UNIVERSITA' DEGLI STUDI DI PARMA

Dottorato di Ricerca in Scienze Medico Veterinarie

Ciclo XXVII

Approccio preliminare alla sindrome cardiorenale nel cane affetto da malattia valvolare mitralica cronica

Preliminary approach to cardiorenal syndrome in dogs affected by chronic mitral valve disease

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Foreword

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Over recent years, the field of human medicine has been challenged by the two epidemics of heart failure and renal insufficiency (Pokhrel 2008). The coexistence of renal and cardiac disease significantly increases mortality and morbidity in human patients (Wencker 2007, Ronco 2010). The heart and kidney are both involved in basic physiology, and their functions are strictly linked, that's why primary disorders of heart or kidney often result in secondary dysfunction or injury to the other organ (Liang 2008, Ronco 2008, Longhini 2010). The presence of the two problems in the same patient is referred as cardiorenal syndrome (CRS). CRS was first described in 1951 but it's over the last decade that there has been a growing interest in it (Ledoux 1951). In veterinary medicine, just little information regarding CRS was available (Haggstrom 1996, Atkins 2002, Nicolle 2007). The most common heart disease affecting dogs and leading to congestive heart failure, is chronic mitral valve disease (CMVD), also known as endocardiosis and myxomatous valve degeneration (Haggstrom 2007, Borgarelli 2010).

This doctoral thesis has been focused over the theme of cardio-renal connection and the coexistence of renal insufficiency and heart failure in dogs affected by CMVD.

State of the Art

State of the Art

Cardiorenal Syndrome

Definition and classification

CRS has been used to define different clinical conditions in which heart and kidney dysfunction overlap. In 2008, during a Consensus Conference, a new definition of the syndrome was proposed by the Acute Dialysis Quality Group (ADQG) (Ronco 2008). The ADQG expanded the previous general definition to five subtypes reflecting the time-frame of the syndrome and the primacy of organ dysfunction (Ronco 2010).

CRS are disorders of the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction of the other

The five subtypes of CRS described in literature were:

CRS type 1 (acute CRS), which is characterized by a rapid worsening of cardiac function, leading to acute kidney injury;

CRS type 2 (chronic CRS), characterized by chronic abnormalities in cardiac function causing progressive chronic kidney disease (CKD);

CRS type 3 (acute renocardiac syndrome), characterized by an abrupt and primary worsening of kidney function, leading to acute cardiac dysfunction;

CRS type 4 (chronic renocardiac syndrome), characterized by a condition of primary CKD contributing to decreased cardiac function, ventricular hypertrophy, diastolic dysfunction and/or increased risk of adverse cardiovascular events;

CRS type 5 (secondary CRS), characterized by the presence of combined cardiac and renal dysfunction due to acute or chronic systemic disorders (Ronco 2008).

Cardiorenal Syndrome

Type 2 and/or 4

Type-2 CRS is characterized by chronic dysfunction of the heart leading to kidney injury or dysfunction, or rather by CKD onset in heart failure patients. Chronic heart failure (CHF) and CKD often coexist but it's still difficult to establish which of these two diseases is primary and which is secondary. CKD has been observed in 45% to 63% of human patients affected by CHF (Ronco 2010). However remains often unclear how to classify patients with concomitant CKD and CHF when information regarding anamnesis are missing (Bongartz 2005, Bock 2010). The coexistence of cardiovascular disease and kidney dysfunction is not enough to diagnose type-2 CRS, and two features were proposed in human literature for the diagnosis:

- CHF and CKD are simultaneously present
- CHF causally underlies occurrence or progression of CKD.

The most important mechanisms proposed to explain the pathophysiology of type-2 CRS are:

- Neurohormonal activation
- Kidney hypoperfusion
- Venous congestion
- Inflammation
- Atherosclerosis
- Oxidative stress
- Recurrent episodes of acute heart and/or kidney decompensation

In particular, kidneys of CHF patients seems to release large amounts of circulating renin. The activation of the renin-angiotensyn-aldosteron system (RAAS) causes efferent arteriolar constriction, increase in oncotic pressure of peritubular capillaries,

nephron-augmented sodium reabsorption, glomerular fibrosis and subsequent pressure and volume overload (DiBartola 2007, Polzin 2007, House 2010, Lazzeri 2011, Colombo 2012, Pierantonazzi 2012, Zatelli 2012).

Type-4 CRS, the chronic renocardiac syndrome, is characterized by cardiovascular involvement in patients affected by CKD at any stage according to the National Kidney Foundation (NKF) classification in human medicine and to the International Renal Interest Society (IRIS) classification in veterinary medicine. The pathophysiologic mechanisms that lead to increased cardiovascular risk in CKD patients are still not completely known, despite a firm connection between the heart and kidney has been established. Loss of kidney function usually leads to the activation of the RAAS and the development of arterial hypertension. Hypertension, together with angiotensin and aldosterone, accelerates left ventricle hypertrophy and cardiac fibrosis. Recent evidence suggests that uremic toxins such as indoxyl sulfate and p-cresol can contribute to cardiac fibrosis in renal patients.

Cardiorenal Syndrome

The diagnosis, still a challenge

The diagnosis of type-2/4 CRS is based on the serologic and instrumental diagnosis of both chronic heart disease and CKD. In human medicine, cardiac function is more widely assessed by NT-proBNP serum levels and echocardiography, whereas the estimate glomerular filtration rate (eGFR), serum urea nitrogen (BUN), serum creatinine (sCr) and proteinuria represent the most widely used test for the evaluation of kidney function.

sCr and BUN

sCr and BUN are extensively used to quantify renal dysfunction both in human and veterinary medicine (Dobre 2012). Considering that in situations of compromised renal blood flow, as such as with cardiac disease, the increased reabsorption of urea from within the renal tubule leads to serum concentrations increasing disproportionately compared to sCr, sCr alone can be elected as a reliable marker of renal dysfunction in dogs with CMVD (Medaille 2004, Boswood 2006, Nicolle 2007).

Worsening renal function (WRF) has been used in human medicine to better control the follow up of hospitalized patient and is defined as elevation in sCr. As reported in human medicine literature WRF could be defined as an absolute sCr elevation ≥0.3 mg/dl or 25% relative elevation from baseline (Smith 2003).

eGFR

Determination of eGFR is a valuable diagnostic tool to investigate renal function in humans and companion small animal (Von Hendy 2011). Despite its superiority for

detecting early renal dysfunction, measurement of GFR remains an underused tool in the diagnosis and management of kidney disease in veterinary medicine. Serum creatinine concentration has replaced GFR measurement in most clinical settings because of its ease and widespred availability; however, because serum creatinine likely does not increase above reference range until approximately 75% of nephrons are nonfunctional, the sensitivity and specificity of creatinine for diagnosis of kidney disease should be considered inferior to actual determination of GFR (Von Hendy 2011).

Proteinuria

In humans the severity of proteinuria is associated with the rate of progression of CKD and is a prognostic indicator in individuals with cardiac disease (Harley 2012). There is no established cause and effect relationship between proteinuria and the progression or development of renal failure in animals. Proteinuria is associated with reduced survival, but it is not clear whether the proteinuria is the marker or the cause. The urine protein/creatinine ratio (UPC) has become the gold standard test for proteinuria and should be run on any patient testing trace or greater on a urine dipstick or positive on sulfosalicylic acid (SSA). Ideally, protein is measured over 24-hour period but this is impractical in animals. The UPC, performed on a single random urine sample, has a close correlation to the 24-hour urine protein quantification (Grauer 2011, Harley 2012).

Ultrasonography

Imaging of the heart and the kidney can provide valuable information in the diagnosis and management of CRS. Ultrasound based imaging is an essential component in the

initial diagnostic workup of CRS; in fact imaging using ultrasound waves is a noninvasive, cost-effective and widely available technology (George 2011). Echocardiography provides information on the structure and function of the heart, and renal ultrasound is useful in differentiating between acute and chronic kidney disease and excluding certain causes of acute kidney injury such as obstructive uropathy.

Echocardiography.

Echocardiography is a safe, noninvasive and reproducible test that provides valuable information on the anatomy and function of the heart. Using echocardiography, the structure of the myocardium and pericardium, global and regional left ventricle function and wall motion can be assessed. Two dimensional, gray-scale, or B mode echocardiography provides real-time images of heart structures and their motion.

Renal Ultrasonography.

Kidney size and the echogenicity of the renal parenchyma provide useful information in the workup of kidney diseases. (Pennink, 2008) Findings related to CKD are: decreased or normal renal size, irregularly shaped kidneys, increased echogenicity of renal tissue and reduced distinction between medulla and cortex.

Radiography of the thorax

Thoracic radiographs provide information about heart size, status of pulmonary vasculature, and changes in the lungs to help differentiate left-sided congestive heart failure from other disease.

Evaluating the size and shape of the heart silhouette on radiographs is a key step in diagnosing and assessing severity of cardiac disease in dogs. The presence of a straight/concave caudal margin of the heart, an elevation of the trachea or an

increased vertebral heart score (VHS) should be used along with clinical signs and physical exam to determine if CHF treatment is indicated (Guglielmini 2009).

Aim of the Study

Aim of the Study

CRS in dogs affected from CMVD was investigated in a retrospective study and in a prospective study.

The retrospective study

Little information concerning the actual prevalence of renal dysfunction in canine patients with naturally occurring heart failure is available (Nicolle 2007). The aims of this study were to assess the prevalence of CKD associated with azotemia complicating CMVD in dogs, to evaluate a possible connection between class of cardiac failure (ACVIM classification) and class of renal failure (IRIS classification) and to investigate the correlation between parameters of renal failure and echocardiographic parameters.

The prospective study

The prospective study is an evaluation of dogs affected by CMVD in ACVIM class B2. Recurrent episodes of acute heart and/or kidney failure are considered one of the causes leading to worsening renal and heart functions in patients affected by CRS type 2/4, the aims of the current study were to assess the influence of heart/kidney failure or worsening (defined on echocardiographic, radiographic and laboratory parameters) on elected parameters of kidney/heart function in a period of three years.

Materials and Methods

Materials and Methods

The retrospective study

Two thousand two hundred seventy-one records of dogs presented at the Cardiology Service of the Department of Veterinary Science and Public Health, University of Milan, between January 2003 and December 2012 were retrospectively evaluated.

The inclusion criteria were: dogs with complete clinical examination (including signalment, anamnesis and physical examination findings), thorax radiographs, a CMVD diagnosis based on echocardiographic examination (presence of mitral leaflets thickening and/or prolapse associated with an abnormal mitral regurgitant jet on Doppler color flow imaging) performed by a trained observer, ECG and serum biochemical analysis, including serum creatinine (sCr) and serum urea nitrogen (BUN) (Atkins 2010). The exclusion criteria were: other heart disease, neoplasm and systemic diseases. Dogs were divided into two groups: dogs receiving therapy for medical management of heart failure (therapy-group) and dogs without therapy (non-therapy-group) and categorized according to the ACVIM class (Fig.1) and IRIS class (Fig.2).

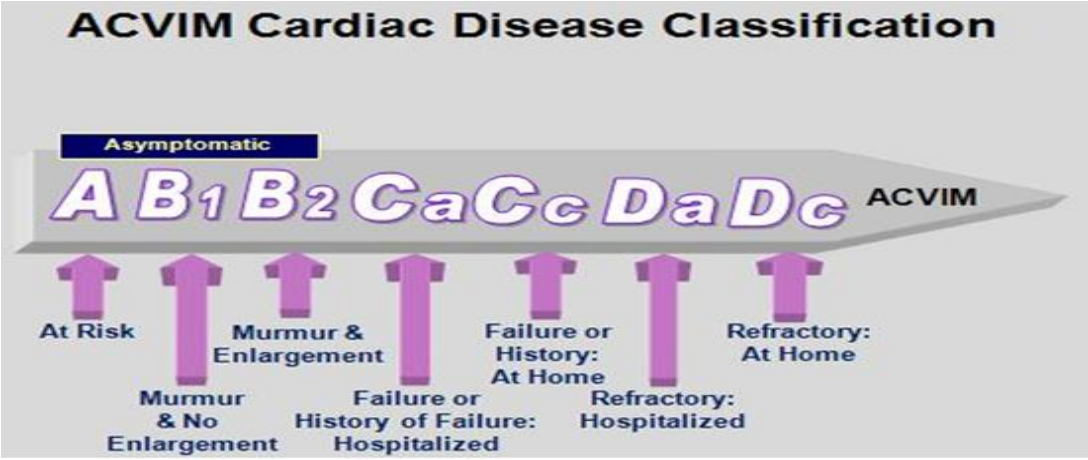


Fig. 1 ACVIM classification (http://cavalierhealth.org/mitral_valve_disease.htm)

Stage	Blood creatinine µmol/l mg/dl		Comments
	Dogs	Cats	
At risk	<125 <1.4	<140 <1.6	History suggests the animal is at increased risk of developing CKD in the future because of a number of factors (e.g., exposure to nephrotoxic drugs, breed, high prevalence of infectious disease in the area, or old age).
1	<125 <1.4	<140 <1.6	Nonazotemic. Some other renal abnormality present (e.g., inadequate urinary concentrating ability without identifiable nonrenal cause, abnormal renal palpation or renal imaging findings, proteinuria of renal origin, abnormal renal biopsy results, increasing blood creatinine concentrations in samples collected serially).
2	125 – 180 1.4 – 2.0	140 – 250 1.6 – 2.8	Mild renal azotemia (lower end of the range lies within reference ranges for many laboratories, but the insensitivity of creatinine concentration as a screening test means that animals with creatinine values close to the upper reference limit often have excretory failure). Clinical signs usually mild or absent.
3	181 – 440 2.1 – 5.0	251 – 440 2.9 – 5.0	Moderate renal azotemia. Many extrarenal clinical signs may be present.
4	>440 >5.0	>440 >5.0	Increasing risk of systemic clinical signs and uraemic crises

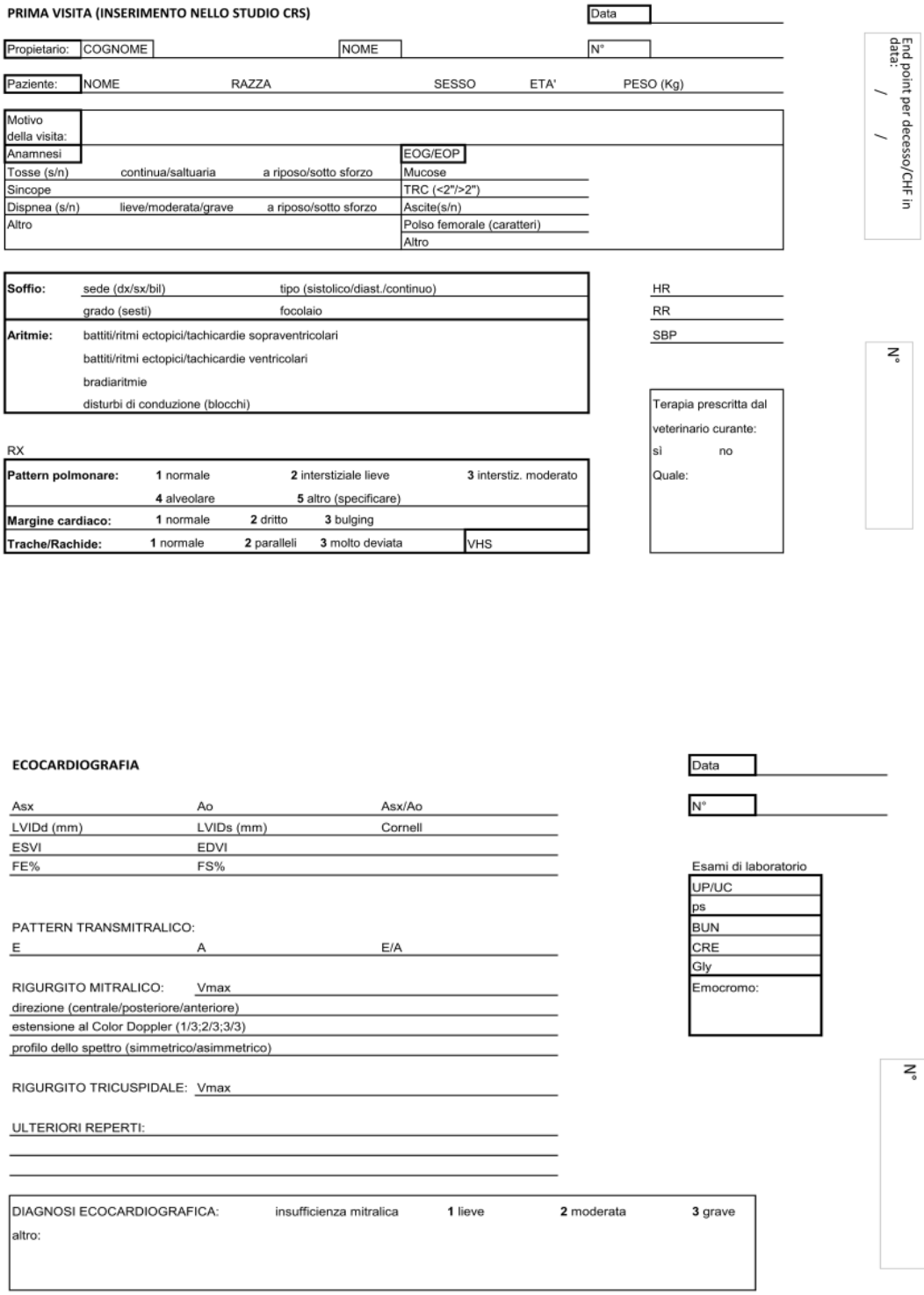
Fig. 2 IRIS classification (http://www.iris-kidney.com/)

The prospective study

Dogs presented at the Cardiology Service of the Department of Veterinary Science and Public Health, University of Milan, and at the Cardiology Service of The Department of Veterinary Science, University of Parma between July 2012 and May 2013 were evaluated. The inclusion criteria (cases) were: dogs with a CMVD diagnosis based on echocardiographic examination (presence of mitral leaflets thickening and/or prolapse associated with an abnormal mitral regurgitant jet on Doppler color flow imaging) performed by trained observers and belonging to ACVIM class B2 (not receiving therapy for medical management of heart failure). The exclusion criteria

(cases) were: heart disease different from CMVD, class of heart failure different from ACVIM B2, neoplasm, systemic diseases, lower urinary tract disease. Healthy adult dogs, older than 6 years, were included in the control group.

Information obtained from the medical records included signalment, anamnesis and physical examination findings. All the dogs (both cases and controls) underwent thorax radiography, ECG, serum biochemical analysis, including assessment of serum creatinine (sCr), serum urea nitrogen(BUN) and glycaemia (GLY), a complete blood count (CBC), urine analysis, urine protein/creatinine ratio (UPC) and indirect systemic blood pressure evaluation by Doppler flow meter. Data were collected in a dedicated datasheet (Fig.3). Dogs were re-evaluated every 6 month until October 2014.



PRIMA VISITA (INSERIMENTO NELLO STUDIO CRS) Data

Propietario: COGNOME NOME N°

Paziente: NOME RAZZA SESSO ETA' PESO (Kg)

End point per decesso/CHF in data: / /

Motivo della visita:

Anamnesi

Tosse (s/n) continua/saltuaria a riposo/sotto sforzo

Sincope

Dispnea (s/n) lieve/moderata/grave a riposo/sotto sforzo

Altro

EOG/EOP

Mucose

TRC (<2"/>2")

Ascite(s/n)

Polso femorale (caratteri)

Altro

Soffio: sede (dx/sx/bil) tipo (sistolico/diast./continuo)

grado (sesti) focolaio

Aritmie: battiti/ritmi ectopici/tachicardie sopraventricolari

battiti/ritmi ectopici/tachicardie ventricolari

bradiaritmie

disturbi di conduzione (blocchi)

HR

RR

SBP

N°

Terapia prescritta dal veterinario curante:

sì no

Quale:

RX

Pattern polmonare: **1** normale **2** interstiziale lieve **3** interstiz. moderato **4** alveolare **5** altro (specificare)

Margine cardiaco: **1** normale **2** dritto **3** bulging

Trache/Rachide: **1** normale **2** paralleli **3** molto deviata VHS

ECOCARDIOGRAFIA Data

N°

Asx Ao Asx/Ao

LVIDd (mm) LVIDs (mm) Cornell

ESVI EDVI

FE% FS%

Esami di laboratorio

UP/UC

ps

BUN

CRE

Gly

Emocromo:

PATTERN TRANSMITRALICO:

E A E/A

RIGURGITO MITRALICO: Vmax

direzione (centrale/posteriore/anteriore)

estensione al Color Doppler (1/3;2/3;3/3)

profilo dello spettro (simmetrico/asimmetrico)

RIGURGITO TRICUSPIDALE: Vmax

ULTERIORI REPERTI:

N°

DIAGNOSI ECOCARDIOGRAFICA: insufficienza mitralica **1** lieve **2** moderata **3** grave

altro:

Fig.3 Datasheet

Echocardiography and ECG

All echocardiographic studies were performed using either a Megas Esaote Cvx ultrasound machine (Esaote Medical System) or an Esaote MyLab50 ultrasound machine (Esaote Medical System), both equipped with 5-7.5 MHz and 2.5-3 MHz, multi-frequency phased array transducers. The dogs were consecutively positioned in right and left recumbency. All the echocardiographic measurements were made on conscious dogs in accordance with the guidelines of the American Society of Echocardiography using the leading-edge to leading-edge method for M-mode measurements and the Hansson's method for the 2D measurements of left atrial (LA) and aortic root (Ao) diameters (Thomas et al. 1993, Hannson 2002).

From the right parasternal short-axis M-mode view at the chordae level, the following parameters were obtained: interventricular septal thickness (IVS), left ventricular internal diameter (LVID), and left ventricular posterior wall thickness (LVPW) in diastole (d) and systole (s). The following parameters were obtained from 2D views: aortic root diameter (Ao) and left atrial diameter (LA) from right parasternal short-axis view. Mitral valve inflow (E peak velocity - EVmax, A peak velocity – Avmax, E/A ratio), aortic peak velocity (AoVmax) and peak gradient (AoGmax), peak velocity of mitral and tricuspid regurgitations (MR and TR) were evaluated using the color Doppler and the spectral Doppler (Boon 2011). No angle corrections were needed as parallel alignment of the Doppler gate was possible in all dogs. The following parameters were calculated: left atrial to aortic root ratio (LA/Ao), fractional shortening (FS%), left ventricular ejection fraction (EF%), Endsystolic volume index (ESVI), Enddiastolic volume index (EDVI) and Cornell Index calculated over the LVIDd (Cornell 2004). The EDVI and ESVI were calculated according to the Teichholz

formula, normalized to body surface area (BSA). A standard 6-lead electrocardiogram was obtained in awake dogs in order to assess heart rate and diagnose cardiac arrhythmias.

Biochemical Analysis

Blood urea nitrogen (BUN), serum creatinine (sCr) and GLY (only in the prospective study) were recorded. Reference intervals for canine BUN and sCr were 20-60 mg/dl and <1,4 mg/dl respectively. Dogs were divided into 2 groups: the azotemic (sCr≥1,4 mg/dl; without reference to BUN values) and the non-azotemic group (sCr<1,4 mg/dl) (www.iris.kidney.com).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 20 and C5.0 [Release 2.07 GPL Edition]. Data were analyzed using simple and multiple linear regression test, simple and multiple ordinal regression test and Chi Squared test as needed. A p value <0.05 was considered significant. In the retrospective study the dependent variables considered were ACVIM class, IRIS class, BUN and sCr; the independent variables considered were all the echocardiographic parameters evaluated, body weight (BW), body surface area (BSA), heart rate (HR), systolic blood pressure (SBP), murmur grade, administration of furosemide *vs* non-therapy and the total dose of furosemide administered in mg/kg/die.

In the prospective study dogs were sorted into groups according to the presence/absence of signs of worsening renal/cardiac function. The variables considered were: WRF, presence/absence of increasing proteinuria levels, radiographic parameters of heart enlargement (presence of a straight/concave caudal

margin of the heart, elevation of the trachea, increased VHS), presence/absence of increasing of furosemide's dose administered, ACVIM class, IRIS class and the echocardiographic parameters LA/Ao, Cornell Index, EVmax.

Results

Results

The retrospective study

Descriptive statistics

Of the 2271 dogs evaluated at the Cardiology Service of the Department of Veterinary Science and Public Health, University of Milan, 48% were affected by CMVD. One hundred and fifty eight dogs of both genders (94 males and 64 females) were included. Of these, the 20% of males and the 65% of females were neutered. They were between 5 and 17 years of age (mean age 11.56 ± 2.51 years), with BW ranging from 2 to 48 kg (mean BW 11.52 ± 8.71 kg). The most represented breeds were mongrel (42%), miniature Poodle (10 %),York Shire Terrier (10 %), Shih -Tzu (6%), Pinscher (5%) and Dachshund (5%). Arrhythmias were described in the 18% of the dogs, with a prevalence of atrial fibrillation (48%) and ventricular premature complexes (20%). In the therapy-group (including the 70.9% of the patients) the 64% received furosemide associated with an ACE-inhibitor (benazepril or enalapril) and the 36% received triple therapy with furosemide, ACE-inhibitor and pimobendan. Echocardiographic values are reported in Table 1. Dogs were classified as follow: 20.9% ACVIM B1 (asymptomatic patients that have no radiographic or echocardiographic evidence of cardiac remodeling in response to CMVD) , 9.5% ACVIM B2 (asymptomatic patients that have radiographic or echocardiographic evidence of cardiac remodeling in response to CMVD), 67.1% ACVIM C (patients with past or current clinical signs of heart failure associated with structural heart disease), 2.5% ACVIM D (patients with end-stage disease with clinical signs of heart failure caused by CMVD that are refractory to ‘‘standard therapy’’); 74.7% normoazotemic

(sCr<1.4 mg/dl), 12.7 % IRIS 2 (early renal failure), 11.4% IRIS 3 (uremic renal failure), 1.3% IRIS 4 (end stage renal failure). The normoazotemic group included: 87.9% of ACVIM B1 dogs, 100% of ACVIM B2 dogs, 67.9% of ACVIM C dogs and 50% of ACVIM D dogs (Fig.4). The normoazotemic-group was larger than the azotemic-group in all the ACVIM class but the frequency of IRIS class 2 and 3 was higher in ACVIM class C and D. The prevalence of CKD associated with azotemia in dogs affected by CMVD was 25%. The prevalence of azotemia was significantly higher in the therapy-group than in the non-therapy-group (32% *vs* 1%, p< 0.001). The mean sCr value and BUN value were significantly higher in the therapy-group than in the non-therapy-group (sCr: mean 1.5 *vs* 1.0, p<0.001; BUN: mean 83.8 *vs* 58.7, p=0.006).

Echocardiographic parameters	min	max	mean	± ds
IVSd	3,40	18,00	7,88	2,17
LVIDd	16,60	74,70	36,43	9,90
LVPWd	4,10	15,10	7,41	1,95
IVSs	4,40	19,80	11,41	2,77
LVIDs	7,90	53,00	21,23	7,57
LVPWs	6,50	18,60	11,35	2,60
EF%	30,10	94,99	72,42	10,86
FS%	14,20	68,83	41,65	8,85
ESVI	3,76	182,59	36,90	28,01
EDVI	24,48	549,60	127,87	68,74
Ao	7,10	30,20	15,66	4,53
LA	11,80	66,00	30,27	10,22
LA/Ao	0,75	4,85	1,98	0,66
E_Vmax	0,42	1,92	1,06	0,37
E/A	0,50	2,95	1,36	0,57
Ao_Vmax	0,67	3,76	1,23	0,36
Ao_Gmax_	1,80	14,14	6,21	2,60
MR	3,08	6,86	5,34	0,63
TR	1,31	5,09	2,96	0,81

Tab. 1 Echocardiographic variables in 158 dogs with chronic mitral valve disease : interventricular septal thickness (IVS), left ventricular internal diameter (LVID), and left ventricular posterior wall thickness (LVPW) in diastole (d) and systole (s), aortic root diameter (Ao), left atrial diameter (LA), left atrial to aortic root ratio (LA/Ao), E peak velocity (EVmax), E/A ratio, aortic peak velocity (AoVmax) and peak gradient (AoGmax), peak velocity of mitral and tricuspid regurgitations (MR and TR), fractional shortening (FS%), left ventricular ejection fraction (EF%), End systolic volume index (ESVI), End diastolic volume index (EDVI)

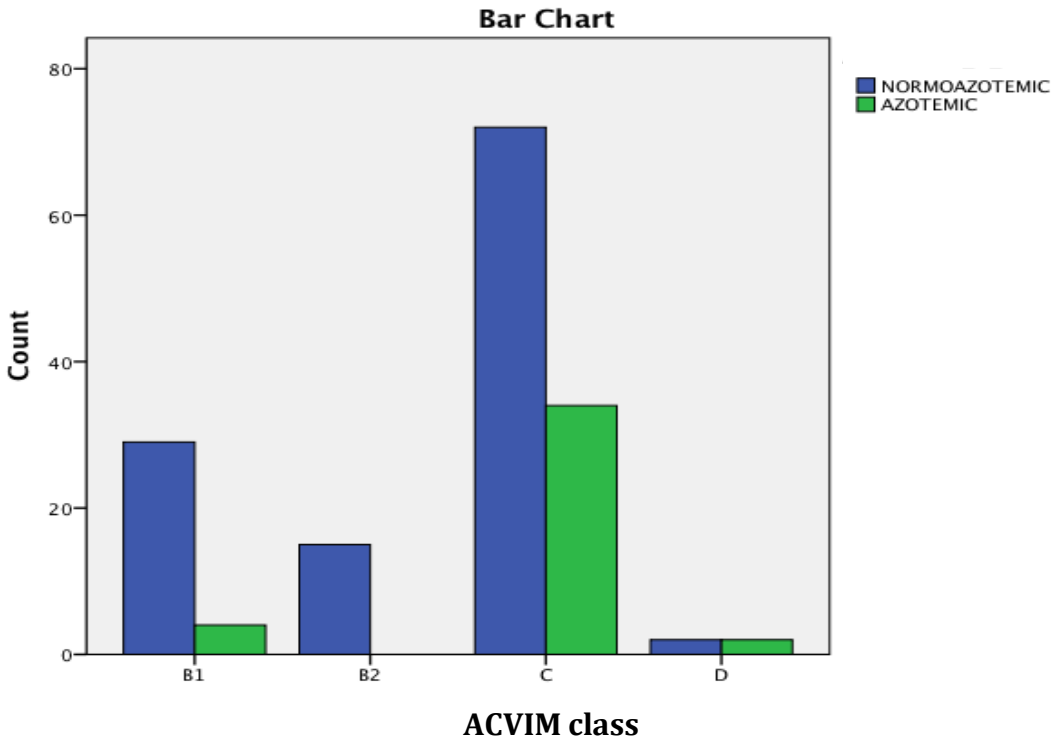


Fig.4 Dog's distribution in ACVIM class: 20.9% ACVIM B1, 9.5% ACVIM B2, 67.1% ACVIM C, 2.5% ACVIM D. Blue: normoazotemic dogs; Green: azotemic dogs.

Inferential statistics

The statistically significant correlations between the considered parameters are reported in Table 2. Simple linear regression confirmed the statistically significant correlation shown by the Chi-square Test and so data have not been reported there.

Simple ordinal regression showed that considering advanced IRIS class it is possible to make a prediction of advanced ACVIM class and vice versa. Multiple linear and ordinal regressions underlined the importance of therapy for medical management of heart failure and of the echocardiographic parameters LA/Ao and Cornell index in defining ACVIM class, IRIS class, BUN and sCr. Clinically interesting rules elaborated by the C5.0 [Release 2.07 GPL Edition] software were reported in Table 3. Just one decision tree elaborated by the same software was considered clinically useful by the authors (Fig. 5).

Correlation	Pearson's Coefficent	P Value
BUN-ACVIM	0.166	0.038
BUN-IRIS	0.735	0.00
BUN-sCr	0.751	0.00
BUN-SBP	0.644	0.032
BUN-Furosemide(mg/kg/die)	0.232	0.02
BUN- Therapy vs NO Therapy	0.174	0.032
sCr-ACVIM	0.186	0.019
sCr-IRIS	0.830	0.00
sCr-BUN	0.751	0.00
sCr-Murmur Grade	0.177	0.039
sCr-SBP	0.645	0.032
sCr- Therapy vs NO Therapy	0.211	0.009
ACVIM-IRIS	0.235	0.003
ACVIM-sCr	0.186	0.019
ACVIM-BUN	0.166	0.038
ACVIM-BSA	(-)0.226	0.004
ACVIM-BW	(-)0.216	0.006
ACVIM-Murmur Grade	0.482	0.00
ACVIM-HR	0.320	0.025
ACVIM-LVIDd	0.260	0.001
ACVIM-LVIDs	0.164	0.043
ACVIM-Ao	(-)0.261	0.001
ACVIM-LA	0.357	0.00
ACVIM-LA/Ao	0.576	0.00
ACVIM-Evmax	0.574	0.00
ACVIM-E/A	0.399	0.00
ACVIM-MR	(-)0.248	0.006
ACVIM-TR	0.234	0.036
ACVIM-Furosemide(mg/kg/die)	0.430	0.00
ACVIM- Therapy vs NO Therapy	0.925	0.00
ACVIM-Cornell Index	0.519	0.00
ACVIM-ESVI	0.314	0.00
ACVIM-EDVI	0.456	0.00
IRIS-ACVIM	0.235	0.003
IRIS-sCr	0.830	0.00
IRIS-BUN	0.735	0.00
IRIS-HR	0.286	0.046
IRIS-IVSd	0.177	0.029
IRIS-LA	0.223	0.006
IRIS-LA/Ao	0.196	0.017

Tab. 2 Statistically significant correlations

Rules	
Assumption	**Effect**
LA/Ao >1,88	ACVIM class C
Murmur Grade >3	ACVIM class C
Cornell Index >1,83	ACVIM class C
BUN <131	normoazotemic

Tab. 3 Rules of clinical interest elaborated by the C5.0 [Release 2.07 GPL Edition] software

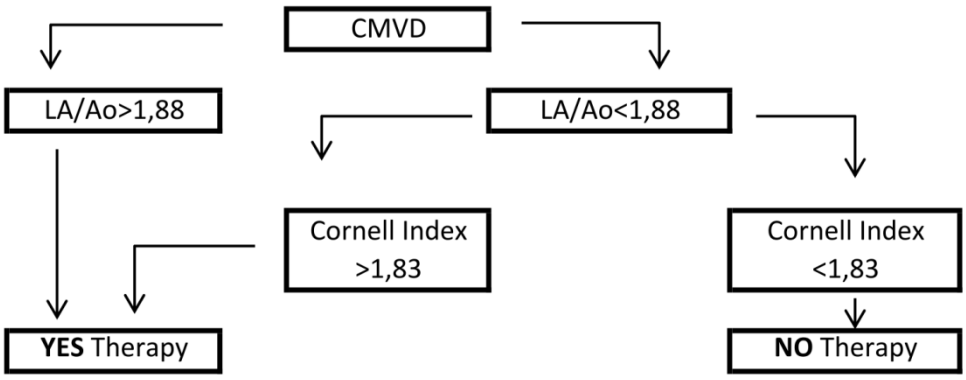


Fig.5 Decision tree: dogs affected by CMVD where sort according to the echocardiographic parameter left atrium to aortic root ratio (LA/Ao) and according to the Cornell Index calculated over the left ventricle internal diameter in diastole

The prospective study

Descriptive statistics

Twenty one dogs affected by CMVD (cases) of both genders (12 males and 9 females) and 20 healthy dogs (controls) of both genders (12 males and 8 females) were included. They were between 6 and 15 years of age (mean age 10.3 ± 2.9 years), with BW ranging from 3.2 to 42 kg (mean BW 16.5 ± 11.2 kg). The most represented breeds were mongrel (58%), Pitbull (10%) and miniature Poodle (7%). Arrhythmias were described in the 19% of cases, with a prevalence of atrial premature complexes (75%). The 77% of cases included in the study were not receiving any drug for medical management of CHF before the inclusion day while the 23% of cases were under treatment with benazepril at presentation since less than one week. The 14% of cases began therapy for medical management of CHF at first evaluation with benazepril 0.5 mg/kg PO BID and furosemide 2 ± 0.3 mg/kg PO BID. The 33% of cases experienced at least one episode of CHF (switching from ACVIM class B2 to ACVIM class C), but none of these patients developed CKD. One case experienced three episodes of CHF in two years while renal function remained unaltered. The 14% of cases developed CKD while remaining in ACVIM class B2. The 5% of controls were affected by CKD at presentation (IRIS class 1) and didn't experience worsening renal function or CHF. No other controls developed CKD or CMVD. Echocardiographic values are reported in Table 4.

Echocardiographic parameters at presentation

	Cases				Controls			
	min	max	mean	ds	min	max	mean	ds
LVIDd	25,40	56,90	36,25	7,35	15,60	52,60	38,09	9,40
LVIDs	12,50	36,20	19,66	5,81	6,70	35,00	23,48	7,18
EF%	74,25	91,70	84,18	4,99	46,82	92,08	75,72	11,63
FS%	36,38	56,38	46,49	5,69	18,98	57,05	39,14	10,02
ESVI	8,59	66,30	30,28	13,72	2,00	39,50	25,03	15,43
EDVI	67,65	191,58	135,61	32,03	16,92	122,30	81,63	32,36
Ao	10,10	22,80	15,00	3,36	12,40	28,40	21,46	4,17
LA	15,80	40,90	25,09	6,01	18,30	37,30	27,10	5,81
LA/Ao	1,43	2,10	1,68	0,17	0,97	1,58	1,27	0,17
E_Vmax	0,59	1,38	0,98	0,23	0,47	0,89	0,69	0,13
A Vmax	0,53	1,12	0,85	0,16	0,56	0,86	0,70	0,11
E/A	0,61	1,88	1,18	0,31	0,70	1,52	1,02	0,29
Cornell Ind	1,46	2,24	1,94	0,20	0,84	1,81	1,45	0,44
MR	4,62	6,67	5,62	0,52				
TR	1,31	3,23	2,42	0,53				

Tab.4 Tab. 1 Echocardiographic variables in 158 daogs with chronic mitral valve disease : left ventricular internal diameter (LVID) in diastole (d) and systole (s), aortic root diameter (Ao), left atrial diameter (LA), left atrial to aortic root ratio (LA/Ao), E peak velocity (EVmax), A peak velocity (AVmax), E/A ratio, peak velocity of mitral and tricuspid regurgitations (MR and TR), fractional shortening (FS%), left ventricular ejection fraction (EF%), Endsystolic volume index (ESVI), Enddiastolic volume index (EDVI), Cornell Index calculated over the LVIDd (Cornell Ind)

Inferential statistics

Considering the sorting of cases according to WRF in two groups (dogs experiencing WRF and dogs without WRF): there wasn't statistically significant difference in the considered echocardiographic parameters or in ACVIM class between the groups. The simple regression test between WRF and the Cornell Index leads to a regression model statistically significant ($p<0.001$). The simple regression test between sCr elevations and the other considered variables did not lead to a significant model.

Considering the sorting of dogs according to UPC levels in two groups (dogs with worsening proteinuria and dogs without proteinuria or with steady proteinuria): there wasn't statistically significant difference in the considered echocardiographic parameters or in ACVIM class between the groups. The simple regression test

between UPC elevations and the considered variables didn't lead to a significant model.

Considering the sorting of dogs according to furosemide administration in two groups (dogs that increase oral administration of diuretics and dogs without therapy or changes in therapy): there wasn't statistically significant difference in parameters of renal impairment between the groups. The simple regression test between furosemide's dose increases and the considered variables didn't lead to a significant model.

Considering the sorting of dogs according to radiographic parameters of heart enlargement in two groups (dogs with cardiac remodeling and dogs without cardiac remodeling): there wasn't statistically significant difference in IRIS class or in ACVIM class between the groups. The simple regression test between parameters of heart enlargement elevations and the considered variables didn't lead to a significant model.

There wasn't any statistically significant difference between cases and controls in survival time.

Discussion

Discussion

The studied population was representative of the general dogs' population affected by CMVD (aged dogs of small and medium size). In this study the prevalence of CKD associated with azotemia complicating CMVD in dogs was assessed. Such prevalence was significantly higher than the prevalence of CKD calculated over the general population of dogs evaluated at the University of Milan (25% *vs* 15%, $p<0.0001$) and higher than the prevalence of CKD reported by colleagues in the dog (0.05% - 5.8%)(Bartlett 2010, O'Neill 2013). This data suggest an influence of CMVD over renal impairment. After more, therapy seems to influence renal function increasing both BUN and sCr even if these results may be affected by the most severe cardiac condition in dogs requiring medical management of HF. The amount of furosemide administered was correlated with BUN but not with IRIS class and sCr. This finding could be explained considering that in situations of compromised renal blood flow, as such as with cardiac disease, the increased reabsorption of BUN from within the renal tubule leads to serum concentrations increasing disproportionately compared to sCr (Medaille 2004, Boswood 2006, Nicolle 2007). The connection between class of cardiac failure and class of renal failure was confirmed by the statistically significant correlation found between the two variables ($p=0.003$). In case of advanced ACVIM class it is possible to make a prediction of advanced IRIS class (and vice versa). This finding, with the statistically significant correlation found between the variable ACVIM class and both BUN and sCr, is of clinical importance in the management of patients with severe heart's condition. Interestingly, IRIS class resulted correlated with the echocardiographic parameters of heart's enlargement LA, LA/Ao and Cornell Index suggesting a direct connection between cardiac remodeling and renal

impairment. ACVIM class showed a statistically significant correlation with a large amount of echocardiographic parameters, as expected, and with murmur grade, as recently reported in veterinary literature (Ljungvall 2014). Rules and decision tree elaborated by the C5.0 [Release 2.07 GPL Edition] software highlighted the impossibility of predicting IRIS class from cardiovascular variables or ACVIM class from parameters of renal insufficiency. However, the decision tree elaborated by the software and the cut off proposed for some echocardiographic parameters (LA/Ao>1.88 and Cornell Index>1.83) could be of clinical interest in the management of HF. The suggested BUN cut off of 131mg/dl may be useful to differentiate renal azotemia than pre-renal azotemia in dogs with CMVD while further studies are needed to confirm this hypothesis. The presence of azotemic dogs (11.1%) in ACVIM class B1, suggests the existence, in some dogs included in the present study, of primary renal damage, not due to HF influence over the renin-angiotensin-aldosterone-system and/or over the neurohormonal systems activation. In these patients renal impairment and CMVD probably just coexisted.

The prospective study assessed the most common used parameters of renal insufficiency sCr, BUN and UPC (associated with urine specific gravity) periodically in a three years period and in well-controlled conditions. It denies the hypothesized cardio-renal connection supported by the retrospective study. None of the cases included experienced both CHF and renal damage in the study period. Considering our expectation, multiple CHF events experienced by some patients should have affected renal function. The persistence of optimal renal condition regardless of CHF events and therapy administration is in contrast with the previous results. The statistical analysis revealed a cause-effect relation between WRF and the Cornell Index calculated over the LVIDd. This data suggest a link between cardiac remodeling,

and in particular left ventricle enlargement, and renal function. To authors 'opinion, the use of WRF, better than single sCr and BUN levels, may highlight the existence of a cardio-renal connection. However the small number of cases included in this prospective study represents a great limit. We consider this work a pilot study.

Limitations

Limitations

The main limitations of this retrospective study are due to the retrospective nature of the study itself. Type 2 and type 4 patients could not be certainly differentiated. The small sample size and the underrepresented ACVIM class B2 could have affect some results. The lack of biomarkers of early renal injury, of urine examination and of abdomen ultrasound made us underestimate the real prevalence of CKD.

The main limitations of this prospective study are related to the small sample size. The restrictive inclusion criteria require a longer period of inclusion. Dogs receiving benazepril at presentation would have needed a withdrawal of therapy before inclusion to reduce the influence of the drugs on UPC and other parameters at baseline.

Conclusions

Conclusions

In conclusion, these results demonstrate that the prevalence of CKD associated with azotemia in dogs affected by CMVD is 25%, higher than the prevalence in the general population. There is a statistically significant direct correlation between ACVIM and IRIS class. Dogs in advanced ACVIM class and receiving therapy for medical management of CHF are commonly affected by concomitant CKD. The Cornell Index, and so left ventricle enlargement, is correlated with WRF. Experiencing CHF seems not to directly affect renal function.

Further investigations are needed to define if CMVD or the administration of drugs for medical management of CHF can directly affect renal function inducing and/or worsening dysfunction of the kidneys.

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