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Bioelectrical impedance analysis in monitoring dogs with myxomatous mitral valve disease: a preliminary study

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ABSTRACT
Bioelectrical Impedance Analysis (BIA) is a simple, non-invasive, real-time diagnostic technique routinely used in human clinical practice to assess body composition and hydration status. BIA raw parameters, particularly the Phase Angle (PhA), are gaining attention as markers of congestive states in humans, as body electrical impedance can be affected by variations in body water content. This study aimed to assess whether changes in BIA raw parameters in dogs with myxomatous mitral valve disease (MMVD) could be linked to disease progression. Nine Cavalier King Charles Spaniels (CKCS) with MMVD were prospectively recruited in a longitudinal study design. During the clinical follow-up of each dog, BIA raw parameters - Impedance (Z), Resistance (R), Reactance (Xc), and PhA - were recorded alongside NT-proBNP assessments at two time points. A reduction in PhA recorded at 50 kHz (P = 0.03) and an increase in NT-proBNP concentrations (P = 0.02) were observed in parallel with the progression of MMVD in this group of CKCS between the two time points. These preliminary findings suggest the potential clinical application of BIA in dogs, emphasizing the need for further research to determine whether PhA could serve as a valuable diagnostic and prognostic marker in cardiac diseases, as has already been established in human patients.

Section: RESEARCH PAPER

Keywords: BIA; bioelectrical impedance analysis; phase angle; MMVD; congestion; dogs; measurement

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1. INTRODUCTION

Bioelectrical impedance analysis (BIA) is a general term that refers to the safe, non-invasive measurement of body impedance (Z), through the application of a painless, low-level alternating current via surface electrodes to the human or animal body. Z is characterized by two components: resistance (R, ohm) and reactance (Xc, ohm), and it depends on body water content, ionic conduction, and body composition [1].

R represents the opposition to the flow of alternating current as it passes through the body, arising from intracellular and extracellular fluids, whereas Xc is primarily due to the capacitive properties of cells and cellular membranes. Changes in body composition and body fluid imbalances are known to affect the electrical properties and conducting characteristics of the body, either enhancing, or reducing the passage of the current.

The use of BIA in human nutrition, based on the distinct electrical behaviours and impedance of fat and lean tissues, is well established. Recently, medical interest has shifted toward using BIA for fluid status assessment and the detection of fluid disorders, based on the theory that fluid overload enhances the conductivity of the electrical current passing through the body, while fluid loss or dehydration reduces this conductivity.

In humans, BIA has been proposed in the management of congestion due to increased total body water content in the context of congestive heart failure (CHF) [2], [3]. BIA raw data, obtained at one or more current frequencies, are used to estimate different body compartments using predictive equations, previously derived from reference intervals established in the human population. However, the use of classical equation-based predictive BIA is controversial in people with altered body fluid status [4], [5], thus there is a growing interest in using BIA raw parameters, particularly the Phase Angle (PhA), for the longitudinal assessment of patients with body fluid imbalances.

The bioelectrical *PhA* obtained through BIA reflects the relationship between *Xc* and *R* of the body and is calculated as the arctangent$[Xc/R] \times [180°/\pi]$. *PhA* is regarded a biological marker of cellular health and hydration, as a higher cell mass volume and robust cell membranes result in delayed signals, leading to a higher *PhA* [6].

Studies in humans demonstrated that *PhA* recorded with a 50kHz bioimpedance device is a powerful marker for disease severity and mortality in different clinical settings including haemodialysis and CHF [7] and, in CHF patients, it has been found closely related to congestion parameters [8].

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs leading to CHF, with some breeds like Cavalier King Charles Spaniel (CKCS) being most frequently and severely affected. The American College of Veterinary Internal Medicine (ACVIM) staging system for MMVD describes 4 basic stages of the disease: stages A, B (subdivided in B1 and B2), C and D [9]. In this staging system clinical examination, echocardiographic and radiographic signs of cardiac remodelling and serum N-terminal propeptide of B-type natriuretic peptide (NTproBNP) concentrations are currently recommended to diagnose and monitor MMVD, as well as to evaluate treatment efficacy and delay the onset or manage clinical signs of CHF.

Therapy is commonly directed to reverse fluid accumulation and the catabolic state that leads to cardiac cachexia. NTproBNP is released from the myocardium in response to cardiac wall stretching, as occurs during volume overload. Elevated NTproBNP concentrations have been associated with cardiac enlargement and increased risk of congestive heart failure and mortality in dogs with MMVD [10].

Up to this time, BIA studies in dogs have primarily focused on nutritional assessment and total body water estimation [11] and research has not yet been conducted on the clinical usefulness of BIA raw parameters in congestive states. The purpose of this study was to perform a longitudinal BIA analysis, recording raw bioelectrical parameters along with NTproBNP assessment, at two time points of the clinical follow-up of a group of CKCS with MMVD, to determine whether disease progression could be associated with changes in raw bioelectrical parameters.

2. MATERIALS AND METHODS

2.1. Study design

This is a prospective longitudinal study.

2.2. Dogs

Nine clients owned CKCS referred to the cardiology section of the University Teaching Hospital of Perugia between May 2021 and September 2022 and diagnosed with MMVD were prospectively enrolled in the study.

Dogs were included based on the clinical and echocardiographic diagnosis of MMVD and classified according to the ACVIM disease consensus [9]. Stage B1 included asymptomatic dogs without echocardiographic evidence of cardiac remodelling. Stage B2 included asymptomatic dogs with echocardiographic evidence of cardiac remodelling based on left atrial (LA) and left ventricular (LV) enlargement (LA/Aortic diameter ≥ 1.6; LV end-diastolic internal diameter normalized for body weight ≥ 1.7 using a scaling exponent of 0.294). Stage C included MMVD dogs with clinical signs of left-sided CHF diagnosed by a combination of history, physical examination (tachypnea, orthopnea, sleeping respiratory rate > 30 breaths/min, crackles), echocardiographic results, thoracic radiology and ultrasound findings, and response to diuretic therapy. Stage D included symptomatic patients with CHF refractory to standard treatment for Stage C. CKCS with concurrent systemic diseases or other cardiac abnormalities were excluded from the study. Any ongoing treatment for MMVD was not considered an exclusion criterion.

All experimental protocols used in this study adhered to European Union guidelines and were approved by the University of Perugia Bioethical Committee (protocol n°67668, 13/04/2021). Informed owner consent was obtained for each patient.

2.3. Bioelectrical Impedance Analysis

Bioelectrical impedance raw parameters were recorded in each dog at study inclusion (1° time point) using a portable, wireless, multifrequency (MF) bioimpedance analyser (Biosmart® EX.516(XX), Eupraxia, Italy). The device emitted an alternating sinusoidal electric current of 800 μA at operating frequencies of 50 and 100 kHz.

A calibration test was performed prior to each measuring session using the provided test circuit, which ensures system and data accuracy. BIA measurements were carried out alongside clinical and echocardiographic examination for each dog.

Measurements were performed on the right body side using a tetrapolar electrode configuration previously validated in dogs [11] (Figure 1).

Dogs were not sedated but gently restrained in a natural standing position on a non-conductive insulating mat making sure that all their four feet were in contact with the mat. To avoid electric interferences during measurements, care was taken to ensure that the dog did not contact any electrically conductive object. For this purpose, any collar or harness was removed, and dogs were handled using latex gloves.

Three sequential BIA measurements were performed. Each measurement lasted approximately 5 seconds and allowed for the real-time acquisition of whole-body BIA raw parameters, including impedance (*Z*, ohm), reactance (*Xc*, ohm), resistance (*R*, ohm) and Phase Angle (*PhA*, °), which was directly calculated from the device software.

The second time point of the study was scheduled according to the MMVD ACVIM consensus follow-up of the disease stage as follows:

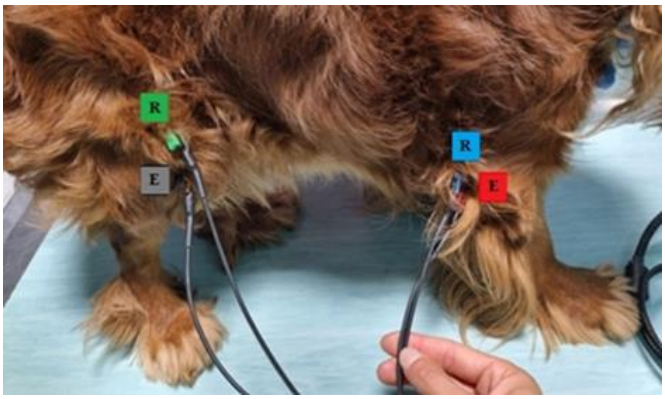

Figure 1. Whole-body right tetrapolar electrode configuration for multifrequency (MFBIA) analysis. Two Emitting current-injecting electrodes (E, grey and red) are placed dorsal to the elbow and dorsal to the patella. Two Receiving voltage-sensing electrodes (R, green and blue) are applied 3.5 dorsally to their respective emitting electrodes.

- Stage B1: 6 months (n2, n3) or 1 year (n1, n9)
- Stage B2: 6 months (n7, n8)
- Stage C /D class: 3 months (n4, n5, n6).

Dogs were treated in accordance with the MMVD ACVIM guidelines, which included the administration of diuretic drugs in stage C and D. The second time point (2°) of BIA measurement was carried out along with the cardiological reassessment of each dog, following the same protocol as the 1° time point.

2.4. NTproBNP

Blood samples were drawn from the cephalic vein or collected via jugular puncture into EDTA-tubes at both 1° and 2° time points. Samples were immediately centrifuged for 15 min at 1.500 g at room temperature to separate and isolate plasma. Plasma samples (1.5 ml) were stored in polypropylene tubes at −70 °C to prevent enzymatic degradation of the analyte until batch analysis. All samples were then packed frozen on dry ice and shipped to IDEXX Laboratories (IDEXX Laboratories Srl, Italy, Milan) for determination of NTproBNP concentrations.

2.5. Statistical Analysis

The three sequential MFBIA measurements obtained for each dog at the two time points were averaged and used for the analysis. Due to the small sample size, the Wilcoxon signed-rank test for non-normally distributed data was used to compare the bioelectrical parameters (*Z*, *R*, *X*c, and *PhA*, recorded at 50 kHz and 100 kHz) and NT-proBNP concentration between the two time points.

P-values less than 0.05 were considered statistically significant. Statistical analysis was performed using commercially available software (GraphPad Prism 5.00, GraphPad Software, San Diego, CA).

3. RESULTS

Nine CKCS (4 neutered males and 5 neutered females) were longitudinally studied. Mean ± SD age was 8.6 ± 2.5 years and mean ± SD body weight was 9.4 ± 2.6 kg. BIA was quick, feasible and well tolerated by all study dogs. Measurements were carried out in non-sedated dogs allowing to obtain in real time bioelectrical parameters.

Clinical progression of MMVD was observed in dog n5 (from C to D) and n8 (from B2 to C) between the two follow-up time points) (Table 1).

Median NTproBNP concentrations were 699 (377-3148) pmol/l at the 1° time point and 755 (414-4882) pmol/l at the 2° and differences were statistically significant between the two time points ($P = 0.02$) and significantly higher in the 2° (Figure 2).

Table 1. CKCS MMVD disease stage follow-up at both time points.

CKCK	Disease Stage	
	1° time point	*2° time point*
n1	B1	B1
n2	B1	B1
n3	B1	B1
n4	C	C
n5	C	D
n6	C	C
n7	B2	B2
n8	B2	C
n9	B2	B1

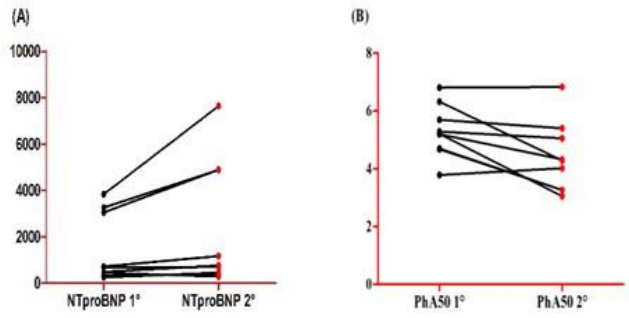

Figure 2. Changes in NTproBNP concentration (A) and PhA (B) between the 1° and 2° time points.

Table 2. Whole-body raw parameters obtained at the 1° and 2° time points. 1° vs 2°: *p*-value less than 0.05 was considered statistically significant (*). Data are expressed as median and interquartile range (IQR).

BIA raw parameters	1° time point	2° time point	Two-tailed *p*-value
	Median (IQR)	Median (IQR)	
Z 50 (Ω)	225 (206-260)	261 (221-285)	0.20
R 50 (Ω)	224 (205-259)	259 (220-285)	0.57
Xc 50 (Ω)	21 (18-25)	21 (14-25)	0.16
PhA 50 (°)	5.2 (4.7-5.2)	4.3 (3.3-5.2)	**0.02***
Z 100 (Ω)	211 (191-244)	248 (208-269)	0.13
R 100 (Ω)	210 (190-243)	246 (208-268)	0.05
Xc 100 (Ω)	19 (14-23)	18 (6.3-22)	0.35
PhA 100 (°)	5.2 (4.2-6)	4 (1.8-4.9)	0.13

Statistically significant differences were not found for raw bioelectrical parameters between the 1° and 2° time point except for the PhA obtained at 50 kHz ($P = 0.03$) (Table 2), which was reduced at the 2° time point (Figure 2).

4. DISCUSSION

CHF is a complex clinical syndrome resulting in fluid accumulation, cardiac remodelling, and cachexia. Due to its complexity and its gradual progression over weeks or months, each diagnostic tool has practical limitations in the early detection of congestive status and the loss of functional body mass. This has led to the development of an integrated diagnostic algorithm that involves clinical examination, diagnostic imaging and biomarkers.

A statistically significant increase in NTproBNP along with a decrease of *PhA* at 50 kHz was observed as MMVD progressed in our cohort of 9 CKCS. The bioelectrical *PhA* similarly showed lower values in human patients in New York Heart Association classes of CHF, with lower *PhA* values in patients in classes III-IV, than in those in classes I-II [12].

PhA was also found significantly smaller in CHF patients with peripheral edema and fluid retention, while it increased soon after therapies for restoration of clinical stability [13].

Indeed, as the main determinants of *PhA* are the fluid and the nutritional status of patients [14], the variations in *PhA* may be considered as the mirror of variations in fluid and nutritional status also in CHF patients. When adjusted for age, haemoglobin levels and diabetes, a *PhA* < 4.2 was identified as an independent predictor of all-cause mortality in people with chronic heart failure [15].

In our group of CKCS affected by MMVD the median *PhA* recorded at the 2° time point was 4.3. Whether this finding could

correspond to a predictive cut-off similar to that in human patients should be determined through long-term survival longitudinal studies. *PhA* values are easy to measure and could be obtained quickly and in real-time. Based on human data, we can hypothesize that *PhA* may be a valuable prognostic marker in dogs with CHF and its usefulness could be extended to the evaluation of decongestive treatments.

Further studies could establish *PhA* cut-off points for monitoring of dogs with heart disease. These *PhA* cut-off values could be integrated into current monitoring protocols to identify dogs at risk of developing CHF and to better guide therapeutic interventions and manage disease follow-up.

This study has several limitations. BIA itself it's a technique that could be affected by various factors [11]. However, we adopted a standardized protocol, a longitudinal study design and only one canine breed minimize potential interferences. The primary limitation of our study is its preliminary nature and the small sample size. In addition, although our study group included only CKCS to reduce morphometric influence on BIA measurements, we analysed CKCS at different stages of MMVD together, which may have affected NTproBNP and bioelectrical results. Despite the morphological homogeneity of our group, *PhA* is known to be influenced by sex, ethnicity, and body composition in humans [6]. Consequently, we did not assess the potential impact of age, sex, or body composition changes on PhA in our group of CKCS. Moreover, reference intervals for bioelectrical raw parameters have not yet been established for dogs, so the lower PhA values recorded at the 2° time point should be interpreted carefully.

5. CONCLUSIONS

The application of BIA technology in dogs is feasible, quick, safe, and well-tolerated. Measurements can be performed without sedation or anaesthesia, and the electrode crocodile clips used in this study were similar to those of an ECG and did not cause any discomfort to the animals. Real-time results, portability, and low cost highlight the potential for point-of-care application of the technique.

A reduction in bioelectrical *PhA* and an increase in NTproBNP were observed in parallel with the progression of MMVD in this group of 9 CKCS. These preliminary findings suggest the potential clinical usefulness of the method, encouraging and underscoring the need for further research to determine whether the *PhA* could serve as a valuable diagnostic and prognostic marker in MMVD and CHF within an integrated diagnostic algorithm.

REFERENCES

[1] H. C. Lukaski, N. Vega Diaz, A. Talluri, L. Nescolarde, Classification of hydration in clinical conditions: indirect and direct approaches using bioimpedance, Nutrients 11(4) (2019), no. 809.
DOI: 10.3390/nu11040809

[2] P. Scicchitano, M. M. Ciccone, M. Iacoviello, P. Guida, M. De Palo, P. Angela, M. Basile, P. Sasanelli, (+ 4 more authors), Respiratory failure and bioelectrical phase angle are independent predictors for long-term survival in acute heart failure, Scand. Cardiovasc. J. 56(1) (2022), pp. 28-34.
DOI: 10.1080/14017431.2022.2060527

[3] T. Sakaguchi, K. Yasumura, H. Nishida, H. Inoue, T. Furukawa, K. Shinouchi, H. Miura, K. Miyazaki, (+ 8 more authors), Quantitative assessment of fluid accumulation using bioelectrical impedance analysis in patients with acute decompensated heart failure, Circ. J. 79(12) (2015), pp. 2616-2622.
DOI: 10.1253/circj.CJ-15-0723

[4] U. G. Kyle, I. Bosaeus, A. D. De Lorenzo, P. Deurenberg, M. Elia, J. M. Gomez, B. Lilienthal Heitmann, L. Kent-Smith, (+ 5 more authors), Bioelectrical impedance analysis - part I: review of principles and methods, Clin. Nutr. 23(5) (2015), pp. 1226-1243.
DOI: 10.1016/j.clnu.2004.06.004

[5] S. Ugras, Evaluating of altered hydration status on effectiveness of body composition analysis using bioelectric impedance analysis, Libyan J. Med. 15(1) (2020), pp. 1741904.
DOI: 10.1080/19932820.2020.1741904

[6] M. C. G. Barbosa-Silva, A. J. D. Barros, Bioelectrical impedance analysis in clinical practice: a new perspective on its use beyond body composition equations, Curr. Opin. Clin. Nutr. Metab. Care. 8(3) (2005), pp. 311-317.
DOI: 10.1097/01.mco.0000165011.69943.39

[7] L. M. Garlini, F. D. Alves, L. B. Ceretta, I. S. Perry, G. C. Souza, N. O. Clausell, Phase angle and mortality: a systematic review, Eur. J. Clin. Nutr. 73(4) (2019), pp. 495-508.
DOI: 10.1038/s41430-018-0159-1

[8] F. D. Alves, G. C. Souza, N. Clausell, A. Biolo, Prognostic role of phase angle in hospitalized patients with acute decompensated heart failure, Clin. Nutr. 35(6) (2016), pp. 1530-1534.
DOI: 10.1016/j.clnu.2016.04.007

[9] B. W. Keene, C. E. Atkins, J. D. Bonagura, P. R. Fox, J. Häggström, V. L Fuentes, M. A. Oyama, J. E. Rush, (+ 2 more authors), ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs, J. Vet. Intern. Med. 33(3) (2021), pp. 1127-1140.
DOI: 10.1111/jvim.15488

[10] M. Ogawa, Y. Hori, N. Kanno, N. Iwasa, T. Toyofuku, N. Isayama, A. Yoshikawa, R. Akabane, (+ 5 more authors), Comparison of N-terminal pro-atrial natriuretic peptide and three cardiac biomarkers for discriminatory ability of clinical stage in dogs with myxomatous mitral valve disease, J. Vet. Med. Sci. vol. 83(4) (2021), pp. 705-7015.
DOI: 10.1292/jvms.20-0629

[11] L. Yaguiyan-Colliard, C. Dumas, S. Bousbiat, M. Jaffrin, P. Cardot, D. Grandjean, N. Priymenko, P. Nguyen, (+ 1 more author), Indirect prediction of total body water content in healthy adult Beagles by single-frequency bioelectrical impedance analysis, Am. J. Vet. Res. 76(6) (2015), pp. 547-553.
DOI: 10.2460/ajvr.76.6.547

[12] L. Castillo Martínez, E. Colín Ramírez, A. Orea Tejeda, E. A. Lafuente, L. P. Bernal Rosales, V. Rebollar González, R. Narváez David, J. Dorantes Garcìa, Bioelectrical impedance and strength measurements in patients with heart failure, Nutrition 23(5) (2007), pp. 412-418.
DOI: 10.1016/j.nut.2007.02.005

[13] F. D. Alves, G. C. Souza, G. Badin Aliti, E. R. Rabelo-Silva, N. Clausell, A. Biolo, Dynamic Changes in bioelectrical impedance vector analysis and phase angle in acute decompensated heart failure, Nutrition 31(1) (2015), pp. 84-89.
DOI: 10.1016/j.nut.2014.05.004

[14] H. C. Lukaski, U. G Kyle, J. Kondrup, Assessment of adult malnutrition and prognosis with bioelectrical impedance analysis: phase angle and impedance ratio, Curr. Opin. Clin. Nutr. Metab. Care 20 (2017), pp. 330-339.
DOI: 10.1097/MCO.0000000000000387

[15] E. Colín-Ramírez, L. Castillo-Martínez, A. Orea-Tejeda, M. Vázquez-Durán, A. E. Rodríguez, C. Keirns-Davis, Bioelectrical impedance phase angle as prognostic marker in chronic heart failure, Nutrition 28(9) (2012), pp. 901-905.
DOI: 10.1016/j.nut.2011.11.033