



## RESEARCH ARTICLE

### Quantification of Mitral Regurgitation in Anatolian Shepherd Dogs with Asymptomatic Degenerative Mitral Valve Disease

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#### ARTICLE HISTORY (16-069)

Received: March 20, 2016  
Revised: May 19, 2016  
Accepted: June 19, 2016  
Published online: August 23, 2016

#### Key words:

Anatolian shepherd dog  
Canine  
Cardiac  
Degenerative mitral valve disease  
Quantification of mitral regurgitation

#### ABSTRACT

Degenerative mitral valvular disease (DMVD) is the most frequent cardiac disease, causing mitral regurgitation (MR) in dogs. The purpose of this study was to compare the ratio of the regurgitant jet area (RJA) to the left atrial area (LAA) (RJA/LAA) with subtracting method to quantify regurgitant volume (RegV) and regurgitant fraction (RF) in asymptomatic Anatolian Shepherd Dogs (ASHs) with DMVD. Thirty-eight ASHs with DMVD were used as experimental group. The control group consisted of 35 healthy ASHs. In 38 ASHs with DMVD (20 B1 dogs and 18 B2 dogs), the severity of MR was assessed by RJA/LAA and subtraction method. No differences were noted between the assays measuring the severity of MR by  $\chi^2$  analysis. The observed agreement between the assays was 81% for RJA/LAA vs RegV and was 73% for RJA/LAA vs RF, and the kappa statistic values for RJA/LAA vs RegV and for RJA/LAA vs RF were 0.63 (substantial agreement) and 0.50 (moderate agreement), respectively. Our results indicate that each quantification method was valuable to estimate the acuteness of the disease in ASHs with MR and all were in good accordance with the echocardiographic heart size and N-terminal Pro-Brain Natriuretic Peptide (NT-proBNP) measurements. Therefore, the each of these non-invasive methods may be functional to serially estimate the acuteness of MR in DMVD in order to monitor the progression of disease. Future studies have to evaluate, if these will be useful to anticipate the risk or time of decompensation in asymptomatic dogs.

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**To Cite This Article:** Turgut K, Koc Y, Guzelbektes H, Naseri A, Ince ME and Sen I, 2016. Quantification of mitral regurgitation in Anatolian Shepherd dogs with asymptomatic degenerative mitral valve disease. Pak Vet J, 36(4): 421-424.

#### INTRODUCTION

Degenerative mitral valvular disease (DMVD) is the most frequent cardiac disease, causing mitral regurgitation (MR) in dogs (Terzo *et al.*, 2009; Borgarelli and Buchanan, 2012; Nakamura *et al.*, 2014). Myocardial function, mostly normal or is mildly compromised because of increased preload and decreased afterload in dogs with MR secondary to DMVD, even in the presence of cardiac failure (Haggstroöm *et al.*, 2009). Therefore, the assessment of the regurgitated blood volume into the left atrium (LA) in systole is the major determinant of the degree of left heart volume overload (Kittleson and William, 2003).

Non-invasive estimation of the acuteness of MR is valuable for therapeutic decisions and monitoring of progression. One method used to assess the semi-

quantification MR severity consists of calculating the ratio of the regurgitant jet area (RJA) to the LA area (LAA) (RJA/LAA) using color-flow Doppler mode. The source and course of the jets are also evaluated by this method (Marcello *et al.*, 2014; Sargent *et al.*, 2015a). The subtraction method, where the regurgitant volume (RegV) is determined by subtracting the effective systemic stroke volume (SV) from the total left ventricular SV is the other method to determine regurgitant volume (RegV) quantitatively (Paiva *et al.*, 2012).

There is a debate about the value of each of these quantification methods and only a few studies have reported the comparative value. The purpose of this study was to compare these methods to quantitate RegV and regurgitant fraction (RF) in asymptomatic Anatolian Shepherd Dogs (ASHs) with DMVD.

## MATERIALS AND METHODS

This study was accepted by ethics committee of the Faculty of Veterinary Medicine (permit number: 2012/053). Adult ASHs dogs (n=38) with DMVD and 35 adult healthy ASHs dogs were used as the materials. All the ASHs dogs were referred to the Small Animal Hospital both for a cardiology consultation and for a regular check-up.

The severity of cardiac disease was assigned as specified by the American College of Veterinary Internal Medicine consensus statement (Matos and Glaus, 2010). The vertebral heart scale (VHS) and echocardiographic left atrial/aortic root (LA/Ao) ratio were used for class B animals to differentiate between class B1 and B2. Dogs (n=18) from the experimental group had both VHS scores >10.5 and LA/Ao ratios >1.7 (stage B2, enlarged atrium and ventricle), whereas 20 had both VHS scores ≤10.5 and LA/Ao ratios ≤1.7 (stage B1, normal cardiac size).

Inclusion criteria in the experimental group were the recognition of mitral valve leaflet thickening and/or mitral valve prolapse (MVP) and the determination of MR. The control dogs were regarded as normal according to the normal findings of cardiologic examinations. Dogs were excluded if they had concomitant non-cardiac diseases or cardiac diseases other than DMVD.

**Clinical and cardiologic examination:** The systolic and diastolic blood pressures of each dog were determined with an oscillometric technique (Ware, 2010). The VHS of each dog was measured, and the cardiac enlargement was graded according to the VHS method (Buchanan and Bucheler, 1995).

**Echocardiography:** A complete transthoracic echocardiographic examination, including 2-D, M-mode and Doppler was performed in all the dogs in accordance with techniques (Boon, 2011). The presence mitral valve lesions and MVP, and LA/Ao, end-diastolic volume index (EDV-I), mitral valve early wave velocities (E) and peak early diastolic velocity in the left ventricle mitral annulus (E') were determined. The transmitral E/E' ratio as a filling pressure index was calculated (Höllmer *et al.*, 2016).

The mitral valve insufficiency jets and its direction (central, eccentric) were assessed using color-flow Doppler study. The severity of mitral valve insufficiency jets (%) was subjectively assessed. The largest regurgitant jet found in any plane was recorded as RJA/LAA. MR was assigned as mild, moderate or severe if the RJA/LAA ratio was <20%, 20-40% and >50%, respectively (Boon, 2011).

The quantitation of RegV and RF were assessed by subtracting method where the RegV is determined by subtracting the effective systemic SV from the total left ventricular SV described by Boon (2011). Double measurements were performed to avoid mismeasurements. The plasma N-terminal Pro-Brain Natriuretic Peptide (NT-proBNP) concentrations were measured by enzyme immunoassay.

**Statistical analysis:** All data are reported as the mean±SE. The values for NTpro-BNP, EDV-I and E/E' were evaluated with ANOVA and Tukey's test. Independent

sample t-test was performed to compare the RJA/LAA, RV and RF in the B1 and B2 dogs. To examine univariate associations among RegV, RF, NTpro-BNP and LA/Ao, Pearson correlation test was used. To examine univariate associations between ARJ/LAA and RegV, along with, RF, NTpro-BNP and LA/Ao, the Spearman rank correlation test was used. The agreement among the methods of assays was assessed by McNemar's chi-square analysis and kappa calculation (SPSS 19.0). Significance level was set P<0.05.

## RESULTS

The cardiologic examination findings including the regurgitant jet properties, ARJ/LAA, RegV and RF in the control and the experimental dogs (the B1 dogs, the B2 dogs) are summarized in Table 1 and 2.

The increase in EDV-I was different (P<0.05) in the B2 dogs when compared with the B1 dogs and the control dogs. There was no difference between the B1 dogs and the control dogs (Table 1). The mean plasma NT- proBNP concentration was more elevated (P<0.05) in the B2 dogs (2985±218pmol/L) when compared with the B1 dogs (1588±212pmol/L) and the control dogs (759±47pmol/L). The differences between the B2 dogs and the B1 dogs were also significant (P<0.05) (Table 1). The E/E' showed no difference among the groups (Table 1).

**Table 1:** Cardiologic examination parameters in the control and experimental (B1, B2) groups of dogs

Parameters	Control group	Experimental group	
	(n= 35)	B1 (n=20)	B2 (n=18)
EDV-I (ml/m <sup>2</sup> )	55.7±2.4	52.2±3.6	75.7±2.2
(mean and range)	(29.9-78.1) <sup>b</sup>	(31.4-73.7) <sup>b</sup>	(54.2-85.2) <sup>a</sup>
NT-proBNP (pmol/L)	759±47	1588±212	2985±218
(mean and range)	(250-2050) <sup>c</sup>	(900-4650) <sup>b</sup>	(1250-4300) <sup>a</sup>
E/E' (septal)	4.84±0.27	5.28±0.49	4.90±0.40
(mean and range)	(2.50-8.00)	(3.20-11.76)	(3.00-7.40)

EDV-I=end-diastolic volume index; NT-proBNP=N-terminal Pro-brain Natriuretic Peptide; E/E'=Mitral valve E wave velocity/peak early diastolic velocity in the left ventricle mitral annulus ratio; <sup>abc</sup>= values in each row marked with different character are statistically different with P<0.05.

The 26 dogs (68%) in the experimental group with mitral valve leaflet thickening presented with central regurgitation jet, whereas the 12 dogs (32%) in the experimental group with MVP presented with eccentric regurgitation jet (Table 2). On the color-flow Doppler examination, all the B1 dogs consisting of 20 dogs (100%) had mild mitral regurgitation (<20%), whereas in the B2 dogs consisting of 18 dogs; 6 of which (33%) had mild (<20%), 11 of which (61%) had moderate (20-40%) and 1 of which (6%) had severe (>50%) MR. The severity of mitral regurgitation was more elevated (P<0.05) in the B2 dogs than the B1 dogs (Table 2). According to the results of subtracting method, the RegV and RF values were significantly (P<0.05) higher in the B2 dogs than the B1 dogs (Table 2).

**Bivariate analyses:** The semi-quantitative determination of MR assessed by RJA/LAA was positively correlated to RegV, RF, LA/Ao and NT-proBNP (Table 3). The quantitative determination of MR assessed by RF was positively correlated to RegV, LA/Ao and NT- proBNP (Table 4). The quantitative determination of MR assessed by RegV was positively correlated to RF, LA/Ao and NT-proBNP (Table 5).

**Table 2:** The regurgitant jet properties, RJA/LAA, RegV and RF in the control and experimental (B1, B2) groups of dogs.

Parameters	Control group (n= 35)	Experimental group		P value
		B1 (n=20)	B2 (n=18)	
Eccentric regurgitant jet (n)(%)	ND	7 (35)	5 (28)	
Central regurgitant jet (n)(%)	ND	13 (65)	13 (72)	
Regurgitant jet volume (<20%)(n)(%)	ND	20 (100)	6 (33)	
Regurgitant jet volume (20-40%)(n)(%)	ND	—	11 (61)	
Regurgitant jet volume (>50%)(n)(%)	ND	—	1 (6)	
RJA/LAA	ND	1±0	1.72±0.14	P<0.001
RegV (ml) (ranges)	ND	20.9±3.33 (10-61)	65.9±6.17 (32-140)	P<0.001
RF (%) (ranges)	ND	24±3 (9-56)	41±3 (17-67)	P<0.002

RJA/LAA=the ratio of the regurgitant jet area (RJA) to the left atrial area (LAA); RegV=regurgitant volume; RF=regurgitant fraction; ND=not determined.

**Table 3:** Spearman rank correlations test with RJA/LAA in the B2 and B1 dogs

Variable	t	P value
RegV	5.155	0.000***
RF	2.794	0.008**
LA/Ao	3.624	0.000***
NT-proBNP	3.199	0.017*

RegV=regurgitant volume; RF=regurgitant fraction; LA/Ao=left atrial to aortic root ratio; NT-proBNP=N-terminal Pro-brain Natriuretic Peptide; \*=P<0.05; \*\*=P<0.01; \*\*\*=P<0.001

**Table 4:** Pearson correlations test with RF in the B2 and B1 dogs

Variable	Pearson test	P value
RegV	0.735	0.00***
LA/Ao	0.388	0.019*
NT-proBNP	0.708	0.000***

RegV=regurgitant volume; LA/Ao=left atrial to aortic root ratio; NT-proBNP=N-terminal Pro-brain Natriuretic Peptide; \*=P<0.05; \*\*=P<0.01; \*\*\*=P<0.001

**Table 5:** Pearson correlations test with RegV in the B2 and B1 dogs

Variable	Pearson test	P value
RF	0.735	0.000***
LA/Ao	0.577	0.000***
NT-proBNP	0.643	0.000***

RF=regurgitant fraction; LA/Ao=left atrial to aortic root ratio; NT-proBNP=N-terminal Pro-brain Natriuretic Peptide; \*\*\*=P<0.001

**Table 6:** Comparison of kappa statistics and observed agreement for RJA/LAA vs RegV and for RJA/LAA vs RF for determining MR severity

Test compared	No. of samples	Observed agreement (%)	$\chi^2$ value <sup>a</sup>	Kappa statistic
ARJ/LAA vs RegV	38	81	1.29 <sup>a</sup>	0.630
ARJ/LAA vs RF	38	73	3.60 <sup>a</sup>	0.500

<sup>a</sup>=McNemars  $\chi^2$  value for correlated proportions. <sup>\*</sup>=insignificant difference (P>0.05). RJA/LAA=the ratio of the regurgitant jet area (RJA) to the left atrial area (LAA); RegV=regurgitant volume; RF=regurgitant fraction.

No differences were noted between the assays measuring the severity of MR by chi-square analysis. The observed agreement between the assays was 81% for RJA/LAA vs RegV and was 73% for RJA/LAA vs RF, and kappa statistic values for RJA/LAA vs RegV and for RJA/LAA vs RF were 0.63 (substantial agreement) and 0.50 (moderate agreement), respectively (Table 6).

## DISCUSSION

The RJA/LAA has both advantage and disadvantage (Marcello *et al.*, 2014; Sargent *et al.*, 2015b). The major advantage of the RJA/LAA is the rapidity, repeatability and

reproducibility. It has also been suggested that the determined RJA/LAA corresponded very well to the different degrees of MR, the severity of the clinical symptoms and the other quantitative values (Mele *et al.*, 1995; Lopez-Alvarez *et al.*, 2014). However, RJA/LAA is a semi-quantitative method to detect the regurgitant volume which limits its application (Marcello *et al.*, 2014). In addition to this, the factors such as systemic blood pressure, LA pressure, pulse repetition frequency, angle between jet and ultrasound beam, gain settings and spatial orientation of the jet may effect RJA/LAA evaluations (Muzzi *et al.*, 2003). All the ASHs dogs in this study had no more than 18 mmHg systolic blood pressure, and the difference for septal E/E' among the groups was not significant (Table 1). The 26 dogs (68%) of the experimental group had central regurgitation jet, whereas the remaining 12 dogs (32%) with MVP had eccentric regurgitation jet (Table 2). We agreed that ARJ/LAA method was rapid and reproducible. The observed ARJ/LAAs were mostly comparable with the stage of heart failure. Because, all the B1 dogs had mild mitral regurgitation (<20%) and most of the B2 dogs had moderate mitral regurgitations (20-40%). So, the severity of mitral regurgitation was higher (P<0.05) in the B2 dogs than the B1 dogs (Table 2). The observed ARJ/LAAs were also comparable with LA/Ao and NT-proBNP (Table 3). In this study, more severe mitral regurgitation found in the B2 dogs might be the result of the left atrial and ventricular dilatation, because of high LA/Ao, VHS, NT-proBNP and EDV-I (Table 1).

The other methods to determine RegV and RF quantitatively are a) Proximal Isovelocity Surface Area method, and b) subtraction method (Chetboul and Tissier, 2012; Paiva *et al.*, 2012; Sargent *et al.*, 2015a). Significant correlation has been obtained between the two methods ( $r^2=0.94$ ) in the determination of RegV (Doiguchi and Takahashi, 2000). RJA/LAA is a semi-quantitative method. However, the quantification of MR severity by calculation of the RegV, and assessment of the RF is the main advantage of subtracting method (Gouni *et al.*, 2007). Calculation of the cross-sectional area of the aorta by measuring diameter is the main disadvantage of the subtracting method. Any error in measurement is compounded in the calculation because of squaring the radius (Kittleson *et al.*, 2003). For this reason, measurements in subtracting method were double checked to avoid mismeasurements, in this study.

Mitral insufficiency is usually graded as moderate and severe, according to RF values higher than 30-50% and 75%, respectively (Chetboul *et al.*, 2009). The results of this study showed that RF for the B1 dogs was 24±3% and for the B2 dogs was 41±3%. The difference for RF between the B1 dogs and the B2 dogs was significant (P<0.002) (Table 2). So, in another word, we may inform that the B1 dogs had mild mitral insufficiency, whereas the B2 dogs had moderate mitral insufficiency.

The studies carried out on dogs with DMVD showed that RF was corresponded well with the stage of the disease (ISACHC class), and the left atrial size (LA/Ao) and systolic pulmonary arterial pressure and, NT-proBNP (Serres *et al.*, 2009). In this study, the quantitative determination of MR assessed by RF and RegV were significantly positively correlated to LA/Ao and NT-proBNP (Table 4, 5). In the meantime, the results of this

study showed that asymptomatic ASHs with left heart enlargement (the B2 dogs) or without left heart enlargement (the B1 dogs) might have mild to moderate MR, indicating that the assessment of MR severity could be relevant at this stage of the disease. Therefore, the RegV and RF values may give meaningful results to make decision in the severity of MR in ASHs dogs even at B stage of the disease.

Correlation of the RJA/LAA in mitral regurgitation with both RegV and RF is controversial. Chetboul and Tissier (2012) suggested that the RJA/LAA method has been found to be significantly correlated to RF. The RJA/LAA method might therefore be used for both to assess MR severity and for follow-up the DMVD progression. It may also compensate some limitations of quantitative methods. Muzzi *et al.* (2003) also informed that the RJA/LAA method in MR correlated with both RegV and RF, determined by subtracting method ( $r=0.81$ ). On the other hand, the studies performed by Grossmann *et al.* (1995) in humans were contrary to this. They have found poor correlation coefficients due to erratum in the Doppler method. It might contribute to the differences between the RJA/LAA and RegV and RF by Doppler method. Kittleson and Brown (2003) informed that this was also true in dogs. Only a portion of the LA can be filled with regurgitant jet in a dog with severe regurgitation and, conversely, complete of the LA can be filled with regurgitant jet in a dog with mild regurgitation. The size of the color-flow Doppler jet not only reflects the blood flow ejected into the LA but also the blood around it. Consequently, the RJA/LAA method correlates poorly with RegV. However, it was determined that RJA/LAA was positively correlated to RegV and RF in this study (Table 3). We also found no differences between the assays by  $\chi^2$  analysis in this study. The observed agreement between the assays was 81% for RJA/LAA vs RegV and 73% for RJA/LAA vs RF, and kappa statistic values for RJA/LAA vs RegV and for RJA/LAA vs RF were 0.63 (substantial agreement) and 0.50 (moderate agreement), respectively (Table 6).

**Conclusions:** The RJA/LAA method was easy to obtain as opposed to subtraction method. Our results indicate that the quantification method was valuable to estimate the acuteness of the disease in ASHs dogs with MR and all were in good accordance with echocardiographic heart size and NT-proBNP levels. Therefore, the each of these non-invasive methods may be useful to serially assess the severity of MR in DMVD in order to monitor the progression of the disease. Future studies are proposed to evaluate, if these will be useful to predict the risk or time of decompensation in asymptomatic dogs.

**Acknowledgements:** This study was supported by both The Scientific and Technical Research Council of Turkey (TUBITAK) and Selcuk University Scientific Research Project Council (SUBAP). We are thankful to Dr. Şeref Inal for his excellent assistance to statistical analyses.

**Author's contribution:** KT performed echocardiography, designed and supervised the study, approved the final version of the manuscript. IS, AN and MEI performed echocardiography and drafted parts of the manuscript. YK

and HG performed statistical analysis of the data, created the figures and tables, drafted parts of the manuscript. All authors approved the manuscript.

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