, Henderson IS, Stewart WK, Belch JJ. Lipid peroxidation and antioxidants in continuous ambulatory dialysis patients. Perit Dial Int. 1992;12:252–256.
4. Kubasova T, Horváth M, Kocsis K, Fenyó M. Effect of visible light on some cellular and immune parameters. Immunol Cell Biol. 1995;73:239–244.
5. Hennig B, Chow CK. Lipid peroxidation and endothelial cell injury: Implications in atherosclerosis. Free Radic Biol Med. 1988;4:99–106.
6. Suematsu M, Tsuchiya M. Microtopographic analysis of oxidative stress in organ microcirculatory units. Adv Exp Med Biol. 1992;316:211–221.
7. Eisel’t J, Racek J, Opatrný K. Jr. Free radicals and extracorporeal renal replacement therapy. Vnitr Lek. 1999;45:319–324.
8. Abad S, Sotomayor G, Vega A, et al. The phase angle of the electrical impedance is a predictor of long-term survival in dialysis patients. Nefrologia. 2011;31:670–676.
9. Allison RD, Ray Lewis A, Liedtke R, Buchmeyer ND, Frank H. Early identification of hypovolemia using total body resistance measurements in long-term care facility residents. Gend Med. 2005;2:19–34.
10. Maggiore Q, Nigrelli S, Ciccarelli C, Grimaldi C, Rossi GA, Michelassi C. Nutritional and prognostic correlates of bioimpedance indexes in hemodialysis patients. Kidney Int. 1996;50:2103–2108.
11. Gupta D, Lammersfeld CA, Vashi PG, et al. Bioelectrical impedance phase angle as a prognostic indicator in breast cancer. BMC Cancer. 2008;8:249.
12. Ott M, Fischer H, Polat H, et al. Bioelectrical impedance analysis as a predictor of survival in patients with human immunodeficiency virus infection. J Acquir Immune Defic Syndr Hum Retrovirology. 1996;9:20–25.
13. Kyle UG, Benten L, Pichard C. Low phase angle determined by bioelectrical impedance analysis is associated with malnutrition and nutritional risk at hospital admission. Clin Nutr. 2013;32:294–299.
14. Charalampidis PS, Veltisitas P, Karkabounas S, Evangelou A. Blue CrOS assay: A novel spectrophotometric method for the evaluation of the antioxidant and oxidant capacity of various biological substances. Eur J Med Chem. 2009;44:4162–4168.
15. Piccoli A, Italian CAPD-BIA Study Group. Bioelectric impedance vector distribution in peritoneal dialysis patients with different hydration status. Kidney Int. 2004;65:1050–1063.
16. Norman K, Stobaus N, Pirlich M, Bosy-Westphal A. Bioelectrical phase angle and impedance vector analysis – Clinical relevance and applicability of impedance parameters. Clin Nutr. 2012;31:854–861.
17. Kyle UG, Bosaues I, De Lorenzo AD, et al. Bioelectrical impedance analysis – Part I: Review of the principles and methods. Clin Nutr. 2004;23:1226–1243.
18. Gupta D, Lammersfeld CA, Burrows JL, et al. Bioelectrical impedance phase angle in clinical practice: Implications for prognosis in advanced colorectal cancer. Am J Clin Nutr. 2004;80:1634–1638.
19. Gupta D, Lis CG, Dahlk SL, Vashi PG, Grutsch JF, Lammersfeld CA. Bioelectrical impedance phase angle as a prognostic indicator in advanced pancreatic cancer. Br J Nutr. 2004;92:957–962.
20. Peres WAF, Lento DF, Baluz K, Ramalho A. Phase angle as a nutritional evaluation tool in all stages of chronic liver disease. Nutr Hosp. 2012;27:2072–2078.
21. Stobaus N, Pirlich M, Valentini L, Schulzke JD, Norman K. Determinants of bioelectrical phase angle in disease. Br J Nutr. 2012;107:1217–1220.
22. Barbosa-Silva MCG, Barros AJD. Bioelectrical impedance analysis in clinical practice: A new perspective on its use beyond body composition equations. Curr Opin Clin Nutr Metab Care. 2005;8:311–317.
23. de Araujo Antunes A, Vannini FD, de Arruda Silveira LV, Barretti P, Martin LC, Caramori JC. Associations between bioelectrical impedance parameters and cardiovascular events in chronic dialysis patients. Int Urol Nephrol. 2012;45:1397–1403.
24. Cupisti A, D’Alessandro C, Morelli E, et al. Nutritional status and dietary manipulation in
predialysis chronic renal failure patients. *J Ren Nutr.* 2004;14:127–133.

25. Dumler F, Kilates C. Prospective nutritional surveillance using bioelectrical impedance in chronic kidney disease patients. *J Ren Nutr.* 2005;15:148–151.

26. Bellizzi V, Scalfi L, Terracciano V, et al. Early changes in bioelectrical estimates of body composition in chronic kidney disease. *J Am Soc Nephrol.* 2006;17:1481–1487.

27. Chertow GM, Lazarus JM, Lew NL, Ma L, Lowrie EG. Bioimpedance norms for the hemodialysis population. *Kidney Int.* 1997;52:1617–1621.

28. Di-Gioia MC, Gallar P, Rodriguez I, et al. Changes in body composition parameters in patients on haemodialysis and peritoneal dialysis. *Nefrologia.* 2012;32:108–113.

29. Di Iorio BR, Scalfi L, Terracciano V, Bellizzi V. A systematic evaluation of bioelectrical impedance measurement after hemodialysis session. *Kidney Int.* 2004;65:2435–2440.

30. Ward RA, Ouseph R, McLeish KR. Effects of high-flux hemodialysis on oxidant stress. *Kidney Int.* 2003;63:353–359.

31. Morena M, Delbosc S, Dupuy AM, Canaud B, Cristol JP. Overproduction of reactive oxygen species in end-stage renal disease patients: A potential component of hemodialysis-associated inflammation. *Hemodial Int.* 2005;9:37–46.

32. Johnson-Davis KL, Fernelius C, Eliason NB, Wilson A, Beddu S, Roberts WL. Blood enzymes and oxidative stress in chronic kidney disease: A cross sectional study. *Ann Clin Lab Sci.* 2011;41:331–339.

33. Spittle MA, Hoenich NA, Handelman GJ, Adhikarla R, Homel P, Levin NW. Oxidative stress and inflammation in hemodialysis patients. *Am J Kidney Dis.* 2001;38:1408–1413.

34. Dakshinamurty KV, Rao PV, Saibaba KS, et al. Oxidative stress in hemodialysis – Postdialytic changes. *Clin Lab.* 2003;49:255–261.

35. Paul JL, Sall ND, Soni T, et al. Lipid peroxidation abnormalities in hemodialyzed patients. *Nephron.* 1993;64:106–109.

36. Lim PS, Wei YH, Yu YL, Kho B. Enhanced oxidative stress in haemodialysis patients receiving intravenous iron therapy. *Nephrol Dial Transplant.* 1999;14:2680–2687.