


Evaluation of the diagnostic value of the renal resistive index as a marker of the subclinical development of cardiorenal syndrome in MMVD dogs

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Barbara Szczepankiewicz¹ , Urszula Paślawska^{1,2},
 Natalia Siwińska¹, Krzysztof Plens³ and Robert Paślawski²

Abstract

Introduction: Myxomatous mitral valve disease (MMVD) in dogs inevitably causes renal dysfunction. These interactions are known as the cardiorenal syndrome (CRS). The main aims of the study were to evaluate whether renal resistive index (RRI) may be useful as a non-invasive marker in subclinical stage of kidney injury in dogs with MMVD and to compare RRI with SDMA and Cyst C.

Methods: Forty-four dogs were divided into two groups: control—15 healthy dogs and the heart group—29 dogs with MMVD (ACVIM class Cc). Study protocol included: anamnesis, clinical examination, electrocardiography, echocardiography, chest radiography, abdominal ultrasonography with measurements of the renal resistive index (RRI), urine, and blood analysis.

Results: The RRI in the heart group was significantly higher 0.725 ± 0.035 versus control group 0.665 ± 0.028 ($p < 0.00085$). The RRI cut-off point in dogs with stable chronic heart failure (CHF) under 8 years is 0.775, in older 0.64. RRI was similar in MMVD dogs treated with ACE-I + furosemide and dogs treated ACE-I + torasemide + pimobendan + spironolactone. There was no correlation between RRI and SDMA or Cyst C.

Conclusion: RRI is more sensitive than creatinine, SDMA and Cyst C to reveal kidney injury in MMVD dogs class Cc younger than 8 years.

Keywords

Canine heart failure, mitral insufficiency, myxomatous mitral valve disease, echocardiography, kidney, ultrasonography, RRI, aldosterone

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Introduction

Secondary renal failure due to primary heart disease is known as the cardiorenal syndrome (CRS).¹ A low cardiac output, caused by heart disease, for example myxomatous mitral valve disease (MMVD) results in deterioration of glomerular filtration, increased urea reabsorption in the tubules, and simultaneous activation of the neurohormonal compensatory mechanism, for example, renin-angiotensin-aldosterone system (RAAS).² Serum urea and creatinine are markers most commonly used to assess kidney function and estimate the glomerular filtration rate (GFR) in small animal clinical practice. However, their increase may be the result of prerenal azotemia.³ In recent years, serum symmetric dimethylarginine (SDMA) and cystatin

C (Cyst C) proved to be a good marker of kidney failure, but due to their relatively high price, they are still not as

¹Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

²Institute of Veterinary Medicine, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Torun, Poland

³KCRI, Krakow, Poland

Corresponding author:

Barbara Szczepankiewicz, Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Pl. Grunwaldzki 47, 50-366 Wrocław, Poland.
 Email: barbara.szczepankiewicz@upwr.edu.pl



often used in practice as urea and creatinine.⁴ Additionally, determination of podocin in canine urine samples was promising for early detection of glomerular injury.⁵⁻⁷ However methods for determining podocin are impractical. Therefore, current diagnostic methods are still largely based on ultrasound techniques.

Renal ultrasonography is an attractive diagnostic tool because it is commonly available and reasonably inexpensive.⁸⁻¹⁰ Moreover, this technique is painless and without adverse effects. The use of B-mode and Doppler ultrasound modalities reveals the effects of different pathophysiological pathways involved in renal disease and may identify a deterioration of renal function prior to an increase in serum creatinine. Hence, these modalities play a key role in early detection of disease.^{11,12}

Recent studies have shown that renal resistive index (RRI) is correlated with glomerular injury and tubulointerstitial lesions (most common kidney diseases in dogs and cats) and vascular lesions in the kidney.^{3,13-15} The first study documenting the increase of RRI in the intrarenal artery in dogs with MMVD was published in 2012.¹⁶ Since then, advances have been made in ultrasound imaging while algorithms used for classification and treatment of MMVD in dogs have changed.¹⁶ The inclusion of torasemide and pimobendan in the treatment guidelines of stage Cc MMVD was one of the most important changes. Torasemide acts less rapidly than furosemide, causes slower and gentler pressure decreases and smaller electrolyte disturbances. Thus, it should interfere with the RRI result to a lesser extent.

Our goal was to compare the RRI in dogs treated with angiotensin converting enzyme inhibitor (ACE-I) + torasemide + pimobendan with the results of a previous study, in which dogs were treated ACE-I and furosemide, and to compare the results with a group of healthy dogs. The second aim was to compare RRI measurements with other markers of early renal failure such as: SDMA and Cyst C. But the most important and the main aim of the study was to evaluate whether the RRI may be a clinically useful, non-invasive marker for the detection of early stage kidney injury in dogs with chronic cardiac failure (CRS).

Materials and methods

Study design

Animals. The study included 44 dogs, which were patients of the Department of Internal Diseases with the Clinic of Horses, Dogs and Cats, Faculty of Veterinary Medicine at the University of Life Sciences in Wrocław. The study was performed under standardized conditions previously described by Chetboul et al.¹⁶ Overnight-fasted dogs underwent a physical examination, blood pressure measurement, electrocardiographic (ECG) and echocardiographic examinations, chest radiographs, abdominal ultrasonography (USG), and blood collection, all performed on the same

day. All procedures were carried out without pharmacological restraint.

Based on the anamnesis and clinical examination, the dogs were divided into two groups: (1) the control group that included 15 healthy dogs and (2) the heart group that included 29 dogs with stable chronic heart failure (CHF).

There were two inclusion criteria in the control group: the absence of any signs of illness in the clinical examination (and in the last 6 months prior to the study) and normal results in all the laboratory tests.

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs and consists of four stages of disease (A, B, C, D according to ACVIM classification) ordered from the least advanced form of the disease.¹⁷ In our work we chose stage C, because dogs in this condition are the most common group of animals during cardiological consultation. Dogs included in the heart group had moderate symptoms of MMVD, that is, CHF—stage Cc according to the ACVIM classification scheme.¹⁷ Dogs in this groups had: myxomatous (degenerative) changes on the mitral valve leaflets, hemodynamically significant mitral regurgitation, dilatation of the left atrium and an enlarged left ventricle (confirmed echocardiographically), mild clinical symptoms, for example, weakness, reduced exercise tolerance, increased breathing after physical exercise, restlessness or agitation while sleeping, sometimes coughing or gagging. Heart murmurs were detected in all the dogs in this group during the clinical examination. These dogs required chronic management of heart failure (HF).¹⁷ All the dogs were treated with pimobendan 0.25–0.3 mg/kg PO q12h, benazepril hydrochloride 0.25 mg/kg POq24h, spironolactone 2 mg/kg POq24h, and torasemide 0.1–0.6 mg/kg POq24h. Torasemide was used in relation to the respiratory symptoms. The presence of abnormally high levels of serum creatinine >1.4 mg/dl, 125 µmol/l was an exclusion criterion.

The exclusion criteria for all the groups were: pregnancy, lactation, periods of growth and convalescence, the presence of diseases that could significantly affect the blood flow in the kidneys, for example, cancer, central nervous system diseases, the presence of an acute inflammatory process, food poisoning or an intake of medication, such as glucocorticosteroids that could significantly alter the kidney blood flow. Dogs with chronic kidney disease and with renal artery stenosis, as well as endocrine and immunological diseases and acute respiratory failure were also excluded from the study. Additionally dogs with advanced HF requiring hospital treatment (acute stage of heart disease) were also excluded from this study.

Clinical examination with measurements of arterial pressure

A full history, including information on previous diseases, was taken and a clinical examination with particular emphasis on the circulatory and urinary systems was performed. The measurement of systolic arterial blood

pressure (SAP) was performed on the common digital artery after a 20 min. rest period using a Doppler Flow Detector (model 811-B, Parks Medical Electronics Inc. USA). An average of 3–5 consecutive SAP measurements was recorded.

Blood sample collection and assays

Blood was collected from the v. saphena or v. cephalica antebrachii into 2 ml EDTA blood tubes. The hematological examination was performed immediately after the blood collection using an IDEXX LaserCyte (IDEXX, Westbrook, Maine, USA) hematology analyzer and a Horiba ABC animal blood counter (Horiba ABX SAS, Montpellier, France). The following parameters were measured: red blood cell count (RBC), white blood cell count (WBC), concentration of hemoglobin (HGB), hematocrit (HT), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution (RDW), mean platelet volume, platelets (PLT) including the number of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The biochemical parameters were assessed in serum, after centrifugation, using the TermoScientific Konelab Prime 30ISE (Thermo Fisher Scientific, Vantaa, Finland) biochemical analyzer in the analytical laboratory of the Department of Internal Diseases of Horses, Dogs and Cats. The concentration of sodium (Na), potassium (K), ionized calcium (Ca^{2+}), magnesium (Mg), iron (Fe), glucose, urea, creatinine, total protein, albumin, aspartate transaminase (AspAT), alanine transaminase (ALT), C-reactive protein (CRP), and aldosterone was also determined. The dogs were considered azotemic if the serum creatinine and urea concentrations were $>132.6 \mu\text{mol/l}$ and $>8.33 \text{ mmol/l}$, respectively. The dogs were considered to have prerenal azotemia if they had increased serum urea concentrations without concomitant increased serum creatinine concentrations.

In addition, serum samples were transported to the IDEXX Ludwigsburg Germany Laboratories to determine concentrations of SDMA and Cyst C. SDMA was determined using an enzyme immunoassay dedicated to dogs. Cyst C was determined by liquid chromatography with mass spectrometry—a nephelometric analysis dedicated to dogs. Reference values for urea, creatinine, Cyst C, SDMA, aldosterone that were used in this study have been published elsewhere.^{3,18,19}

Examination of urine

Morning urine was collected by the owner into a sterile container during spontaneous urination. The urine examination was carried out immediately after receiving the urine samples. A physicochemical examination of urine included an analysis of its color, transparency, specific gravity, pH, protein, albumin, creatinine, glucose, blood, acetone, and urobilinogen levels. Urine albumin, protein,

and creatinine were measured in urine supernatant using the TermoScientific Konelab Prime 30ISE (Finland) biochemical analyser in the analytical laboratory. Urine albumin was measured using calibration kit-SpeciCal and control-SpeciTrol and SpeciTrol High. UPC and UAC were calculated by dividing the protein and albumin concentration (mg/dl) by the urine creatinine concentration (mg/dl). The result was a unitless ratio (dimensionless).

Chest X-ray examination

Right lateral, left lateral, and dorso-ventral thoracic radiographic images were obtained using a Gierth CHF200 (X-ray tube Toshiba, Japan) digital device. The lung field, dilation of the pulmonary artery and vein, elevation of the distal part of trachea toward the spine and the presence of cardiomegaly were assessed.²⁰ The size of the heart was determined using the vertebral heart scale (VHS scale).²⁰

Abdominal ultrasonography

Prior to performing a transabdominal ultrasound of the kidneys, the hair from the adjacent areas was shaved and a water-soluble coupling gel was applied. A standard abdominal ultrasound examination was carried out using the Hitachi Aloka F37 (Hitachi Aloka Medical, Ltd., Tokyo, Japan) machine with a 5–10 MHz linear probe. After imaging the kidney in the sagittal or parasagittal projection, its size, appearance, and echogenicity using gray-scale ultrasonography were evaluated.²¹ Intrarenal vessels were localized using color Doppler techniques, and the arcuate arteries were visualized on the longest possible kidney surface. Flow spectrum was obtained using the spectral pulsed Doppler technique. Waveforms were measured on the narrowest frequency range possible without aliasing. The gate size was 2 mm with a wall filter of 50 Hz. RRI was measured in the arcuate artery of the left (RIL) and right (RIR) kidney until three adequate consecutive spectral waveforms were obtained. RRI was calculated on the basis of the ratio between peak systolic velocity (PSV) and end diastolic velocity (EDV), according to the formula: $\text{RRI} = (\text{PSV} - \text{EDV}) / \text{PSV}$.^{22,23} RRI value was measured automatically by the ultrasound machine software. The average RRI value for each kidney was calculated from three measurements obtained from the arcuate arteries. Care was taken to obtain the best visualization of the ultrasound beam and its diagnostic value was assessed by multiple measurements of central renal arcuate artery flow. The Doppler study was abandoned in some dogs with severe dyspnea and accelerated respiratory rate associated with MMVD.

Echocardiographic with Doppler examination and electrocardiography

Standard thoracic parasternal echocardiography was performed with a simultaneous ECG recording using a Hitachi

Table 1. Clinical data of the dogs in the control group, heart group (dogs with mitral regurgitation, ACVIM Class Cc).

Characteristics	Control group	Heart group	p Value
Number of dogs	N=15	N=29	
Female/male	8/7	12/17	
Age (years)	5.4 ± 2.6	11.0 ± 2.0	0.0001
Body weight (kg)	14.3 ± 7.0	10.3 ± 4.0	0.04
Breed of dogs	Cross breed (N=4), Beagle (N=4), Cavalier King Charles Spaniel (N=1), Miniature Schnauzer (N=2), Shi Tzu (N=1), Wire Fox Terrier (N=1), Whippet (N=1), Border Terrier (N=1).	Cross breed (N=13), Cavalier King Charles Spaniel (N=4), Miniature Schnauzer (N=4), Shi-tzu (N=2), Yorkshire terrier (N=2), Dachshund (N=2), Chihuahua (N=1), Cocker Spaniel (N=1)	

Numbers or mean ± standard deviation.

Aloka F37 (Hitachi Aloka Medical, Ltd., Tokyo, Japan) echocardiograph and a 5–7.5 MHz sector probe. The aorta diameter (Ao), left atrium size (LA), end-diastolic (RVIDd) internal diameter of the right ventricle, end-diastolic (LVIDd) and end-systolic (LVIDs) internal diameter of the left ventricle, thickness of the interventricular septum at end diastole (IVSd) and end systole (IVSs), end-diastolic (LVFWd) and end-systolic (LVFWs) thickness of the free wall of the left ventricle were measured. The left atrium-to-aorta ratio LA/Ao and shortening fraction (FS) of the left ventricle (using the Teicholz formula) was calculated. Mitral regurgitation (MR) was calculated as a mean value from the measurements obtained using the continuous wave Doppler technique using the left parasternal two- or four-chamber view. Echocardiography was based on previously based values.¹⁷

Statistical analysis

Statistical analyses was performed using StatSoft Statistica PL 12.0 software. Data were expressed as mean and standard deviation (±SD) or median (depending on the distribution of variables). Data distributed normally was analysed using the Kolmogorov-Smirnov test, followed by the ANOVA and two-sample *t*-test. The U-Man test was used for non-normally distributed variables. Simple or multiple ordinal regression tests were used to describe the relationships between variables. A *p* value <0.05 was considered statistically significant. Multivariate logistic models were fitted using forward stepwise regression. Due to a limited number of observations, a maximum of two covariates were included in the model. Sensitivity comparison was calculated by McNemar.

This research does not require the consent of the ethics committee as the experimental procedures did not go beyond the framework of clinical diagnostics in dogs.

Results

The results of the clinical, hematological, biochemical, and urine analysis are summarized in Tables 1 to 5.

Table 2. Hematological results of dogs in the control group, heart group (dogs with mitral regurgitation ACVIM Class Cc).

Variables	Control group	Heart group	p Value
RBC T/l	6.9 ± 0.6	6.9 ± 0.9	NS
HGB mmol/l	10.4 ± 2.0	11.5 ± 3.0	NS
HT %	48.0 ± 5.0	48.0 ± 6.0	NS
WBC G/l	8.4 ± 2.9	9.7 ± 3.6	NS

HGB: concentration of hemoglobin; HT: hematocrit; RBC: red blood cell count; WBC: white blood cell count; NS: not statistically significant. Average ± standard deviation.

Dogs in the heart group were statistically significantly older and lighter than the healthy dogs (Table 1).

There were no statistically significant differences in the MCV, MCH, MCHC, RDW, MPV, PLT, lymphocyte, monocyte, granulocyte, and reticulocyte count between groups, and all the values were within the reference range (Table 2).

According to the study objectives, all the dogs in the control group had normal values of urea, creatinine, Cyst C, and SDMA. We found that all the dogs in the heart group had serum creatinine values in the reference range. Urea concentration was elevated in four dog in the heart group. These azothemic dogs had a urea concentration that ranged from 9.7 to 18.1 mmol/l, (two of them had a normal urinalysis and two had an increased albumin concentration). One azothemic dog had an elevated serum SDMA and serum Cyst C concentration while another dog had an elevated serum SDMA and urine albumin concentration. The CHF dogs had lower iron and chloride blood serum concentrations, an elevated ALAT activity and elevated levels of CRP compared to healthy dogs. Despite the fact that the average level of aldosterone was visibly higher in the heart versus the control group, the large individual variations made the difference statistically insignificant (Table 3).

The urine specific gravity and urine creatinine were significantly lower in the heart group compared to the control group. Urine protein, UPC and UAC were higher in the heart group in comparison to the healthy dogs (Table 4).

Table 3. Blood biochemistry of dogs in the group of healthy dogs (control group), the dogs with mitral regurgitation ACVIM Class Cc (heart group).

Variables	Control group	Heart group	p Value
Urea mmol/l	5.2 ± 1.3	7.5 ± 4.4	NS
Creatinine µmol/l	86.5 ± 18.0	87.2 ± 29.0	NS
SDMA µg/dl	9.9 ± 1.8	14.0 ± 5.5	0.001
Cyst C mg/l	0.9 ± 0.1	1.5 ± 0.6	0.0009
Total protein g/l	59.4 ± 3.7	61.0 ± 7.0	NS
Albumin g/l	31.5 ± 2.0	32.0 ± 4.0	NS
Mg mmol/l	0.8 ± 0.1	0.8 ± 0.2	NS
Na mmol/l	145.0 ± 2.6	146.0 ± 2.7	NS
K mmol/l	4.6 ± 0.3	4.7 ± 0.5	NS
Cl mmol/l	110.4 ± 1.5	108.0 ± 3.4	0.02
Ca ²⁺ mmol/l	1.27 ± 0.04	1.35 ± 0.25	NS
Fe µmol/l	33.0 ± 7.6	25.0 ± 7.0	0.006
CRP mg/l	1.8 ± 0.3	2.8 ± 1.5	0.0009
Glucose mmol/l	5.0 ± 0.4	5.5 ± 0.9	NS
AspAt U/l	25.5 ± 5.0	31.5 ± 15.0	NS
AIAT U/l	40.0 ± 18.0	73.4 ± 55.7	0.017
Aldosteron pg/ml	128.10 ± 89.66	145.36 ± 135.00 ± 135.00	NS

AIAT: alanine transaminase; AspAT: aspartate transaminase; Ca²⁺: ionized calcium; Cl: chlorine; CRP: C-reactive protein; Cyst C: cystatin C; Fe: iron; K: potassium; Na: sodium; Mg: magnesium; SDMA: symmetric dimethylarginine; NS: not statistically significant. Average ± standard deviation.

Table 4. Urinary examination of dogs in the control group, the group of dogs with mitral regurgitation ACVIM Class Cc (heart group).

Variable	Control group	Heart group	p Value
Specific gravity	1.1 ± 0.15	1.04 ± 0.1	0.0009
PH	6.5 ± 0.6	6.2 ± 0.7	NS
Urine protein g/l	0.01 ± 0.05	0.3 ± 0.38	0.002
UPC	0.15 ± 0.12	0.43 ± 0.5	0.0016
Urine albumin mg/l	13.2 ± 16.5	34.5 ± 36.0	NS
UAC mg/dl	5.4 ± 6.6	55.0 ± 71.0	0.00005
Urine creatinine mmol/l	21.8 ± 5.7	9.5 ± 5.9	0.00001

UAC: urine albumin/creatinine ratio; UPC: urine protein/creatinine ratio; NS: not statistically significant. Mean ± standard deviation.

Heart rate (HR) was the lower in healthy dogs but the average of this parameter did not differ significantly. The LA/Ao, EF, RIL, and RIR was statistically higher in the heart group versus healthy group. Three dogs had elevated RIL and RIR in the control group. In heart group 25 dogs had elevated RIL and 27 had elevated RIR (Table 5).

Ultrasound measurements in the arcuate RRI in the heart group was statistically higher for both kidneys compared to the previous research by Chetboul et al.¹⁶ (Table 6).

There was a tight correlation between the RRI in the left and right renal arteries in the MMVD dogs $r^s = +0.84$ (Figure 1). The RRI value was negatively correlated with proteinuria RIL $r^s = -0.5$ and RIL $r^s = -0.4$ in the heart group (Figure 2).

The multivariate logistic regression (Figure 3) showed that the RRI cut-off point in dogs with CHF above 8 years of age exceeded 0.64, while in dogs younger than 8 years

old it exceeded 0.775 and these values were used to differentiate the control group from the heart group.

Discussion

Our research showed that the RRI value was increased in dogs with CHF (Table 5). The RRI values of the left and right kidneys in this study did not differ significantly. Both observation are in line with previous studies^{13,15} (Figure 1). The upper physiological reference value of the RRI for adult dogs and cats ranges from 0.70^{23,24} to 0.73.²⁵ The RRI for animals under 4 months of age is higher than that for older animals and reaches 0.75⁹ in dogs and 0.71 in cats.²⁵ A similar phenomenon of elevated RRI has been observed in children.^{7,9,26} Age related concentration has been also noted in SDMA concentration, which is also higher in young animals compared to older ones (16 µg/dl

Table 5. Ultrasound measurements in the control group, the group of dogs with mitral regurgitation ACVIM Class Cc (heart group).

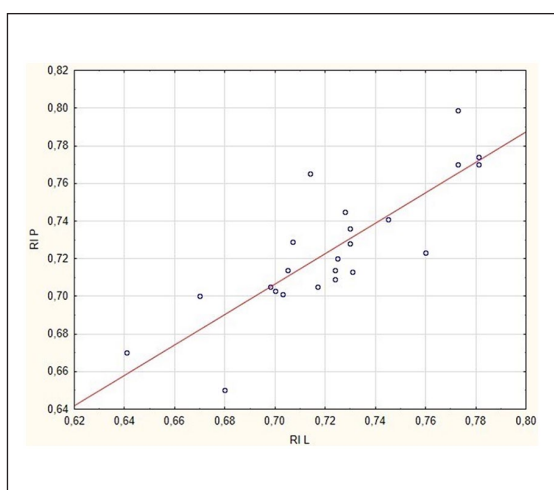
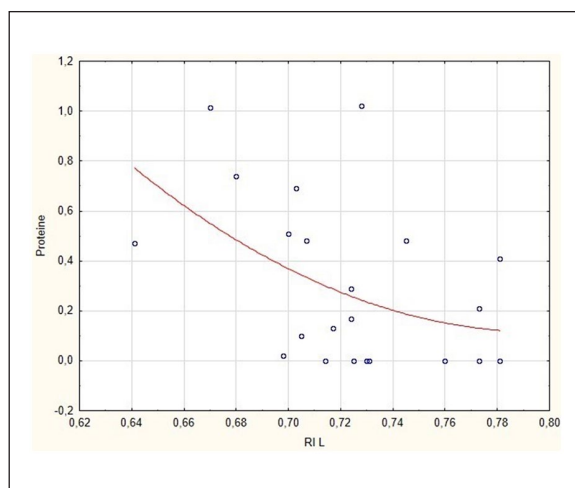
Variable	Control group	Heart group	p Value
LA/Ao	1.35 ± 0.09	2.02 ± 0.50	0.002
LVIDd mm	31.0 ± 6.7	37.0 ± 7.9	NS
LVIDs mm	20.5 ± 7.8	21.0 ± 8.0	NS
EF	61.9 ± 15.6	75.0 ± 10.5	0.04
FS	37.0 ± 13.5	43.8 ± 10.0	NS
HR bpm	134.0 ± 28.0	139.0 ± 30.0	NS
SAP mmHg	129.0 ± 10.0	160.0 ± 28.0	NS
RRIL	0.66 ± 0.05	0.72 ± 0.04	0.001
RRIR	0.67 ± 0.05	0.73 ± 0.03	0.0007

EF: ejection fraction of left ventricle; FS: fractional shortening of left ventricle; HR: heart rate; LA/Ao: left atrium-to-aorta ratio; LVIDd: left ventricular end-diastolic diameter; LVIDs: left ventricular end-systolic diameter; SAP: systolic arterial pressure; RRIL: renal resistive index of left kidney; RRIR: renal resistive index of the right kidney; NS: not statistically significant. Average ± standard deviation.

Table 6. Ultrasound measurements in arcuate RI in heart group compared to previous research.

	Szczepankiewicz et al. ⁵⁻⁷	Chetboul et al. ¹⁶	p Value
RRIL	0.72 ± 0.04	0.65 ± 0.08	0.0014
RRIR	0.73 ± 0.03	0.64 ± 0.08	<0.0001

RRIL: renal resistive index of left kidney; RRIR: renal resistive index of the right kidney.

**Figure 1.** Scatter plot for RRI of left kidney (RIL) and right kidney (RRIP) in dogs with stage Cc cardiac failure.**Figure 2.** Scatter plot for RI of left kidney (RIL) and proteinuria in dogs with stage Cc cardiac failure.

for dogs under the age of 12 months vs 14 µg/dl for dogs older the age of 12 months).^{3,9,27}

Age in dogs with MMVD and in healthy dogs—significantly affects the RRI values, an analysis of this effect was carried out. The cut off point for an age-related elevation of RRI in dogs was set at 8 years (Figure 3). The RIL cut-off point in dogs with CHF younger than 8 years old is ≥ 0.775 and in 8 years and older is ≥ 0.64 . The RRI cut-off point for healthy dogs younger than 8 years is ≤ 0.775 and

in 8 years and older is ≤ 0.64 . In this two groups of dogs (8 years and older with CHF and in healthy younger than 8 years) the value as a marker is useless because the top of physiological range is < 0.7 . In contrast to this RRI may be valuable tool for dogs with CHF younger than 8 years and healthy dogs older than 8 years (Figure 3).

In human medicine, it has been proven that the level of RRI is influenced not only by age, but by other factors such as: HR and irregular rhythm, respiratory rate, and

SAP.^{2,9,28,29} In the present study there was no correlation between the RRI value versus HR and SAP, which corresponds to the findings in previous studies carried out on animals (pigs, horses)^{9,30} In human and animals, suddenly and long-term rhythm changes decrease in HR causes an increase in the RRI for that reason such findings suggest that these parameters should be interpreted together in animals suffer from paroxysmal bradycardia, tachycardia or arrhythmia and with long-term rhythm changes.³¹ In our study, healthy dogs and those in the stage Cc did not have bradycardia, tachycardia or arrhythmia. Dogs in the early stage Cc also do not have rhythm changes, that is, atrial fibrillation appears in a more advanced stage of the heart disease. The intriguing observation is the lack of correlation between RRI and HR in dogs in contrast to reports from human medicine. These and other differences, such as the aforementioned lack of correlation between HR and CHF, show that the results obtained in human medicine cannot be easily compared with veterinary medicine.¹⁵

Literature data suggested that RRI may be a useful marker to monitor renal function during CHF due to MMVD, as it does not respond to ACE-I (benazepril hydrochloride) and diuretics, unlike creatinine (sensitivity is significantly different for RIL vs creatinine).^{32–35} The comparison of RRI value in the heart group for both kidneys of dogs (treated with ACE-I + torasemide + pimobendan + spironolactone) shows a higher RRI level with a smaller standard deviation in comparison to earlier research (dogs treated using ACE-I + furosemide). The effect of cardiovascular drugs on RRI values is not fully understood. Scientific studies conducted on these groups of drugs indicate that pimobendan has a double effect: increases the contractility of the heart muscle and causes vasodilation.³⁶ It is generally accepted that the vasodilatory effect compensates for the positive inotropic effect. However, studies show that pimobendan causes an increase in blood flow through the kidneys, an increase in the GFR after just 2 weeks of use.³⁶

ACE-I has been shown to reduce serum aldosterone levels at the beginning of MMVD, however during development of MMVD aldosterone level can continue to rise in patients receiving an ACE-I and pimobendan. For that reason the aldosterone receptor blocker, spironolacton, was included in our study (which is in line with the ACVIM recommendations).^{17,34}

The RAAS is one of the most important mechanisms of regulating blood pressure, homeostasis of the body as well as the water and electrolyte balance. RAAS is also one of the key elements in the development of hypertension, both through the direct effect of angiotensin II on the cardiovascular system and stimulation of cardiac and vascular fibrosis and pro-inflammatory effects^{35,36} of the kidneys, resulting in gradual damage of the nephron function.^{25,37–39} Finally, neurohormonal hyperactivity expressed as elevated RAAS leads to increased sodium reabsorption in the

Henle loop, which results in dilatation of glomerular arterioles causing glomerular hyperperfusion.⁴⁰ This phenomenon occurs secondary to an increase in RRI, which is followed by increase in serum urea, creatinine, SDMA, and Cyst C as well as albuminuria.^{40,41} We found that the RRI was correlated with albuminuria, which was consistent with other studies (Figure 2). There was no correlation between RRI and serum SDMA or Cyst C, which was surprising. Numerous studies have reported that the RRI may be a significant predictor of cardiorenal outcomes and is a good marker of renal function because it reflects kidney disorders arising from mitral valve damage and associated cardiac disorders.¹⁴ Chetboul and co-workers determined that RRI increases with a dilatation of the left atrium, an elevation of the serum urea and creatinine concentration in dogs with MMVD.¹⁶ These correlations were not confirmed in the present study. This discrepancy may be a result of a higher homogeneity of the heart group, especially the same stage of heart failure (Tables 1–5). The average WBC count, serum urea and creatinine concentration remained at the higher end of the physiological range in the heart group (no significant differences compared to healthy dogs). This was in contrast to SDMA and Cyst C, which were significantly elevated (Tables 2 and 3). This seems to reflect GFR, and may be used as indirect biomarker of GFR. However, Lorin and Choi found that there was no increase in SDMA in dogs with CHF, contrary to the reports in humans.^{40,41} This is consistent with our findings, where only two of four dogs with MMVD had increased SDMA levels. The difference in the SDMA elevation recorded in humans and dogs is most likely caused by the differences in the etiology of the heart disease. In humans, ischemic HF is mainly associated with an impaired systolic function. In dogs with MMVD systolic function is preserved for a long period.

Aldosterone was positively correlated with Cyst C but not with SDMA in the heart group. Although a correlation between aldosterone and RRI was expected in dogs with MMVD, an increased RRI was not associated with a higher aldosterone level in this study. Moreover there were no significant aldosterone differences between the healthy group and cardiac group. The mean aldosterone concentration was visibly higher in the heart group but individual variations made the difference statistically insignificant. In previous studies, dogs with MMVD exhibited significantly higher aldosterone values than our heart group.^{20,21} Similarly, we expected that SAP would be higher in the heart group versus the control group, as chronic activation of RAAS in CRS results in hypertension (over 160 mmHg according to ACVIM consensus).⁴² Although visible difference between heart (160 mmHg) versus control group (129 mmHg), the differences did not reach statistical significance and did not exceed the limit of 160 mmHg for systolic pressure. In this study we analysed only SPB because Doppler method do not allow to measure diastolic

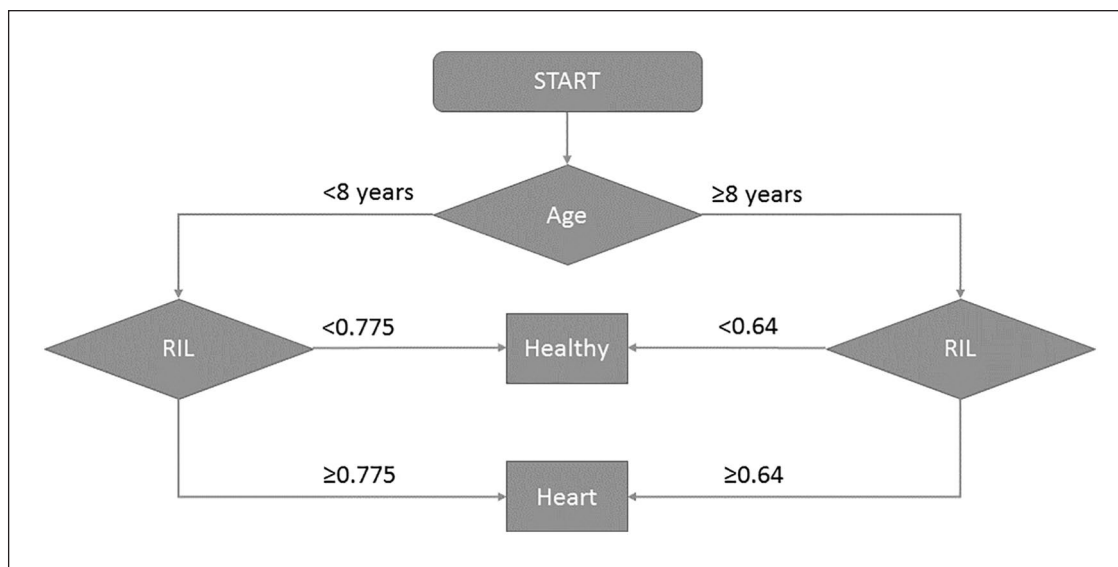


Figure 3. Multiple logistic regression (control vs heart) for RRI.

pressure, but is most useful in non-hypertensive (normotensive or hypotensive) dog.⁴²

Obtained results SPB measurements indicate correct treatment of dogs with drugs blocking the RAAS: ACE-I and spironolactone. A similar SBP level was determined by Chetboul and co-workers in stage 2 MMVD dogs according to the ISCHAC classification.¹⁶ This stage is similar to stage Cc in the ACVIM classification scheme.¹⁷

The division of groups into dogs from the heart group and dogs from the control group was mainly based on echocardiographic tests. In the assessment of heart disease in dogs, is based first of all on the increased LA/Ao and LVIDd markers of left sided overload. The another typical parameter measured is preserved FS and ejection fraction (EF).¹⁸

Main goal of this study was to show that at an early stage of heart disease, even before azotemia occurs, glomerulus are slowly destroyed, which is compounded by increased RRI parameters in most dogs from the heart group. In this group of dogs late parameters of kidney injury such as creatinine and urine in serum were in reference range. According to Szczepankiewicz et al.^{5,6} during MMVD occurs for the detachment of podocytes from the glomeruli and their entry into the urine. Podocytes are highly specialized cells of the glomerular capsule, crucial from the point of view of selective plasma filtration and primary urine production. These cells appear in the urine at the time of the active phase of glomerular damage, much earlier than the markers indicating kidney damage to date (e.g. urea, creatinine, as well as Cyst C and SDMA). Determining the number of podocytes in the urine is of great diagnostic importance, as it can be regarded as a reflection of irreversible changes in the glomeruli.^{5,6}

In conclusion, the search for new relevant biomarkers to better stratify patients with CRS according to the risk of progression, morbidity, and mortality is underway. An extremely important issue is a multidisciplinary approach to the care of patients with the cardiovascular syndrome and to elaborate guidelines for the management of worsening kidney function in dogs suffering from MMVD. It seems that new guidelines for the treatment of dogs suffering from MMVD satisfactorily inhibit RAAS, as evidenced by the lack of a significant increase in aldosterone in a group of dogs with heart disease. On the other hand, a slightly higher RRI was found in this group of dogs than in previous studies, suggesting that more detailed studies of the effects of pimobendan on intrarenal vascular resistance are needed.

The main aim of the study was to check whether RRI could be a useful, non-invasive marker for the detection very early changes in glomeruli and assess early stages of kidney injury in dogs with CHF—CRS. The present study indicated that RRI may be useful for the evaluation of very early renal damage and may be more sensitive than creatinine. The RRI cut-off point in dogs with CHF is 0.775 in dogs younger than 8 years old and 0.64 in older dogs. RRI may be used to estimate CRS in dogs with CHF due to MMVD and is more sensitive than creatinine.

Limitation

Due to the fact that literature reports indicate that both RRI¹⁶ and albuminuria increase as HF progresses—we expected a positive correlation between these parameters. However, a negative correlation was observed. Therefore, it seems that the ultimate effect on the amount of albumin

in urine is the result of many, partly opposite effects. The results of the studies on dogs selected during one particular phase of HF indicate that RRI elevation is associated with chronic renal failure and GFR reduction.¹⁴

As Lubas et al.⁴³ pointed out, one of the features limiting the usefulness of RRI is a single measurement made in and well-visualized artery, which results in obvious omission of vessels with lower flow and often overestimates the result. Another limitation of this method is the fact that RRI are not only specific markers of kidney damage and indicators of renal functional prognosis but are also always clearly and strongly linked with systemic circulation. Factors which mainly affect peak systolic velocity in renal arteries in dogs are: pulse pressure, left ventricular out-flow, HF. Factors affecting end diastolic velocity in dogs are: HR, HF, chronic nephropathies, and according to some authors diuretics (there is no consensus on whether diuretics affect RRI).⁴⁴

Studies on dogs with kidney disease show that another drawback of this method is the lack of histopathologic confirmation of the RRI values.¹² This examination also requires high skill, because the kidneys move constantly during breathing, which is particularly marked in patients with left ventricular HF that present with signs of reduced respiratory capacity. Visualization of the left kidney is easier than the right kidney. This is important as dogs must lie on their backs for this examination, which is not only uncomfortable for them, but also intensifies breathing problems. Therefore, the longer the examination lasts—the more the animal moves.

An additional limitation of this study was that dogs in the control and heart group were not homogenous. This was a result of the predisposition of older small breed dogs to heart disease, causing differences in the body weight and age between the groups. Furthermore, male dogs are more likely to get MMVD than females, causing the males to be predominant in the heart group.

The evidence presented does not allow one to say whether or not RRI can distinguish between dogs with or without renal dysfunction among all dogs with HF, which would seem to be the goal.

During the selection of animals for research groups, animals with diseases affecting RI increase were excluded, despite the fact that statistically significant values of aldosterone for dogs with MMVD could not be determined but the fact is that the average level of aldosterone was visibly higher in the heart versus the control group, the large individual variations made the difference statistically insignificant. Therefore, we cannot say with certainty that the epidemiology of elevated RRI results from elevated aldosterone, however, the fact of exclusion of other systemic diseases speaks precisely for this pathomechanism.

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ORCID iD

Barbara Szczepankiewicz  <https://orcid.org/0000-0001-7483-6909>

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