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Speckle-Tracking Echocardiography in Dogs with Patent Ductus Arteriosus

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Index

Foreword

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Structure and function are intrinsically correlated in any organ, making anatomical issues the cornerstone of understanding physiology during health and disease.[1,2]

Cardiology has witnessed in the latest decades an incredible flourishing of theories and technologies to help deepen our knowledge of cardiac morphology and function.[3]

The helical ventricular myocardial band of Torrent Guasp is the first revolution that has shaken cardiology by describing the anatomy of the heart not simply based on "chambers", but identifying for the first time over centuries the exact fiber disposition and correlating it perfectly with function.[1,2]

Starting from the need for validating the new anatomical theory, greater interest has grown also on assessing cardiac function, and new echocardiographic techniques have been developed to study as minimally invasive as possible cardiac motion and its premiere characteristic, contractility.[3,4]

Speckle tracking echocardiography (STE) is the latest echocardiographic tool available in clinical cardiology to assess cardiac function non-invasively. It provides data about myocardial deformation as accurate as tagged magnetic resonance imaging (MRI). The great potential of this technique lies in the possibility to evaluate directly myocardial contractility by tracking the movement of ultrasound reflectors within myocardial tissues (the so-called "speckles") and providing the percentage (strain) or the velocity (strain rate) of deformation of the myocardial fibers during the cardiac cycle.[3,4]

Torsion of the LV is another ground-breaking finding in cardiology, as heart contraction is not restricted to longitudinal, radial or circumferential motion, but also on a counter directed rotation of the heart base and apex, which contributes greatly on ejection and filling of the LV. [5]

Through the advent of minimally invasive percutaneous techniques, new complex palliative or definitive interventions have been made possible without surgery, requiring echocardiography to guide perioperative and intraoperative non coronary cardiac procedures. The success of percutaneous techniques relies on the accurate assessment of defect size, rim lengths and relationship with nearby structures, enhancing decision making, shortening fluoroscopy times and reducing procedural complication rates.[3]

Patent ductus arteriosus (PDA) is one of the most common congenital heart defect in dogs and has benefitted of the increasing experience veterinary cardiologists have taken from human interventional cardiology and imaging, as nowadays is possible to close the defect by means of minimally-invasive techniques with low complication rates, high closure success rates and long term survival.[6]

PDA is not as common in human cardiology as it is in veterinary medicine and stems from a different etiology, thus dogs with PDA might represent an interesting animal model in order to help unraveling further cardiac mechanics in congenital heart disease.

State of the Art

State of the Art

Patent Ductus Arteriosus

Definition, Incidence and Etiology

The ductus arteriosus is a vascular structure normally present during fetal life which connects the ascending aorta and the main pulmonary artery and allows right ventricular output to bypass the nonventilated fetal lungs.[7,8]

During birth and few hours later, following the physiological changes of the cardio-pulmonary system, the ductus undergoes functional closure, followed by anatomical closure over the next several days; in the adult it can be seen as a fibrous structure called ligamentum arteriosus.

The ductus has to close by the first week of life. Ductal constriction is mediated by different physiological and neurohormonal factors which all contribute to smooth muscle contraction and prevent ductal patency[8]. (Fig 1)

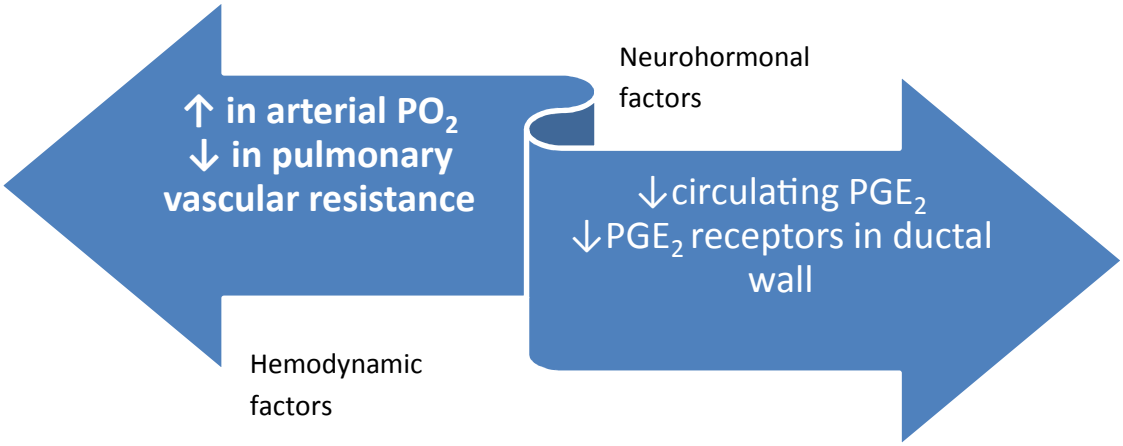


Fig 1 : simplified scheme of mechanism regulating ductal constriction[8]

In human medicine the etiology of PDA can be divided in three distinct categories: a congenital condition in term infants in which PDA is an isolated finding (nonsyndromic PDA), part of more complex congenital malformations (syndromic PDA) or a complication arising in preterm infants.[9]

The prevalence of PDA in term infants is approximately 2-8 per 10.000 live births, while in preterm infants the incidence of a PDA increases most linearly with decreasing gestational age and the overall incidence in infants weighing <1.5kg is approximately 37%. [9]

In dogs, PDA as a complication in preterm puppies has not yet been reported. PDA as single cardiac malformation is the most common presentation and the prevalence of PDA in different university teaching hospitals is around 3.1-4.7 over 1000 examined dogs.[10,11] PDA is one of the three most common cardiac malformation in veterinary medicine, accounting for 21 to 32% of congenital heart diseases. [10-12] Subaortic stenosis and pulmonic stenosis are the most common congenital heart defects in dogs presenting with multiple cardiac malformation and PDA (8.8-15 % of dogs with PDA)[12,13]

The pattern of inheritance does not follow a simple mendelian trait and is thought to be a polygenic threshold trait with high heritability and low environmental influence : as the proportion of defective genes received from the progenitors increases, the proportion of dogs in the same litter to present incomplete closure increases in frequency and in the severity of disease. [14]

There is a clear sex predisposition, with female dogs outnumbering males with a 1.5-3:1 ratio.[10,11,12,14]

Breed predisposition is mostly dependent on geographical location, but generally speaking small breed dogs (Bichon Frisé, Poodle, Maltese, Bolognese, Chihuahua, Yorkshire Terrier, Dachshund and Cavalier King Charles), some shepherd breed dogs (German Shepherd, Collie, Shetland Sheepdog, Australian shepherd) and other large breed dogs (Newfoundland, Dobermann) are prone to develop the disease.[10-14] (Tab1)

	Breed Predisposition	Odds ratio
Patterson, 1953-1965	Poodle Collie Pomeranian	5 - -
Buchanan, 1986-1990	Maltese Toy & Miniature Poodle Bichon Frisé Pomeranian Yorkshire Terrier English Springer Spaniel Shetland Sheepdog Cocker Spaniel	12.4 6.7 & 5.9 5.5 4.6 4.2 4 3.9 2.6
Oliveira et al, 2011	German Sheperd Newfoundland Maltese Dobermann Cavalier King Charles Spaniel	5.2 4.65 4.14 2.8 3.7

Tab 1: breed predisposition and estimated relative risks (odds ratio) by breed in distinct surveys.

Pathogenesis

The failure in ductal closure in dogs with PDA is always secondary to the inability of the ductus to contract and ensure complete functional closure during postnatal period.[8]

Canine ductus arteriosus is normally constituted by smooth muscle cells (constituting 98% of ductal wall), which encircle the entire ductal diameter and length.

The cause for patency is due to an insufficient ductal muscle mass and to the presence of aorta-like elastic tissue in the ductal wall : both these anomalies unable the ductus to exert enough strength to stop blood flow during the delicate transition between fetal and postnatal circulation. Furthermore, it has been shown by serial histological sectioning of the ductus that muscle loss and ductal length were inversely related: the shorter the ductus, the less ductal muscle mass was present.[15]

This finding is not proportionally related to the severity of disease, except for dogs with reversed flow (right to left shunt) in which the histologic alterations were the most severe (>50% ductal diameter constituted by elastic wall tissue).

Generally, the remaining smooth muscle cells are located near the pulmonary artery and this histological characteristic is reflected by the general appearance in macroscopic anatomy of the ductus, which is funnel shaped with the narrowest segment adjacent to the pulmonary artery. A *forme fruste* of PDA is the ductus diverticulum, where the ductus arteriosus is closed at the pulmonary ostium but the ductal ampulla is dilated. This condition is not associated with clinical disease but is identified during angiography or necropsy and indicates the dog is carrying some genes related with disease.[10,15]

Pathophysiology

The persistence of a communication between two vessels causes blood to shunt depending on pressure gradients and vascular resistance.

In the case of a physiological postnatal circulation, PDA patients present generally with a left to right shunt.

This condition is the most frequent and accounts for 96-97% of total PDA cases, as the reversion of the flow due to pulmonary vascular remodeling is uncommon (3-4% reported in veterinary literature).[10,11,14,16]

Left to Right Shunt (L-R PDA)

Blood shunting from the ascending aorta increases pulmonary flow and finally reaches the left ventricle, thus the typical manifestation of L-R PDA is a volume-overloaded left ventricle with pulmonary overcirculation.

Blood flow in this case is also dependent on ductal diameter and resistance to flow through the defect, with small ductus causing little ventricular volume loading and large ductus determining left ventricular and atrial enlargement with highest chance of clinical signs.

The main effect of PDA on cardiovascular hemodynamics is primarily a volume-overloading action on the left ventricle. It determines an increase in end diastolic volume and pressure (increase in preload), which, following starling's law, induces increased contractility, which stimulates myocardial growth in order to supply for effective ejection of an increased amount of blood during the cardiac cycle. [10,11]

Nonetheless, even if stroke volume is increased and contractility is enhanced by these mechanisms, end systolic volumes are also increased in dogs with PDA, because the heart is not able to pump efficiently on a single beat all the extra blood received, thus afterload is increased too. As more blood is pumped in every cardiac cycle, an increase in aortic velocity is seen (relative aortic stenosis).[10,11]

Aortic systolic pressures are higher than normal, while diastolic pressures are decreased, due to the continuous leakage of blood into the pulmonary artery. This finding is appreciated on peripheral pulse: dogs with PDA have frequently a "water-hammer" or hyperkinetic pulse.
When volume overload is severe, atrial dilation may be present .Another clinical condition frequently associated with PDA is pulmonary edema, when atrial pressure rises and causes venous congestion and fluid retention in the alveolar bed.[10,11]

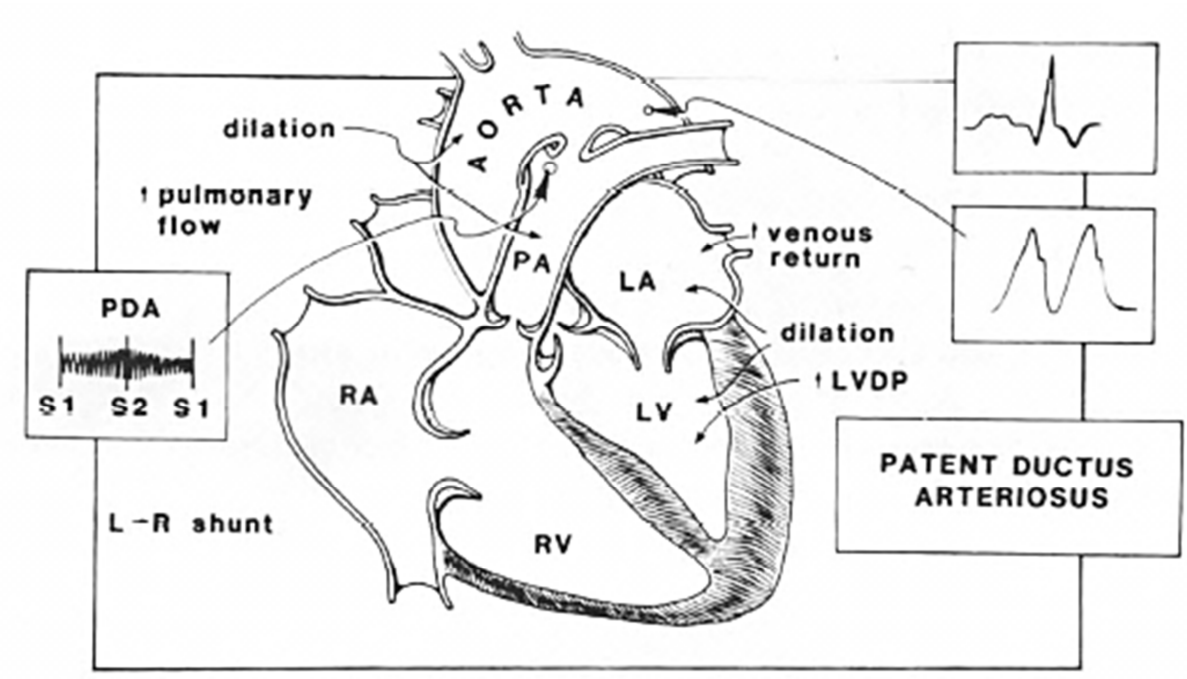


Fig 2: a schematic summary of pathophysiology and genesis of clinical findings in L-R PDA (from Fox PR, Sisson D, Moise N, eds Textbook of canine and feline cardiology : principles and clinical practice, 1999, WB Saunders)

Right to Left Shunt (R-L PDA, reversed PDA)

Note: As the main objective of the experimental work will be related only on L-R PDA, some clinical and therapeutic findings on R-L PDAs will be explained briefly in this section.

In tubular-shaped PDA, where little ductal smooth muscle is present (histological grading 6 following Buchanan's classification) and no stricture at the pulmonary end is present, the dramatic increase in pulmonary blood flow determines early pulmonary vascular remodeling in an attempt to stop or reduce blood flow through the lungs.[15]

This is associated with an increase in pulmonary vascular resistance and in the onset of pulmonary hypertension, thus reverting PDA flow (Eisenmenger's physiology).

The vascular lesions secondary to vascular remodeling are advanced and irreversible and generally happen few weeks after birth. In a colony of dogs bred by Patterson et al, some puppies were followed from birth up to the first 3 months of life and those with R-L PDA experienced shunt reversal between 2 to 5 week of age.[14]

The continuous murmur, pathognomonic for PDA, generally disappears as the flow reversed, and the typical clinical manifestation is differential cyanosis, weakness and syncope. As a result of persistent hypoxia, polycythemia with hyperviscosity syndrome occurs. [14]

Therapeutic strategies are focused in increasing quality of life and in alleviating hypoxia; this includes phlebotomy or the use of bone-marrow suppressant agents (hydroxyurea or cyclophosphamide) in order to decrease packed cells volume (PCV) when severe polycythemia is present and PDE-5 inhibitor sildenafil citrate in order to increase quality of life and decrease erythrocythosis.

The most important consequence of flow reversal is the impossibility to close the defect, as this would decrease blood flow to the lungs. Few data are available concerning survival, however dogs with rPDA are supposed to live 2-5 years.[10,11,17]

Clinical and instrumental findings

Dogs with L-R PDA are most of the time asymptomatic, as the only alteration identified during clinical examination is a continuous murmur located on the heart base. It is sometimes possible to hear a second murmur, located on the mitral orifice, and this is secondary to mitral annular stretching due to volume-overloading.

Cardiac thrill may be localized or easily palpated in the left emithorax in most of the patients. Femoral pulse might be hyperkinetic or bounding .

Clinical signs related to PDA are stunted growth, exercise intolerance, cough, dyspnea and, in dogs with atrial fibrillation or right to left shunting, ascites.

ECG findings can show left ventricular and atrial enlargement patterns, ventricular or atrial premature complexes or atrial fibrillation mostly in medium to large breed dogs.

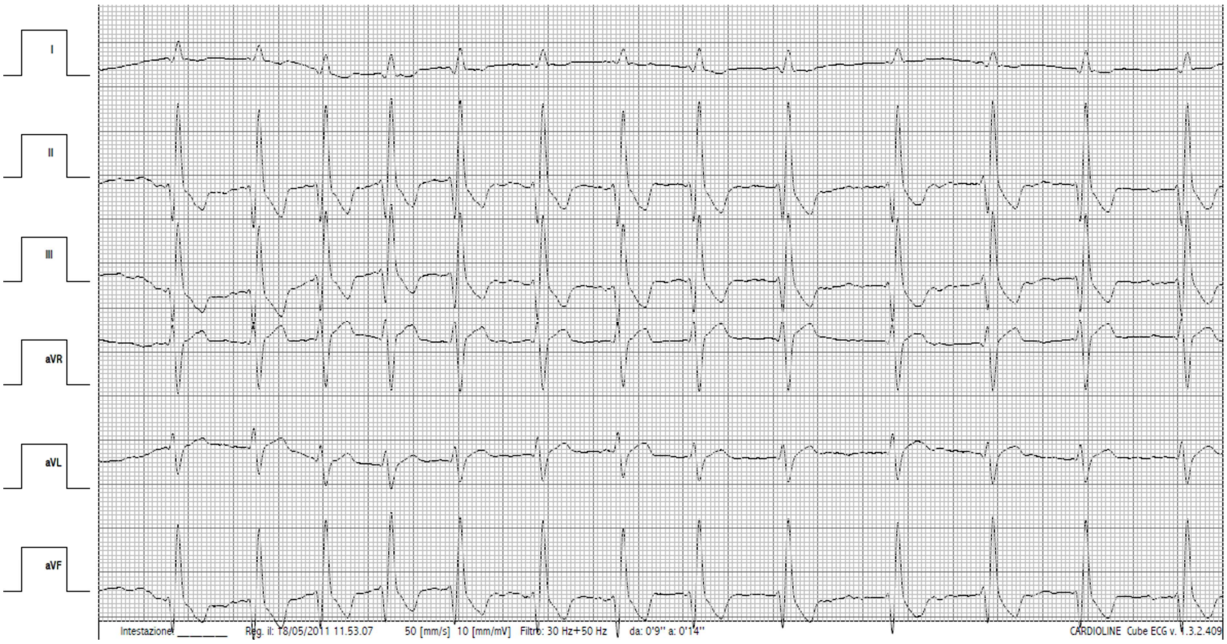


Fig 3.Electrocardiogram recorded in a patient with L-R PDA and exercise intolerance. QRS complexes are enlarged and irregularly irregular, with absent P waves. The ECG finding is consistent with atrial fibrillation with a left bundle branch block morphology (ventricular enlargement pattern)

Radiographs generally show varying degrees of left-sided cardiomegaly, ductal aneurysm, pulmonary overcirculation with increased diameter of both venous and arterial pulmonary veins. Dogs with left sided congestive heart failure also present with pulmonary congestion and an interstitial-alveolar pulmonary pattern.

Transthoracic Echocardiography (TTE) is generally the cornerstone in the diagnosis of PDA, which is almost always identified on both right and left views.[18] The ductus is seen on the pulmonary side and a continuous blood flow seen both on Color and Continuous wave Doppler confirms the clinical suspicion. Spectral Doppler shows the classic flow pattern of a continuous flow with the highest velocity recorded during systole and a decrescendo slope on the diastolic phase.[10,11]

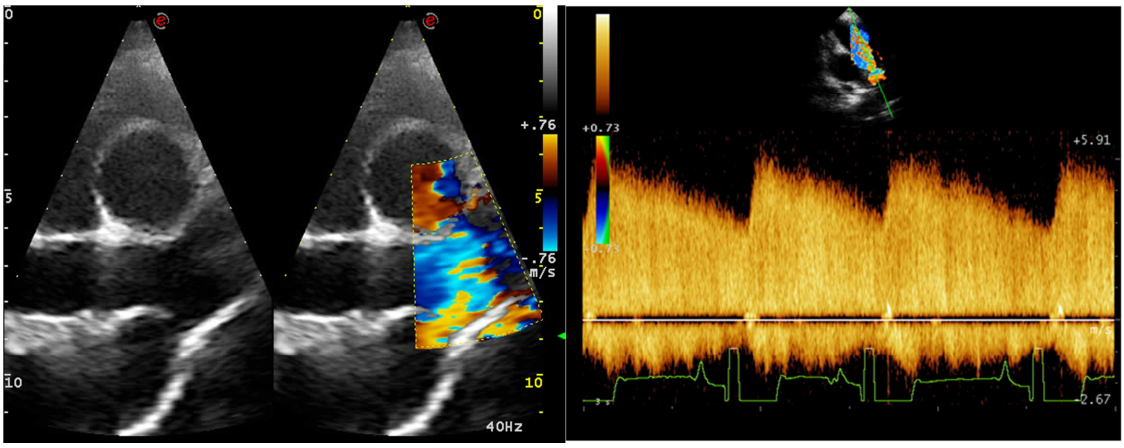


Fig 4.Continuous wave Doppler tracing showing the typical continuous flow through the ductus.

TTE echocardiography can provide adequate measurement of ductal dimension and can quantify left ventricular overload/left atrial enlargement. The quantification of systolic function based on fractional shortening (FS) and ejection fraction has been routinely used in veterinary and human medicine.[16,20-23]

The accuracy of TTE on ductal sizing for cardiovascular interventional procedures however is lower as compared to angiography[18,19] or transesophageal echocardiography, with a general overestimation of minimal ductal diameters with TTE[24,25].

Transesophageal echocardiography (TEE) can provide accurate, radiation-free technology to assess ductal diameters and monitor minimally-invasive procedures.[3, 24-26]

Few consistent studies demonstrated its usefulness in providing one of the best assessment of PDA sizing and morphology, with a general superior image quality as compared to TTE (due to anatomical proximity to the heart base), thus becoming one of the leading technique to rely on PDA device selection and deployment.[24-26]

TEE consists of a multiplanar, phased-array transducer mounted at the tip of a modified flexible endoscope, which can allow ventral (anteroflexion) or dorsal (retroflexion) flection of the tip and can permit rotation of the phased array from 0° to 180°, thus allowing multiple views from the same esophageal position. The standard image for ductal visualization is obtained from cranial longitudinal views.

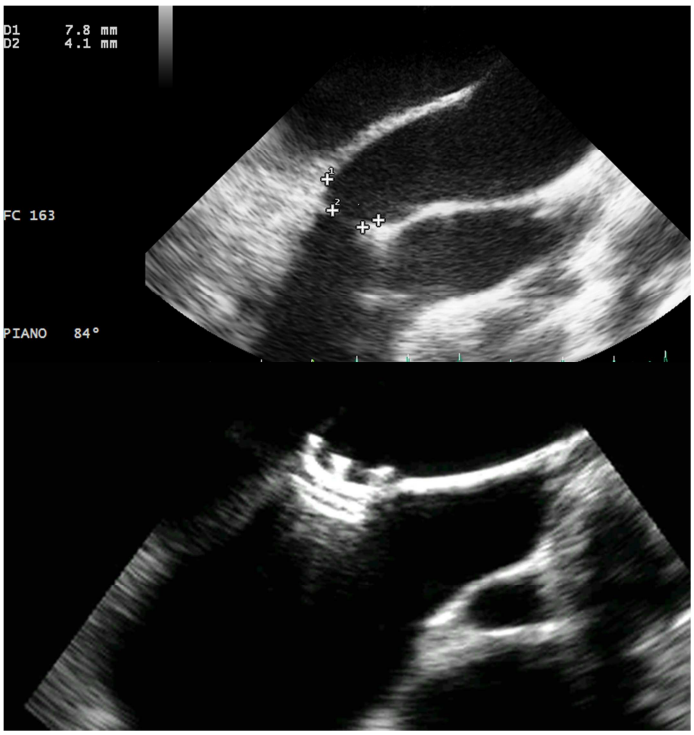


Fig 5 Intraoperative assessment of PDA size and ACDO in position during intraoperative monitoring with TEE.

Angiography has been considered the gold standard in confirming the diagnosis of PDA long before the clinical use of echocardiography and has been since considered as the elective technique to assess PDA morphology and dimensions during interventional cardiology.
The increasing enthusiasm on TEE for morphological assessment of PDA has limited, but not eliminated the usefulness of angiography during heart catheterization.
The standard classification of ductal morphology comes from angiographic measurements and is currently used to classify ductal morphology, which is of outmost importance due to the fact that different techniques for PDA closure have to be considered depending on PDA shape: minimally-invasive techniques can provide efficient ductal closure only on type I, IIa and IIb, while type III PDA should undergo surgical closure, due to the lack of a restriction on the pulmonary side necessary for device stability.
The classification of veterinary PDA comes from the Krichenko's classification of human PDAs.[18,27]

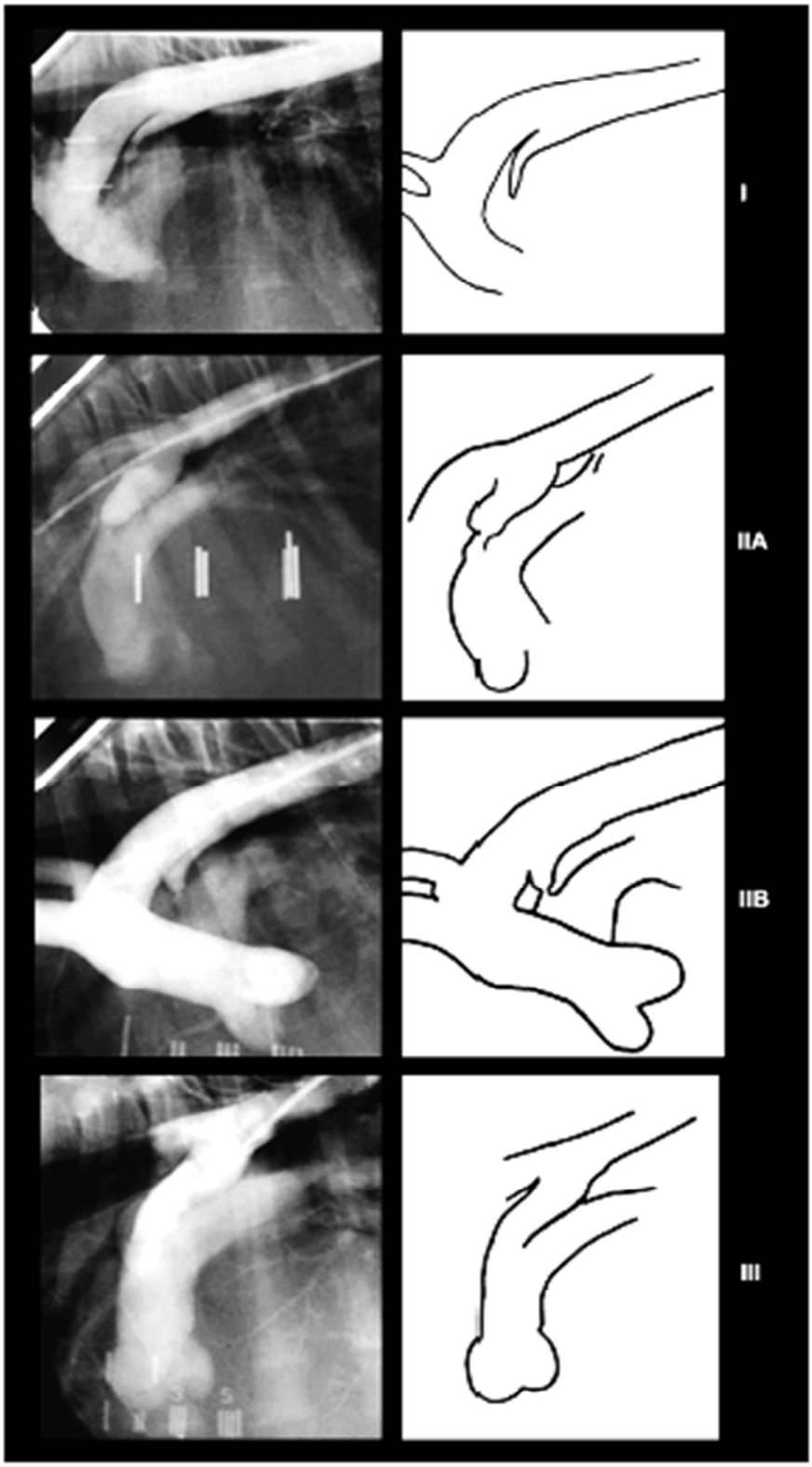


Fig 6: angiographic classification of PDA morphology [27]

Natural history and therapeutic options

Few data are available concerning the natural history of dogs with PDA because as early as in the 50s surgical closure was already performed by leading surgeons in veterinary university teaching hospitals.

The first report in which a comparison was made between untreated vs surgically treated dogs dates back in the late 70s by Eyster et al[28]. The reported survival in dogs with PDA in which the owner refused surgery was as high as 65% by the first year of age, with the remaining dogs lost to follow up. However, it must be said that the group of untreated dogs was made up by only 14 cases and that few of the drugs now available were then commercialized.

More recently, a study by Van Israel and colleagues identified longer survival time (cardiac death, mean 87months, range 26-114months) in a smaller group of dogs not undergoing PDA closure, however severity was not assessed in this subgroup of patients, thus higher survival might be related to small, hemodynamically irrelevant PDA.[20]

The most recent study to take into account dogs not undergoing PDA closure was published last year by Saunders and colleagues[13]. Even if the number of untreated dogs was higher than other reports, most of the dogs were euthanised shortly after diagnosis (37/64 survival time <1day);nonetheless a marked difference in median survival time between dogs which underwent treatment and those which did not was observed: PDA closure adds 10 years to the median survival time to the median lifespan (12 years treated vs 2 years median survival time in the untreated group).

Thus, closure of PDA is of outmost importance and should always be performed.

Different techniques can be used in order to achieve ductal closure, but the most important classification divides all the techniques into surgical and percutaneous ones.

Surgical closure

Surgical closure of the PDA was the only therapeutic option since minimally invasive techniques were developed. The standard surgical technique is performed from a left 4th intercostal thoracotomy.
The ductus is dissected from surrounding tissue by blinded gentle cranial and caudal dissection by use of right angled forceps, then suture strands are passed caudo-cranially at the aortic and pulmonary ends.
A double ligation is accomplished and the ductus is closed.

The complications mainly reported with the standard surgical technique are tearing of the PDA and hemorrhage, mostly in older dogs (due to more friable ductal tissue) and in dogs weighing more than 23 kg.

The Jackson-Henderson ligation was developed in order to reduce the risk of ductal tearing and rupture by the blinded dissection of the standard method. It is the suggested technique when bleeding or rupture associated with the standard technique occurs.

The Jackson-Henderson technique involves dissection of the dorsal aspect of the aorta and not on the medial aspect of the ductus, while the aortic arch is elevated. The ligatures are tightened from the dorsomedial aspect of the aorta to the cranial aspect of the ductus, and a second strand is passed to the caudal aspect of the ductus. Both are tied, one more dorsal and the other more ventral, achieving ductal ligation.

This technique involves more soft tissue to be incorporated with the ductus, with higher prevalence of residual flow due to improper tightening of the sutures around the PDA.

WHEN TO PERFORM : Surgical ligation of PDA is still effective and experienced surgeons achieve low mortality and complication rates. Thus it must be considered when percutaneous methods are not recommended, i.e. dogs with large, tunnel-shaped PDAs (type III) and in small dogs with large PDAs, where a catheter-based approach is not feasible due to small peripheral vessel dimensions.

Percutaneous techniques and devices

Minimally invasive vascular procedures have been sought and investigated intensively since early 90s both in human and veterinary medicine in order to minimize complications and mortality related to general anesthesia, thoracotomy and in order to reduce postoperative care and pain. Percutaneous techniques allow heart catheterization from a peripheral vascular access (i.e. femoral or jugular vein, femoral artery). The vessel is catheterized by Seldinger technique or by surgical cut-down/modified Seldinger technique. An introducer is then inserted, which allows patency of the vessel and catheters to pass through the introducer and reach the target vessel (for PDA closure it is generally the ascending aorta and the ductal ampulla).

Since 1994, numerous devices have been used in order to achieve PDA closure, both from transvenous and transarterial routes.

A brief description of the percutaneous devices and its indication of use will be provided. It should be stressed that a percutaneous approach should always be considered as a first line treatment in most of the dogs with PDA, due to the low mortality rates and the low rate of complication reported, although variable depending on the device used.[29]

Coils

Coils are the first device developed for ductal closure in both human and veterinary medicine : as the name suggests, coils are made of metal and synthetic fibers and, when released or detached, they arrange in a spiral shape, which has to be adjusted from the main pulmonary artery (1 loop) into the minimal ductal diameter and in the ampulla, in order to induce controlled coagulation.

A wide variety of detachable and controlled-release coils are available and some of them can be deployed with very small catheters (as small as 3F), thus enhancing the feasibility of percutaneous ductal closure also in very small dogs (<3kg). Accurate minimal ductal sizing is of outmost importance, as the most common complication related to coils is pulmonary embolization due to instability of the device (reported incidence of 3%-15%).

Two routes of coils embolization have been described: the transarterial and the transvenous[29-31]. The first technique aims at reaching the ductus through the femoral artery, thus catheters and coils are deployed from the ductal ampulla, while the transvenous technique, most commonly used in humans, deploys coils from a retrograde way, as catheters are inserted through the jugular vein, pass through the right ventricle, pulmonary artery and finally enter into the ductus. Generally, the transarterial route is the most commonly adopted in veterinary medicine.

The use of multiple coils of different diameters and length are generally necessary in order to achieve complete PDA closure, as residual flow is commonly reported in up to 66% of patients in the immediate perioperative period. Another complication related to coils is transient hemolysis, induced by the presence of large coils and residual flow, which causes mechanical damage to erythrocytes passing through the ductus.

WHEN TO USE :The recommended use of coils in the latest years is in small patients (<2.5kg) with small PDAs (minimal ductal diameter smaller than 2mm or <4mm depending on authors' opinions).[29,32]

Amplatzer Ductal Occluder

The ADO is the counterpart of ACDO in human interventional cardiology, designed to fit the human PDA. It has been used in large PDAs (>6 mm diameter) in veterinary medicine when the ACDO was not yet available.[32]

Amplatzer Vascular Plug

The Amplatzer Vascular Plug is a nitinol mesh self-expandable device routinely used for arterial controlled embolization in human medicine. It was not designed for PDA closure, however represented an alternative to coils in veterinary medicine when the ACDO was not available(before 2007)[33]. Owing to the double umbrella-like appearance, it was suggested it might be of use in closing the PDA and one study reported safety and efficient PDA closure in 93% of patient, with lower residual flows as compared to coils. The major complication in AVP device is the risk for embolization if device sizing is not accurate, probably due to the fact that AVP morphology was not intended for canine PDA.

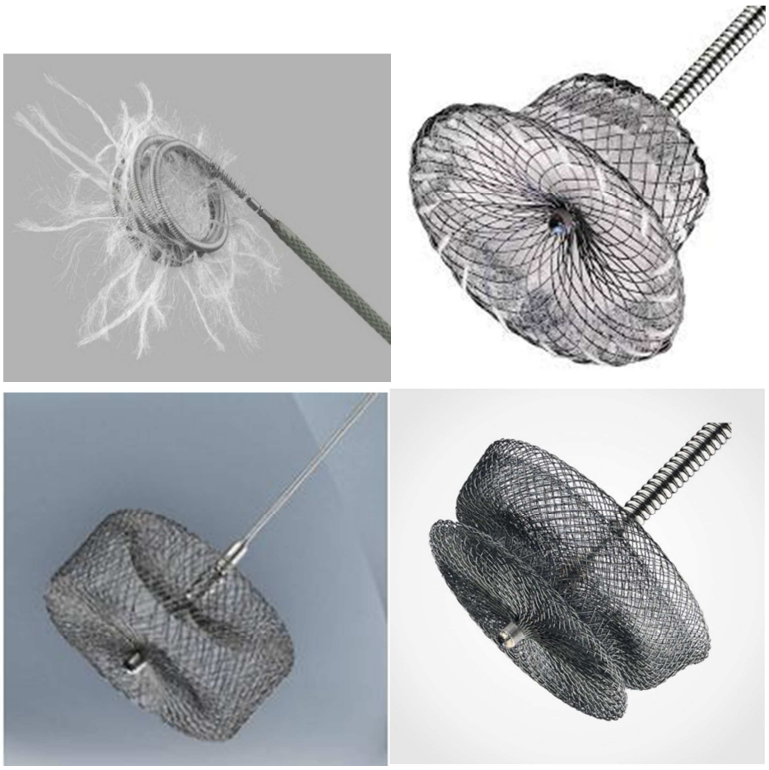


Fig 7. Percutaneous device available for PDA closure.From top left: detachable coils, Amplatzer Ductal Occluder, Amplatzer Vascular Plug, Amplatzer Canine Duct Occluder

Amplatzer Canine Duct Occluder (ACDO©)

The ACDO is a controlled release, self-expanding nitinol-mesh device . It has been designed to conform to the morphology of canine PDAs and is currently considered the gold standard in percutaneous closure, as it is easy to use, safe and efficient with lowest residual shunt rates as compared to coils or other Amplatz devices[29]. It is constituted by two discs, the first is designed to be deployed at the pulmonary ductal end (flat disc), the waist is then placed in the ductal minimal diameter, while the second, cup-like, disc has to adjust into the ductal ampulla[6]. This morphology confers the ACDO great stability, provided the minimal ductal diameter is circular and there is enough membrane at the pulmonary end (type I, IIa and IIb angiographic PDA classification)[24,25].

The ACDO is released through a delivery cable , which can be carried by a guiding catheter, and the transarterial approach is the recommended catheterization approach.

Device sizing has been referred to be 2 times the minimal ductal diameter, however lower device sizing has been used [24-25,33](1.8 silva et al)

WHEN TO USE : The ACDO is considered the gold standard in PDA closure and should be the first-line treatment option in all dogs.

A comparison between techniques and devices for PDA closure

The comparison between techniques and thus the definitive suggestions as whether one approach is preferred to another relies only on few studies.

Most of these considered not only perioperative death as a complication, but generally divided the complications into major (in which patient's life might be at risk) and minor (no risk for patient's life)[30].

In the first study in which a comparison between surgical vs percutaneous technique (coils) was made, perioperative mortality rates (death within 14 days) were not statistically significant in the two group of dogs; however, major complications (12% of surgical patients) were more commonly reported in surgically treated dogs as compared to percutaneous one and minor complications were most commonly present in dogs with a percutaneous approach (26%)[30].

When comparing different devices used for percutaneous closure of PDAs, the most important factor to take into account is the possibility to close the ductus and the presence of residual flow. Coils (26% by transarterial and 33% by transvenous approach) and AVP (26%) have higher complication rates as compared to ACDO (3% of complication). ACDO fluoroscopy times were lower as compared to other techniques and also residual flow was detected in fewer cases and was most of the time trivial.[29]

Study and technique used	Major complication (%)	Major complication description	Minor complication (%)	Minor complication description
Goodrich et al Surgical vs Coils	12% surgical 4,3% coil	Hemorrhage, lung damage, mesenteric torsion, embolization of coils into the systemic vasculature	12% surgical 26% coil	Limb lameness, suture reaction or seroma, coil embolization into pulmonary vasculature, transient hemolysis
Singh et al Coils, AVP, ACDO	3% ACDO 26% transarterial coil 26%AVP 33%transvenous coil	-no PDA closure (Type III) -pulmonary embolization and femoral artery tearing -device instability delayed recanalization -femoral vein laceration, vena cava laceration and no PDA closure	2.8% 45% 39% 23%	Residual flow 24hr post

Tab 2: reported incidence of complication with different techniques and devices.[29,30]

From these data it can be suggested that the percutaneous approach has few major complications and the ACDO has excellent closure rates with low complications and zero perioperative death rate. ACDO should be the first line treatment for dogs with type I, IIa and IIb PDA. The use of coils should be limited to small patients with a small minimal ductal diameter but with femoral arteries large enough to accommodate catheter sheaths. Very small dogs or dogs with type III PDA should always be referred to surgeons in order to achieve ductal closure.[29,30,32]

Quantifying PDA shunt: the role of echocardiography

Techniques and criteria available in veterinary medicine to evaluate PDA hemodynamics.

The presence of a PDA on term puppies determines left ventricular volume overload with increased preload and afterload; it might be accompanied in some cases by systolic dysfunction. Atrial fibrillation may compromise further left ventricular output and thus increase systolic dysfunction by a tachycardia-induced mechanism.

All these findings are well-known, however different tools are available to quantify specific aspects of PDA physiology (left ventricular overload, contractiilty, cardiac output, degree of hypertrophy) but generally focus only on one aspect and lack of a general view.[10,11]

The definition of hemodynamically significant PDA in human medicine is still a controversial issue and the classifications provided rely on ductal dimensions and personal evaluation rather than being based on consensus statement.[23]

In veterinary cardiology, dogs with a continuous murmur are generally referred for closure, regardless of PDA dimensions, shunts or LV overload. Echocardiography is the tool generally used in the clinical setting to evaluate PDA hemodynamics, as invasive measurements by heart catheterization or MRI-derived indices of function are generally used under research settings.

The presence of a PDA alters normal cardiovascular function, but also PDA closure is associated with a change in cardiovascular hemodynamics: postclosure changes are related to a decrease in preload and an increase in afterload, due to the elimination of the shunt.[10,11,16,34]

Echocardiography shows an immediate decrease in end-diastolic volumes and a slight decrease of end systolic volumes, with a reduction in left atrium to aorta ratio immediately after PDA closure. It has also been reported a decrease in aortic flow velocities and in some patients a decrease in fractional shortening, thus the presence of systolic dysfunction has been advocated, however no influence was found on survival times. [16,34]

The assessment of PDA-related changes by echocardiography can be thus divided into three main topics:

1. Left ventricular volume overload quantification
2. Shunt quantification
3. Indices of contractility/ systolic function

Left ventricular geometry and volume quantification

The left ventricle is a V-shaped structure (bullet-like) responsible for the ejection of blood in the systemic circulation. Shape and function are strictly correlated, so that a change in shape might be related to a change in cardiac function and vice versa. Volume quantification in systole and diastole provides information about both systolic and diastolic function and also evaluates chamber dilation and hypertrophy.

Assessing cardiac volumes may present some limitation due to different factors:

- All assessments of cardiac volumes are obtained by mathematical formulas, all of which come from a simplification of a 3D cardiac geometry into an M-mode/2D structure.
  The equations are based on the assumption that a normal left ventricle can be compared to a geometrical shape, thus making possible evaluating areas and volumes from it;
- The formulas were extrapolated from normal hearts, however this might not correlate as well as in diseased, dilated hearts; thus the accuracy may decrease during chamber dilation;
- The correlation between different methods has not been established, and currently there are no conversion tables available;
- Cardiac volumes are assessed both in systole and diastole, however veterinary patients have generally higher heart rates as compared to humans, thus high temporal and spatial resolutions are required, because imaging quality is of outmost importance in assessing/tracking cardiac volumes.
- Body surface area (BSA) is required in most of these equations to compare animals with different body weights, but in obese or malnourished animals this can be over- or underestimated, thus the accuracy can be lowered.

In veterinary medicine, four methods are currently reported to assess cardiac diameters and volume.[35]

M-mode derived Teicholz formula

The Teicholz formula is based on the assumption that the left ventricle is an ellipse and is as follows:

LV diastolic volume (LVVd) = $\frac{(7x\,(LVIDd)^3)}{(2.4+LVIDd)}$

LV systolic volume (LVVs) = $\frac{(7x\,(LVIDs)^3)}{(2.4+LVIDs)}$

The most commonly Teicholz-derived formulas are the end diastolic volume index ($EDVI_M$) and end systolic volume index ($ESVI_M$), which are obtained dividing LVVd/s by the BSA.

An M-mode derived EDVI less than 100ml/m^2 and ESVI less than 30ml/m^2 are considered normal in all breeds and sizes of dogs.

From the right parasternal short axis view at the level of the chordae tendineae the beam is directed perpendicular to the left ventricular walls and should bisect into equal halves the left ventricle. Measurements are made in diastole (beginning of the QRS) and systole (peak downward point of septal motion-wave/end of T wave), and these provide diastolic and systolic left ventricular dimensions (LVIDd/LVIDs).

The Teicholz method was the first echocardiography-based equation to assess ventricular volumes, however when compared from 2D images-derived indices of volumes it has lower accuracy, mostly in a dilated heart. It is intuitive to consider that extrapolating LV morphology from a mono dimensional image of the heart is less accurate than a 2D image; furthermore, when the LV undergoes dilation, the Teicholz formulas are associated with an overestimation of the volumes. [36]

M-mode derived allometric scaling (Cornell's index)

The allometric scaling was proposed in order to predict chamber size based on the assumption that cardiac dimensions are related to weight and other constants (allometric equation is $Y=aM^b$).

The final equation proposed to calculate an indexed value from M-mode derived end diastolic and systolic diameters was based on 494 healthy dogs from different breeds and weights (2.2kg -95kg)as a reference population.ref cornell

The indexed value is as follows:

AlloD = M-mode LVIDd/$BW^{0.294}$

AlloS= M-mode LVIDs/$BW^{0.315}$

Values of AlloD between 1. 27 and 1. 85 were predictive of a diastolic index being in the 95 % percentile of the referenced population, while AlloS value between 0.71 and 1.26 were predictive of systolic index of normality.[35]

The allometric scaling system can be used to predict deviation from normal values, however it is based on the extrapolation of data from dogs of different breeds considered to be normal by echocardiography and physical examination, thus it does not refer to specific breeds and to a certain population of dogs.

One of the major limitation is that allometric scaling still relies on M-mode derived values, with all the issues related to obtaining a good mono-dimensional image from a 3D structure, thus lacking in a way in providing accurate assessment of cardiac chambers.

2D derived Area-Length/ monoplanar Simpson's rule of discs

The Area-Length method is based on the modified monoplanar Simpson's method, under the assumption that the LV volume can be quantified by the sum of the area of small discs (summation of discs method). One view in veterinary medicine can be considered accurate, in contrast with human medicine, because the presence of regional motion wall abnormalities is uncommon.

The area length method implies calculating the length of the left ventricle and calculates the area by tracking endocardial borders; the equation is as follows:

Volume= 0.85 $Area^2$/Length

It can be obtained both from right parasternal 4 chamber long axis view or from apical left 4chamber view. The volume is calculated both in diastole and systole and it can be divided by BSA ,providing $EDVI_B$ and $ESVI_B$. A diastolic volume index <70ml/m^2 and a systolic volume index <30ml/m^2 are considered normal in dogs.

The major limitation of the area-length method is related to image quality rather than equation limitations : to achieve accurate measurements it is of outmost importance to obtain a good image of the left ventricle and not to shorten the ventricular chambers, which in particular cases is challenging.

2D derived biplanar Simpson's rule of discs

This formula implies calculating the volume of the left ventricle as the sum of at least a stack of 20 discs in two imaging planes, generally the left apical 4chamber and 2 chamber views. Both views should maximize length and width and can provide a more accurate measurement of the LV.

The formula for biplane Simpson's is as follows:

$$\text{Volume} = \frac{\pi}{4} \sum_{i=1}^{20} a_i b_i \frac{L}{20}$$

Like the monoplanar version of the modified Simpson's rule, dividing the volume for BSA provides $EDVI_B$ and $EDVI_B$.

The apical 2 chamber view is sometimes subject to drop-out artifacts and cannot provide a satisfactory apex visualization, thus this can be considered the main limitation of Simpson's biplanar equation in veterinary medicine. The coefficient of variation within-day and between-day for a single trained operator is acceptable (<11%CV)

Spericity Index

The sphericity index (SI) is based on the concept that the dilation of the left ventricle secondary to volume overload or systolic dysfunction will determine the LV to assume a rounder, spherical shape.

It is calculated dividing left ventricular length from a 4 chamber view to the M-mode measurement of a diastolic dimension. Normal sphericity is 1.78±0.16.

Values of SI < 1.65 can be considered suggestive of LV dilation and the European Society of Veterinary Cardiology has proposed this method as one of the criteria to rely on when making diagnosis of dilated cardiomyopathy (DCM), and a low SI has been found as predictor of DCM in dogs. [35] The variability of SI has been validated by Chetboul et al and can be considered satisfactory (max 6.2% coefficient of variation)

PDA and the quantification of volume-overloaded left ventricle.

From the advent of echocardiography most PDA studies identified LV overload associated with aortic annulus dilation and main pulmonary artery dilation.

Generally, most of the studies referred M-mode derived indices of diastolic and systolic dimensions ($EDVI_M$ and $ESVI_M$) and on the left atrium to aorta ratio (LA/Ao) to identify left atrial enlargement.

After PDA closure, a decrease in $EDVI_M$ and LA/Ao was immediately appreciated in post-closure evaluation, while $ESVI_M$ remained high up until one year after the procedure in some patients (Tab 3).

Mitral regurgitation as the effect of annular stretching induced by LV overload disappeared in some cases, although some older dogs experienced mitral valve endocardiosis in a younger age as expected.[20]

Some of the dogs, depending on papers, also had significative residual shunt, thus the effect of it on the LV must be considered (Tab 3).

	Diastolic volumes	Systolic volumes	Left atrial dimension	Residual shunt
Van Israel et al 2002 before closure *91dogs*	N in 18%, ↑82% M-mode derived ($EDVI_M$)	N 16%, ↑84% M-mode derived ($ESVI_M$)	↑in 33%, severe 10% M-mode and 2D derived	43% MR 40%before
Van Israel et al 2003 12mo after closure *11 dogs*	N in all dogs M-mode derived ($EDVI_M$)	↑100% M-mode derived ($ESVI_M$)	↑in 32%, severe 2.7% M-mode and 2D derived	43%
Campbell et al 2006 before closure 24hrs after closure	AlloD 2.0±0.3 1.7±0.3	AlloS 1.3±0.3 1.3±0.3	LA/Ao Allometric 1.6±0.5 1.4±0.2	66% residual ductal flow
Saunders et al 2013 before closure 12mo after closure *71 dogs*	AlloD 2.15(IQR 1.85-2.37) 1.50 (IQR 1.4-1.7)	AlloS 1.33(IQR 1.02-1.59) 1 (IQR 0.87-1.20)	LA/Ao Allometric 1.46 (IQR 1.17-1.62) 1.23 (IQR 1.11-1.31)	14.5% MR 24hrs 62%, Severe MR 10%
Stauthammer &al 2013 before closure after closure (24hrs) 12mo after closure *24dogs*	AlloD 2.12±0.05 1.88±0.05 1.64±0.05	AlloS 1.36±0.04 1.34±0.04 1.17±0.04	atrial diameter (cm) 1.11±0.03, 0.99±0.03 0.78±0.03	No residual flow

Tab 3. Summary of echocardiographic characteristics of dogs with PDA as reported in veterinary literature

Shunt quantification

PDA determines shunting of blood from the ascending aorta to the main pulmonary artery, thus one of the ways to calculate PDA severity can imply calculating the difference in blood distribution.
The Qp:Qs value is based on the ratio between blood passing through the systemic and pulmonary flow.
For intracardiac shunts, the ratio is between pulmonary flow to systemic one, while for PDA quantification is reversed:

$$Qp/Qs = \frac{AoSV}{PASV}$$

where AoSV is the aortic stroke volume and PASV is the stroke volume of the pulmonary artery.[35]

In normal dogs the ratio between aortic and pulmonary flow should be 1. The hemodynamic importance of PDA could be related to the increasing quantity of blood shunting, and thus the increasing value of Qp/Qs. Even if interesting, no studies validated this technique in dogs with PDA .

Chetboul et al validated Qp/Qs in normal dogs, assessing intra-observer within-day and between-day repeatability and providing normal values. The overall intraobserver variability was low(4.2-6.6% of variation), and the mean Qp/Qs in healthy normal dogs was 1.00±0.15, however values ranged from 0.68 to 1.26, and most of the dogs (57%) had values > 1, thus dogs with small PDAs cannot be well assessed by Qp/Qs, due to the wide normality ranges identified in standing, awake dogs.[37]

One of the major limitations of Qp/Qs is related to the fact that this method is based on 3D orifice calculation based on 2D images and thus is subjected to over and underestimation.

Contractility indices/ systolic function

Systolic function relates to the ability of the heart to pump an adequate amount of blood into the systemic bed. A variety of factors influence systolic function: preload, afterload, contractility, coordinated contraction and heart rate.[11,35]

Several indices have been studied to provide information about systolic function both in human and veterinary medicine. Every technique has its own limitations and strength. In human medicine, EF probably is still considered the premiere indicator of systolic function, due to its ease of application, good reproducibility and extensive documentation on its clinical utility in different cardiac disease[38].

Most of veterinary studies are mainly focused on FS and ESVI and, in some instances, take into account EF too[20,34]. Some cases of successfully closed PDA presented an important decrease in FS and an unchanged ESVI postclosure, thus depending on the studies, the presence of systolic dysfunction has been claimed. It should however be stressed that all these indexes are indirect measurements of myocardial contractility, thus they might be influenced by several factors like loading condition, heart rate and neurohormonal activation and thus it might be argued that in some cases systolic dysfunction is a misleading conclusion from indirect findings.

Routinely used indices of global ventricular function	Human medicine	Veterinary cardiology
Fractional Shortening	++	++++
Ejection Fraction	++++	++
End systolic volume index (ESVI)	+++	+++
Velocity of circumferential fiber shortening(Vcf)	+	0
Systolic time intervals	++	+
Tei Index	+	0
TDI/STE	++	+

Tab 4: a summary of selected indices of global ventricular function and their frequency of use in human and vete+rinary medicine.

Fractional Shortening

Fractional shortening is the percentage change in left ventricular size between filling and emptying:

$$FS= \frac{LVIDd-LVIDs}{LVIDd} \text{ x100}$$

FS is not a direct measure of contractility, as left ventricular dimensions are both contractility and load dependent measurements.
For example, an increase in preload is supposed to increase FS due to Frank Starling's mechanism, however if FS is normal in an increased preload patient, this might be due to an increased afterload (as is the case of PDA) or to a decrease in contractility. On the other hand, if preload decreases (ie secondary to PDA closure), FS is supposed to decrease, as it is the case in all dogs after PDA closure, due to the fact that there is less myofiber stretching as compared to the previous overloading condition.

Fractional shortening has been regarded as poor if less than 20-25% depending on dogs' breeds and this cut-off values have been recommended by the European society of veterinary cardiology guidelines for the diagnosis of dilated cardiomyopathy (DCM). Most of the studies in veterinary medicine refer to 25% as cut-off value to diagnose systolic dysfunction. [35]

In dogs with PDA, Van Israel proposed a cut-off value of FS<25%, while Stauthammer used FS <23% to define systolic dysfunction.

Ejection Fraction

Ejection Fraction is the ratio of stroke volume to end diastolic volume:

$$EF= \frac{LVVd-LVVs}{LVVd} x100$$

Normal EF reported in human cardiology are 55-75% and older patients are reported to have lower EF as compared to young patients. An acute increase in afterload can decrease EF up to 45%, but values less than 45% always indicate myocardial impairment and thus are considered abnormal. In veterinary medicine some reference ranges have been published depending on dogs' breeds and generally speaking an EF <40% is considered low.ref mcewan

Ejection fraction is still load and contractility dependent, so that in particular circumstances a normal value can mislead the clinician[38].

Ejection fraction can be obtained both from Teicholz-derived M-mode values and from 2D Simpson's mono and biplanar methods. The coefficient of variation of these techniques has been investigated also in veterinary medicine and the intra-operator within-day and between-day variability can be considered good (<10%).

Few reports about dogs with PDA report EF, which is in most of the patients normal (>40%) and only in a some cases (20% in Van Israel et al) depressed, with low FS. It is noteworthy to say that 46% of the dogs of this study had FS values below the reference range of 25%, and of these half (47%) also presented EF values below 40%. Interestingly, no difference was found in survival time depending on FS less than 30% before PDA closure.

Velocity of circumferential fiber shortening (Vcf)

This index has been used only under research condition, but is considered to be preload independent and can be heart-rate corrected, even if it is afterload dependent.[35]

It is of particular value when evaluating myocardial contractility in patients with valvular regurgitation (increased preload, no alteration in afterload). Vcf is inversely and linearly correlated with myocardial wall stress. [22]

To the authors' knowledge, no publication has been done on Vcf and dogs with PDA.

Systolic time intervals

Systolic time intervals can be measured from aortic and pulmonary Doppler flow profiles, and on experimental animals it has been shown that the rate of acceleration during ventricular ejection is an indicator of systolic function[35]. Systolic time intervals are heart rate dependent, thus the values might need to be averaged between different measurements.

Systolic time intervals can be measured as the ratio between the pre-ejection period and the ejection time (PEP/ET). PEP can be measured from the onset of QRS complex to the onset of systolic flow, while ET is measured from the onset of flow to the end of flow at baseline. Acceleration time is another parameter that can be measured (from the onset of the flow to the point of maximal velocity), but it is of major interests in pulmonary flow evaluation of pulmonary hypertension [35].

A PEP/ET ratio of 0.32 of aortic flow is considered normal in the dog. Dogs with PDA have reported systolic time intervals of greater than 0.44, indicating longer time intervals and thus less efficient systolic function. However, only one study investigated systolic time intervals post closure at late follow up in dogs with PDA [20].

Tei index (myocardial performance index)

The Tei index provides assessment of global myocardial function, taking into account both systolic and diastolic time intervals[35].

It is based on the following equation:

$$\text{LV MPI} = \frac{\text{IVRT} + \text{IVCT}}{\text{LVET}} = \frac{\text{MCO} - \text{LVET}}{\text{LVET}}$$

Where IVRT and IVCT are the isovolumic relaxation time and the isovolumic contraction time, LVET is left ventricular ejection time, MCO is mitral closure to opening time.

A good correlation with systolic and diastolic function, and its usefulness in veterinary medicine has been related to diagnosing subclinical DCM, evaluating myocardial dysfunction in dogs with tricuspid regurgitation, mitral regurgitation and pulmonary hypertension (ref boon197-199).

Increasing values of MPI are related to worsening of LV function; it is preload, heart rate and blood pressure independent and is sensitive to acute changes in afterload.

The myocardial performance index can be derived also from Tissue Doppler taken at the level of interventricular septal or free wall; this permits diastolic and systolic flows to be recorded in the same heart cycle, thus reducing heart rate variations.

Limitations of this technique are mainly related to the need for similar timing and heart rate if inflow and outflow are not taken simultaneously. This technique is not an immediate tool, and generally requires particular views . The intraoperator variability has not been assessed until now in veterinary medicine.

Advanced echocardiographic techniques

Definition, technique and actual field of application

Congestive heart failure is a clinical syndrome with high mortality rates and is one of the most common cause of death in the developed countries. It is generally related to the failure of the LV to pump adequately blood , thus it might be said that it is generally considered to be a systolic failure in most of the cases, although recently the role of diastolic dysfunction as the primary cause of congestive heart failure has been found as high as in 50% of all cases of congestive heart failure. The evaluation of cardiac function is thus of outmost importance, as it is the key in diagnosing early cardiac impairment.

The evaluation of systolic function by means of standard echocardiography is routinely used and is based on several indices of myocardial function , which are nevertheless based on an indirect assessment of myocardial function and are dependent on many factors, which could alter the final result.[38]

Considering these limitations, cardiologists have sought to investigate new techniques in order to evaluate cardiac function by measuring cardiac contractility, which is related to intrinsic cardiac geometry and 3D fiber orientation. It is from these basis that the concept of myocardial motion and deformation imaging started.

Advanced echocardiographic techniques are therefore echocardiography-based techniques, which aim at quantifying myocardial contractility and deformation in different planes.[3, 39]

Myocardial motion and deformation

LV fiber contraction during the cardiac cycle is a complex entity, because the myofiber segments undergo deformation in 3 planes and are strongly correlated to adjacent myofiber segments (tethering), which may influence the neighboring myofibers contraction.

The term *strain* is related to the deformation of an object (in this case, of a myocardial segment) normalized to its original shape. The velocity at which this happens is defined as the *strain rate.*[3,4,35,39-43]

If one dimension is considered, then the strain formula is the following:

$$\varepsilon = \frac{L-Lo}{Lo} = \varepsilon(t) = \frac{L(t)-L(t_0)}{L(t_0)} = \varepsilon_N(t) = \int_{t0}^{t} d\varepsilon_N$$

Where L represents the length of the object after deformation and L_0 its original length. The second equation refers to Lagrangian strain, where an instantaneous strain is measured, while the latter takes into account the deformation during a small period of time (natural or Eulerian strain). [40-42]

By convention, a positive strain indicates lengthening and negative strain indicates shortening.[4]

When we consider a 3D object, the deformation occurs in 3 planes and two components can be identified, the normal strain values (in the x, y and z-axes, ie longitudinal, radial and circumferential strain), and the displacement of the surface borders relative to each others , so called shear values (base-apex twisting, epi-endo longitudinal shear or epi-endo circumferential shear)[4,39-43] Fig 8

A 3D object provides 3 normal strain values and 6 shear strains, but of the latter each shear value can be measured relative to two different orthogonal axes, thus in the end 3 shears can be measured.

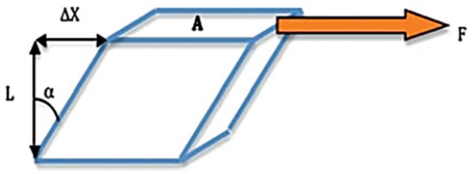


Fig 8. Shear strain. A, surface area; F force; Δx border shift; L height; α shear angle[4]

The three normal strain values can be associated with a cardiac axis system:

- Radial axis: perpendicular to the epicardium, pointing outward, ie away from the cavity;
- Longitudinal axis: perpendicular to the radial axis, tangent to the epicardium, pointing toward the base of the LV, away from the apex;
- Circumferential axis: perpendicular to both radial and longitudinal axis, organized in a right-handed coordinate system, thus directed anti-clockwise in the short axis view.

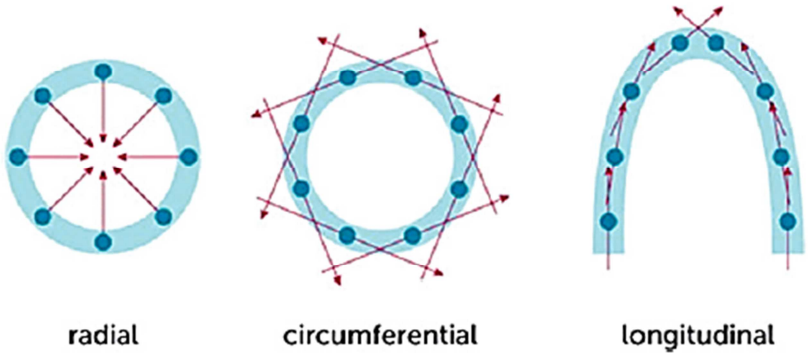


Fig 9. Schematic representation of different types of left ventricular strains [4]

The three normal strains can be quantified by different techniques.

Recently, a fourth parameter has been taken into account when evaluating cardiovascular patho-physiology and is related to the torsional mechanics of the LV (see further chapters)[4].

Techniques to calculate Strain and Strain rate

Tissue Doppler (TDI)

The first technique to assess myocardial motion was Doppler-derived and aimed at evaluating myocardial velocities recorded in small regions of interest in the LV wall. [3,35,39,40]

The general Doppler flow settings should detect low frequency, high amplitude flow signals, wheras TDI settings need high frequency and low amplitude signals, thus in the latter case a filter is applied to remove Doppler shift frequencies from that are reflected from the blood [3,35,39]. TDI produces primarily a myocardial time-velocity curve, and the velocity profiles recorded include a systolic wave (S') and two diastolic waves (E' and A'). From the myocardial velocity strain rate values can be extrapolated and also strain can be measured.[3,4,39]

There are three main Tissue Doppler (TDI) techniques: the Pulsed Wave, the M-mode derived and the Color TDI. The first can display the myocardial velocities by placing the sample area of the Pulsed Wave in the region of interest, generally on the LV walls (Fig 10 A); the M-mode derived TDI displays myocardial displacements by a scanning line, like an anatomical M-mode (Fig 10 B). Color-coded TDI allows the superimposition of color TDI on 2D images(Fig 10 C) and the image stored can be then post-processed to calculate regional strain and strain rate. Color coded TDI is the only Doppler-based technique to contemporarily evaluate different myocardial areas from the same image, thus enabling a regional evaluation of myocardial motion. [39]

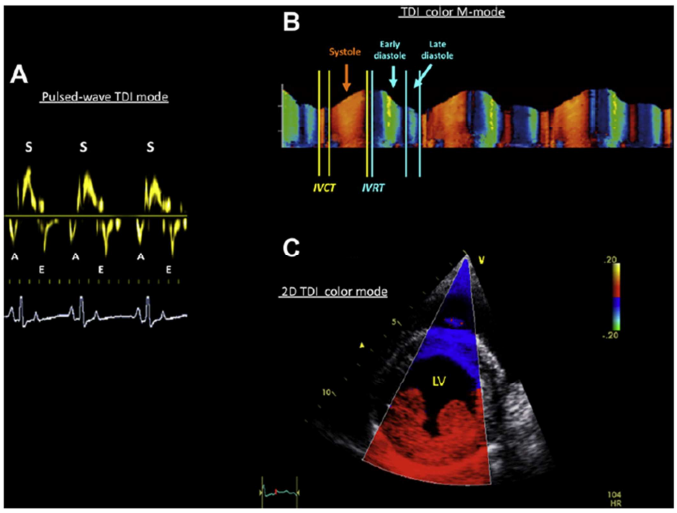


Fig 10.Different types of TDI. A. Pulsed wave TDI, with peak systolic S and diastolic E and A wave B.Color M-mode, with colored systolic and diastolic velocities within the entire wall thickness. Myocardial velocities toward the transducer are encoded in red, and those moving away in blue. C. Color coded TDI. Velocities toward the transducer are colored in red whereas those away from the transducer are colored in blue.[39]

TDI can generate longitudinal and radial strain, however, being a Doppler-derived technique, its main limitation is based on angle-dependency, thus with angles greater than 20° of disalignement the values are not accurate. The apex of the LV cannot be well displayed from this point of view, because the alignment is suboptimal. This limitation is much more important with cardiac disease, above all in patients with dilated hearts, where correct alignment is not possible[3,4,39-43].

Generally, TDI can be obtained from the short axis at the papillary muscle level (providing radial strain) and from the long axis at the mitral and tricuspid annulus, from both interventricular septum or lateral free wall. From this view, mitral inflow is of particular interest in evaluating diastolic function of the LV[35,39].

Clinical application of TDI

As a first deformation imaging technique, reference values were both obtained in human and veterinary medicine and various cardiac disease have been studied by TDI, among them cardiac resynchronization therapy, hypertensive cardiomyopathy, hypertrophic and dilated cardiomyopathy[3,42,43].

In veterinary medicine, the utility and validation protocols have been intensively investigated by Chetboul et al, whose main interest was validation of the technique and assessment of normal reference ranges; furthermore, TDI proved to be an early indicator of systolic dysfunction in a canine model of Duchenne's cardiomyopathy and proved to determine focal areas of segmental myocardial wall motion abnormalities in cats with mutation in myosin-binding protein but no obvious LV hypertrophy. Another application was the detection of right ventricular hypokinesia in dogs with mild pulmonary hypertension.[39]

The operator variability was acceptable, where performed by a single trained operator.

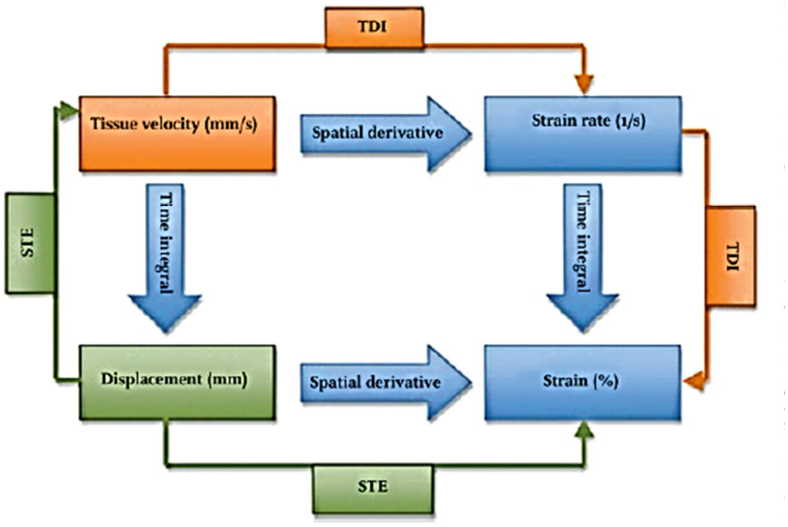


Fig 11: Mathematical relationship between different deformation parameters and mode of calculation for TDI and STE[4].

Speckle-tracking echocardiography (STE)

A more recent echocardiographic technique to assess strain and strain rate is speckle-tracking echocardiography (STE), which is based on post-processed gray scale images based on a tracking algorithm for speckles in the myocardium[3,4,39-43]. By placing the selected region of interest in the myocardium, the software is able to divide the area into blocks of pixels, and tracks the motion of these speckles during the cardiac cycle. The location shift of these acoustic markers from frame to frame representing tissue movement provides the spatial and temporal data used to calculate velocity vectors, thus providing regional curve analysis of strain and strain rate.

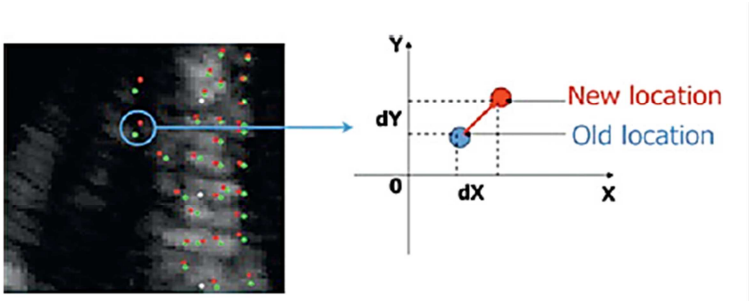


Fig 12.Displacement of acoustic markers from frame to frame. Green dots represent the initial position and red the final position of the speckles[4].

Not being an angle-dependent technique, STE can provide segmental and global longitudinal, circumferential and radial strain and strain rate. Another important acquisition is the quantification of LV twist by comparing endocardial velocity rotations from the cardiac apex and base.[4]

The main limitation related to STE is image quality as frame rates need to be high with a good spatial and temporal resolution. Another important issue is the out of plane motion of the speckles and software issue, which differ depending on the ultrasound machine used (intervendor comparability of values). There are currently different software available, most of them are developed to evaluate speckle tracking motion in the myocardial wall, while few of them can enable the endo- and epicardial borders to be tracked to provide strain and strain rate values (border-tracking echocardiography).

First available as 2D STE, it is now available 3D STE, which provides better spatial information, by providing strain and strainrate values obtained by different orthogonal views and presented as a bull's eye plot.

Clinical application of STE

Even if most people consider STE as a research tool, it was shown to be an accurate predictor of decrease systolic function in patients with dilated cardiomyopathy, hypertrophic cardiomyopathy, regional myocardial infarcts, transplant anti-rejection treatment, resynchronization therapy and congenital heart disease.[42]

In veterinary medicine, the clinical application of STE has been seeing an increase interest, as shown by the increase in publication in the last couple of years [44-54] (Tab 5).

The main application of STE in veterinary medicine was aimed at establishing normality reference ranges[44-48] and study under experimental field the effect of volume overload or hypokinesia (tachycardia-induced cardiomyopathy or drug-induced)[49,50], while the main application in clinical cardiology was the investigation of mitral valve degenerative disease(MVD) progression, in order to evaluate early markers of systolic dysfunction[51-54]. Most of the modifications seen in MVD by STE are associated with end-stage cardiovascular remodeling in dogs with congestive heart failure. STE has not been proven as an useful early indicator of myocardial dysfunction or poorer survival[51-54]. Slight variations in the results are present, with a statistical significant difference between mild vs severe MVD groups. Generally, dogs with severe MVD (class C ACVIM or ISACHC III) showed an increase in all if not most STE-derived strain values (increase longitudinal, circumferential and radial Strain and Strain Rate), which is most representative of chronic and severe LV overload secondary to mitral valve regurgitation. Systolic dysfunction was not identified in none of the case series published.

Few studies analysed the effect of STE on an acquired, tachycardia-induced cardiomyopathy dog model and identified an homogeneous decrease in all strain and strainrate values[50].

No studies were carried on dogs with PDA.

Normal reference ranges	Longitudinal Strain(%) Longitudinal Sr (1/sec)	Circumferential Strain (%) Circuferential Sr (1/sec)	Radial Strain (%) Radial Sr (1/sec)
Chetboul et al 2007 mixed breeds			46.7±12.2 2.69±0.76
Culwell et al 2011 Foxhounds	-16.61±2.21 -1.53±0.28		28.73±7.10 2.18±0.44
Wess et al 2012 Small breed dogs	-15.9 ±5.2 -1.9±0.95		
Smith et al 2012 Small breed dogs		-20.9±3.15	43.9±8.54 3.04±0.47
Zois et al 2012 Beagle	-18.8±1.1 -2.0±0.3		53.1±10 2.9±0.6
Zois et al 2013 Beagle		-19.5 (-17.2 to -21.2) -2.1 (-1.8to -2.4)	
Carnabuci et al 2013 Labrador	-14.8±1.6 -1.3±0.2	-20.4±4.5 -2.1±0.7	27.5±10 2.2±0.8
Kusunose et al 2013 Mongrel	-19.5±5	-17±7	41±19
Suzuki et al 2013 Young dogs	-14.8±3.1 -1.7±0.3	-19.4±4.4 -2.3±0.4	52.4±11.1 3.2±0.6

Tab 5. Reference ranges in normal dogs in veterinary literature

Cardiac torsional mechanics

Definition and description of the anatomical basis

Torsion of the LV is the wringing motion of the ventricle around its long axis, so that the base and apex rotate in opposite directions[4,55-56]. However, different definitions have been provided in human literature: LV torsion can be described as the difference in the rotation (ϕ) between base and apex, or as a normalized twist, where the twist angle is divided by the distance (D) between the measured locations of base and apex, normalized by the mean radius of base and apex. This latter definition is more complex, but would directly relate to longitudinal-circumferential shear[55].

The twist deformation of the heart has been matter of interest since 1669,when Lower first identified the torsion of the heart and suggested an anatomical basis. However, only in 1957 Torrent Guasp was able to provide an accurate description of the helical ventricular myocardial band (HVMB) by simple hand dissection[1,2] (Fig 14). It has been suggested that anatomical fiber disposition is crucial in order to obtain efficient LV ejection: ventricular thickening increases around 50% for only 13% myocyte shortening, and 40% of the entire stroke volume is accomplished by LV torsion[5].

Based on Torrent Guasp anatomical description, the LV is organized in a ropelike model, in which myocardial fibers are organized in a figure of eight into two loops, the transverse basal and oblique apical; the latter is then divided in a descending and ascending segment that conforms to the right and left- handed helical arrangement that form a vortex at the cardiac apex[5].

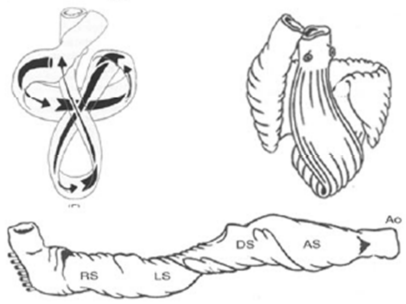
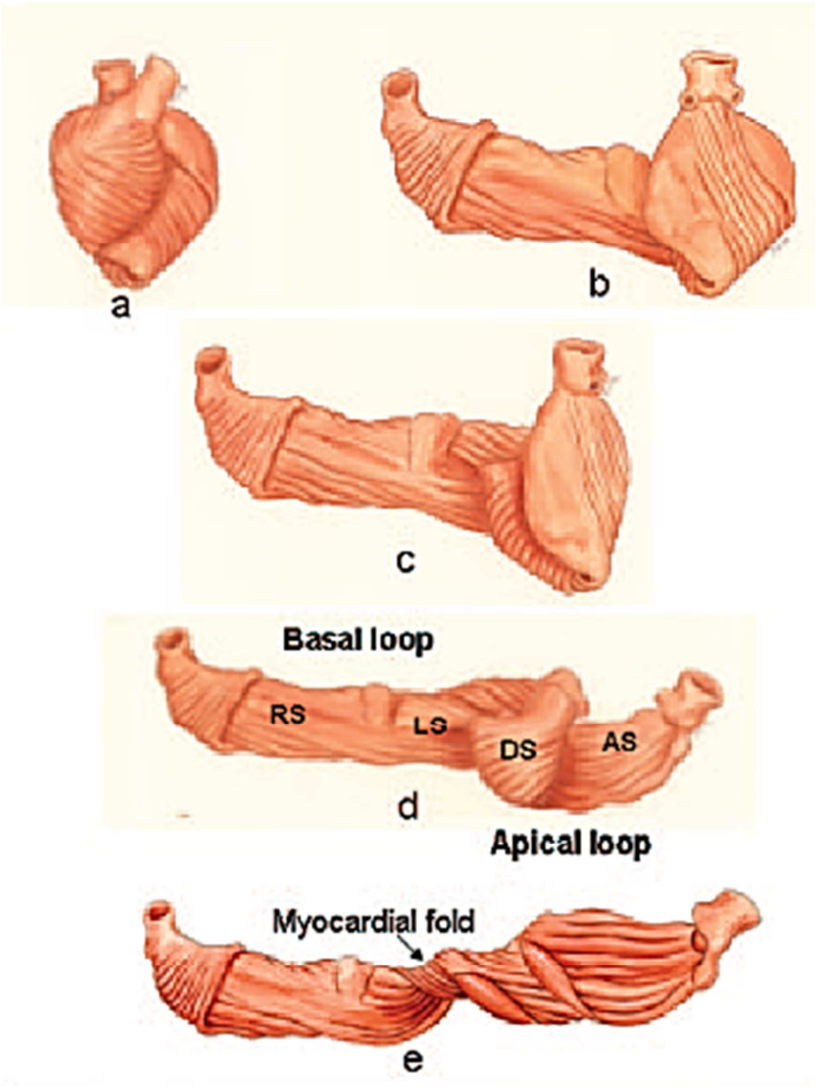


Fig 13. The rope-like model and the figure of eight. At the bottom, the unfolded helical ventricular myocardial band[1,2,5]



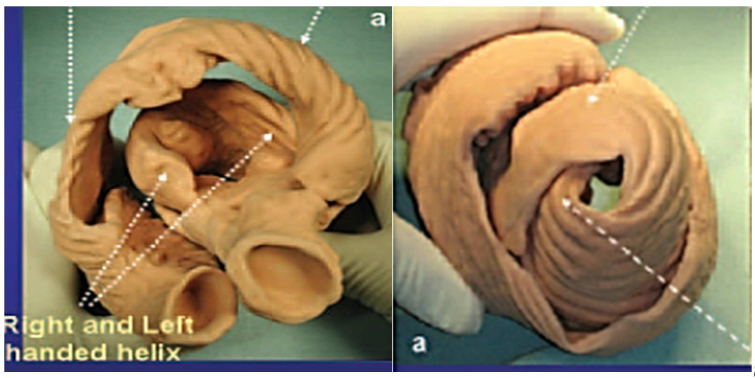


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Fig 14. Schematic drawing of Torrent Guasp helical ventricular myocardial band with close up to the basal and apical fiber disposition. RS, right basal segment, LS, left basal segment, AS, ascending segment (right handed helix), DS, descending segment (left handed helix).[1,2,5]

During the pre-ejection phase, the predominant segment acting on the LV is that of the circumferential fibers belonging to the basal loop, which contract and stretch the two helices like a stiff outer shell which promotes LV elongation and determines a first, counterclockwise rotation of the entire heart[5].

During ejection a more global activation of all myocardial segments is observed and the influence of all segments based on the dominant pathway provides the final global rotation. The circumferential fibers continue to shorten, together with the right and left-handed helices that provide LV thickening and contraction. The circumferential fibers provide stability to heart base as the main ejective force is determined by the coordinated contraction of the ascending (left handed helix or epicardium) and descending segment(right-handed helix or endocardium), which determine a clockwise contraction of the base and a counterclockwise rotation of the LV apex. The subendocardial layer is dominant and responsible for basal twist, while apical rotation is secondary to the subepicardial layer- left-handed helix which provides a larger radius of curvature[5].

After ejection the right-handed helix stops contracting but does not relax until later, in order to provide stiffness and tension to the chamber as the left-handed helix continues to contract (it is the only force acting during the beginning of isovolumic phase), thus the predominant motion is rapid clockwise untwisting of the apex in a clockwise direction and a more accentuated clockwise rotation of the base. The circumferential fibers stop contracting too, helping the initial recoil of the LV apex.
This early untwisting of the apex together with basal loop widening before valve openings results in a negative pressure in the LV that progresses during early cavity filling and provides suction forces to favor LV filling[5].

Clinical application of LV torsion.

The most common technique in the clinical field to assess LV torsion is STE-derived, and data are generally presented as the net difference of apical and basal rotation; however, different methods have been used to study and identify torsion, from tagged MRI to TDI and STE .[57-59]

MRI is currently considered the most accurate technique, however it is more expensive, takes longer times and, in veterinary patients, requires anesthesia[3,55,56].

Many disease conditions in human medicine are associated with altered twisting and untwisting (the latter being suggestive of diastolic dysfunction), while in veterinary medicine few studies were published to identify normal reference values and to assess torsional mechanics in dogs with degenerative mitral valve disease[52,60].

Torsion is load dependent (pre- and afterload) and is affected by age, as children and infants have a different torsional mechanics as compared to adult and older patients: infants and children have lower twist as compared to adolescents and adults and both basal and apical segments present a counterclockwise rotation during systole. It has been reported that there is a shift from counterclockwise to clockwise rotation at the base in patients from 10-15 years on[60]. Older patient have increase untwisting rates, as a result of an increased stiffness of the LV.

Cardiac disease	Net twist	Basal and apical rotation	Time to peak untwist
Aortic stenosis	Increased	Increased apical	Increased
Hypertrophic cardiomyopathy	=	=	Decreased
Dilated cardiomyopathy	Decreased	Decreased both	Increased
Heart failure	Decreased	Decreased both	-

Tab 6.Different heart disease conditions and rotational mechanics (ref philips)

A single study evaluated accuracy of repeatability and reproducibility in a cohort of healthy dogs, and identified an acceptable within-day coefficient of variation (16%) for torsion values, while lower coefficient of variations were determined for within-day absolute peak basal and apical rotation (9% and 11% respectively). However, the main limitation of this study is related to the high reference values identified (normal mean torsion at end systole : 8.4 ± 3.8°, min 2.5° max 18°)[60].

Concerning pathologic findings in the dog, two studies were performed in order to assess LV torsional mechanics in dogs affected by mitral valve disease (MVD), a progressive volume overloading disease, and found conflictive results[51,61].

Zois et al demonstrated an increase in the magnitude of LV twist and time to peak untwist in dogs with congestive heart failure secondary to MVD (8.3°minimal MVD vs 11.9 in symptomatic dogs), while Suzuki et al identified a decrease in peak systolic torsion as MVD disease progresses (15.3° in healthy dogs, 10.0 in dogs with mild MVD and 9.7 in dogs with severe and symptomatic MVD).

No study analysed torsional mechanics in dogs with PDA.

Aim of the Study

Aim of the Study

The aim of the study was to apply advanced echocardiographic techniques for the evaluation of cardiac mechanics in dogs affected by PDA and included:

Part 1

- Determination of normal values of STE-derived strain and strain rate in a population of healthy young dogs;

-Comparison of STE-derived strain and strain rate values (longitudinal, radial and circumferential) in dogs with naturally occurring PDA vs healthy normal young dogs of same weight;

-The identification of a correlation between standard echocardiographic techniques and advanced echocardiographic techniques;

Part 2

-Comparison of preoperative and postoperative STE-derived strain and strain rate values (longitudinal, radial and circumferential) in dogs with naturally occurring PDA referred for percutaneous closure;

-Comparison of standard echocardiographic techniques and advanced echocardiographic techniques;

Part 3

-Evaluation of torsional mechanics of dogs with naturally occurring PDA before and after percutaneous PDA closure.

The investigation was carried out in collaboration with Clinica Veterinaria Gran Sasso (MI), where PDA closure and echocardiographic evaluation was performed. Healthy young dogs were recruited from the University of Milan.

Materials and Methods

Materials and Methods

Inclusion and exclusion criterias

Dogs were prospectively recruited in the study from March 2010 until June 2013. STE processing was performed from off-line analysis with XStrain software© (Esaote, Florence, Italy).

The dogs were prospectively recruited in the study based on the inclusion criterias stated below:

Inclusion criteria

Healthy young dogs:

-Dogs of any breed and sex;

-Dogs must be younger than 18 months of age;

-Dogs were considered to be healthy based on general assessment and blood sampling; Dogs were auscultated, underwent echocardiography and were included only if the echocardiography and the clinical exam were considered unremarkable;

-Complete echocardiographic evaluation, including the cineloops required for STE analysis.

Dogs with PDA / Part I

- Dogs of any breed, sex and age;

- Dogs had to be diagnosed of PDA by board certified or board eligible cardiologists by a complete echocardiography ;

-The echocardiographic exam must include a complete standard echocardiographic evaluation, including the cineloops required for STE analysis.
Among these, it was of outmost importance to acquire :

1. M-mode at the level of the chordae tendineae for $EDVI_M$, $ESVI_M$ and Allometric scaling

2. 4chamber long axis cineloop suitable for AL evaluation

3. Aorta and pulmonary valvular annulus measurement and Doppler flow profile for Qp/Qs analysis

4. Short axis views at the mitral valve level (MV), papillary muscle (PM) and apical (Apex) level acquired for STE analysis

5.Left apical 4 chamber view acquired for STE analysis (4ch)

6. PDA ductal dimensions

-For STE cineloops it was necessary to have good quality images with high spatial and temporal resolution

Dogs with PDA / Part II

-All the above stated inclusion criterias plus:

-Complete preoperative and postoperative PDA echocardiographic evaluation; the postoperative echocardiographic evaluation had to be performed by 24 hours after PDA closure;

-Absence of residual shunting

Dogs with PDA / Part III

-All the above stated inclusion criterias for dogs with PDA part 1&2

- Of outmost importance to acquire both basal and apical view to determine torsion. It should be desirable to have similar frame rates and heart rates to compare the views.

Exclusion criteria:

-Healthy control dogs older than 18 months;

- PDA dogs with incomplete echocardiography or in which PDA closure was not performed.

- Dogs with multiple congenital malformations hemodynamically significant (responsible for pressure or volume overload of the heart)

-STE derived cineloops not properly stored for this purpose (absence of ECG, incomplete timing of systolic-diastolic period, absence of MV or Apex views in order to obtain torsional values)

Standard echocardiographic parameters

For each patient, breed, sex, weight and age were added in the data sheet. Furthermore, the standard echocardiographic measurements included:

- EDVI and ESVI measured both from M-mode derived Teicholz and AL method ($EDVI_{M-B}$, $ESVI_{M-B}$)

-Allometric scaling M-mode derived values (AlloD, AlloS)

-Ejection fraction, obtained by M-mode or B-mode (EF_{M-B})

-Fractional shortening, M-mode derived (FS_M)

-Qp/Qs, calculated as Aortic flow/pulmonary flow

These values were calculated following the indications of the American echocardiographic society and the referenced indications in veterinary medicine[35]

Dogs undergoing preoperative and postoperative comparison (study part II)

-All the standard echocardiographic measurements were repeated on pre- and postclosure echocardiography;

-Sphericity index was also calculated

Protocol of STE acquisition and processing

-Cineloops must be of adequate image quality and frame rate.
-ECG must be present and the QRS complex must be positive.
-The measurements must be repeated 3 times each and a mean of the repeated measurements for each segment is the final value considered. Generally speaking, the values presented are global strain and strain rate values, which means they are means of all segments.
-Echocardiographic acquisition and values obtained by each view:

1. *Mitral valve (MV) level*: the short axis view at the mitral valve must be circular, the endocardial borders must be clear and well defined and the valve leaflet should not interfere with the endocardial borders. The outflow tract should never be imaged during the cardiac cycle, and the least possible overlapping of transversal images must be obtained.

At this level, the endocardial border is traced and circumferential strain and strain rate (Circ St/Sr) can be obtained. Systolic peak circumferential strain is expressed as a negative percentage.

Systolic peak circumferential strain rate is expressed as a negative value. From this view, the rotational displacement of the heart base can be obtained, which is always a negative value (expressed as °)

2. *Papillary muscle (PM) level*: the short axis view must be circular and should show the papillary muscles, which must be symmetrical and of equal dimensions.

At this level, the endocardial and epicardial border is traced and radial strain and strain rate (Rad St/Sr) can be obtained. Systolic peak radial strain is expressed as a positive percentage. Systolic peak radial strain rate is expressed as a positive value.

Endocardial and epicardial circumferential strain and strain rate are also obtained from this view.

3.*Apical level (Apex)*: this view can be obtained tilting the probe more toward the sternum from the PM view. No papillary muscles must be seen, and a counterclockwise rotation of the myocardial fibers is sometimes appreciated. The liver is sometimes seen next to the apex.

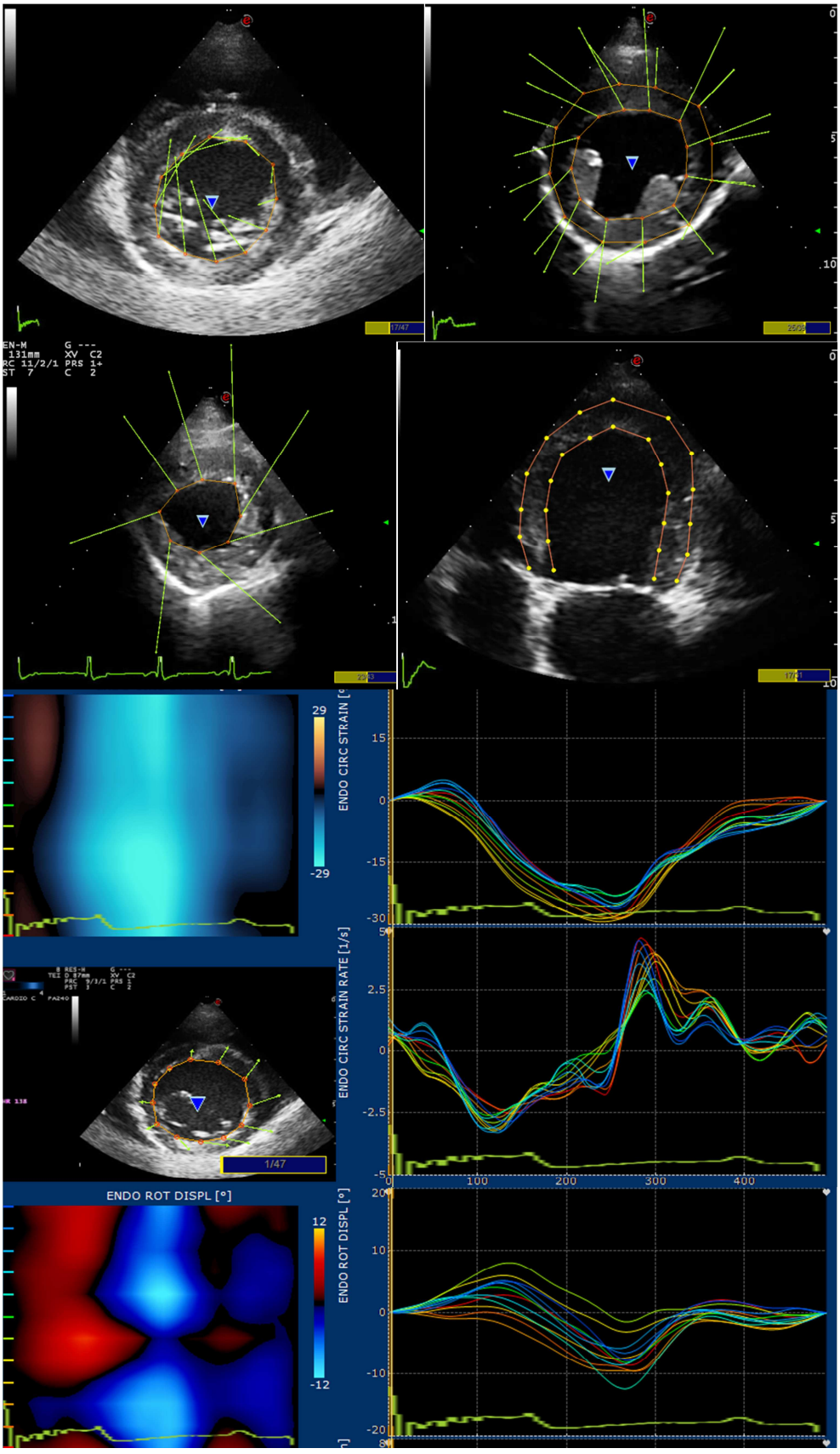
At this level, the endomyocardial border is traced and rotational displacement of the apex can be obtained, which is always positive (expressed as °).

4.*Left apical 4 chambers view*: this view should optimize the LV in its length and dimensions, the endomyocardial border must be clear and well-defined, and the epicardial border should be included. In some cases, the width must be opened up to 90° in order to better visualize the myocardium. No outflow tract must be included during the entire cardiac cycle. The mitral valve must be clearly identified during the entire cardiac cycle.

From this view, the endomyocardial and eventually epicardial borders are traced and longitudinal and transversal strain and strainrate are obtained (Long st/sr, Transv st/sr). Endocardial and epicardial strain and strainrate are obtained.
Longitudinal peak systolic strain values are expressed as a negative percentage, while Transv st as a positive percentage. Longitudinal peak systolic strain rate is expressed as a negative value (1/sec), while transversal strain rate as a positive value (1/sec).

Concerning the torsional mechanics of the LV (part 3 of the study), both rotational displacement of base and apex have been separately considered and the net difference of apical and basal rotation (overall torsion, apex – basal rotation)

All the data obtained were exported to Microsoft Excel© data sheet and were subsequently prepared for statistical analysis.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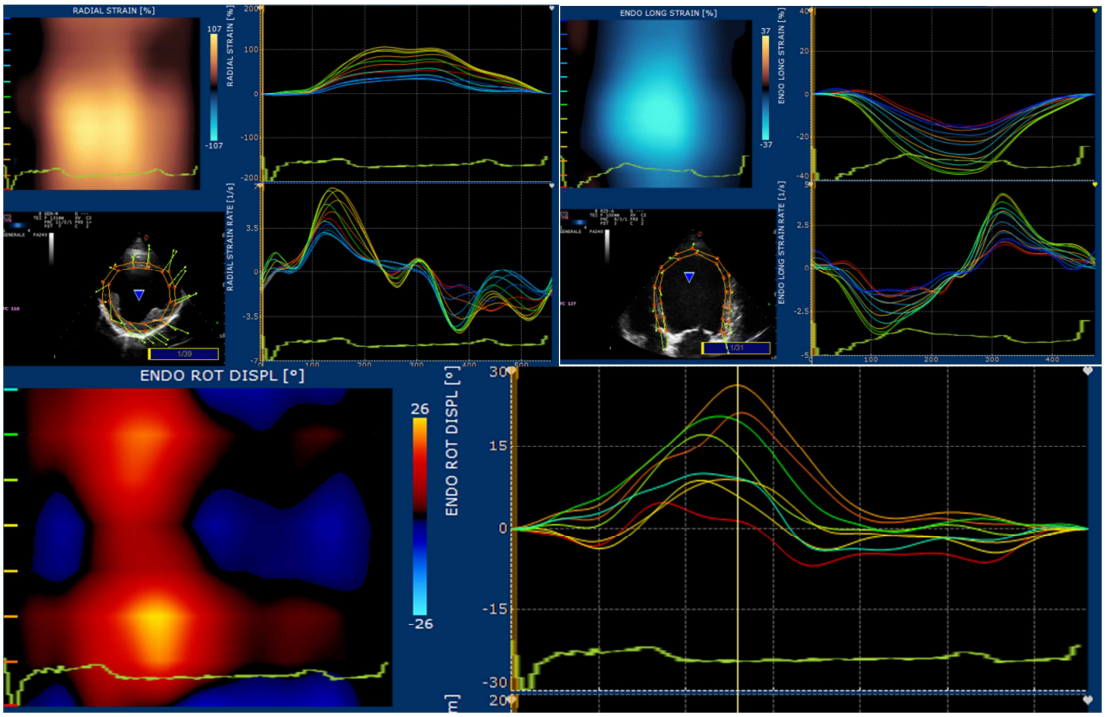

Fig 15 some examples of cineloops and regional curve analysis for STE. From the top, short and long axis views during border tracking and processing. Circumferential curves, followed by basal rotation. On this page: radial and longitudinal curves, followed by apical rotation.

Statistical Analysis

Normally distributed data are presented as mean ± standard deviation, and where necessary, minimum and maximum values are reported. Not normally distributed data are presented as median and minimum-maximum values. Data were tested for normal distribution by the use of Shapiro-Wilk test. Normally distributed data were compared by a one-way anova and if more than two variables were compared, Tukey-Kramer test was performed. If data were not normally distributed, Mann-Whitney-U test was performed Correlation between variables was tested by Pearson's coefficient and Spearman's rho. For perioperative comparison, a two tailed paired T-test was used for normally distributed data, while for not normally distributed data, a Wilcoxon test was performed.

For all test a p value <0.05 was set.
The statistical analysis was performed by different statistical softwares (Q©, SPSS ©).

Results

Results

Part I: Cardiac mechanics in dogs with PDA VS healthy dogs

The population taking part into this study comprises 10 healthy young dogs and 20 dogs affected by PDA (pre-closure evaluation).

Mean age of the population is 12.5 months, with dogs undergoing PDA closure being younger than healthy control dogs ($p<0.05$).

The two population of dogs are weight-matched, as no statistical difference was observed between healthy dogs (16±6.9 Kg) and dogs with PDA (12.4±9.6 Kg).

Female dogs are overrepresented in both population, as 16/20 PDA dogs and 6/10 healthy young dogs are female.

The overall population comprises 9 mongrel dogs, 3 newfoundland dogs, 2 corgi, 2 galgo español and one dog each of Labrador, Shetland Sheepdog, Breton, German Shepherd, Maltese, Poodle, Bichon Frisé, Dobermann, Italian hound, west highland white terrier, cane Corso, Jack Russell Terrier, Cocker Spaniel, Airdale breeds.

	Overall Population	Healthy young dogs	PDA dogs
Nr of patients	30	10	20
Mean age ± SD (min-max)	12.8 ±9.7 mo (3-36mo)	18.5± 6.9 mo* (7-28mo)	10±9.6* (3-36mo)
Sex	22♀ / 8♂	6♀/ 4♂	16♀/4♂
Mean Weight ± SD (min-max)	13.7±8.3 Kg (1.8-40)	16±6.9 Kg (6-40)	12.4±9.6 Kg (1.8-28)

Tab 7. Population characteristic in study I. * indicates statistically significant difference between groups.

Standard echocardiographic parameters

The standard echocardiographic parameters are listed in table 8. A statistically significant difference was found in all but two standard echocardiographic values in the two populations.

Dogs with PDA showed an increase in all LV markers of overload, with increased EDVI and ESVI both on M- and B-mode, allometric indexed values above the reference range ($p<0.05$). On the other hand, EF_M and FS_M were not statistically different between the two population ($p>0.05$).

Qp/Qs was calculated in the two group of dogs, and a statistically higher Qp/Qs was found in dogs with PDA (Qp/Qs =1.68, $p<0.05$) as compared to healthy young dogs (Qp/Qs=1).

	Healthy young dogs	**Dogs with PDA**	Reference values	**P value**
$EDVI_M$	88.40 ± 24 ml/m^2	184.21± 75 ml/m^2	<100ml/m^2	<0.001
$ESVI_M$	56.07± 23 ml/m^2	71.05 ± 37ml/m^2	<30ml/m^2	<0.001
$EDVI_B$	30.87± 10ml/m^2	114.28±52ml/m^2	<70ml/m^2	<0.001
$ESVI_B$	21.64± 11ml/m^2	45.29 ± 21ml/m^2	<30 ml/m^2	<0.001
Allo D	1.62 ± 0.19	2.18 ± 0.36	1.27-1.85	<0.001
Allo S	1.03 ± 0.16	1.40 ± 0.31	0.7-1.26	<0.001
EF_M	61.60 %	61.94 %	45-75%	NS
FS_M	32.90 %	33.66 %	>25%	NS
Qp/Qs	1	1.68	0.8-1.1	<0.002

Tab 8. Standard echocardiographic parameters in dogs with PDA vs healthy control dogs.

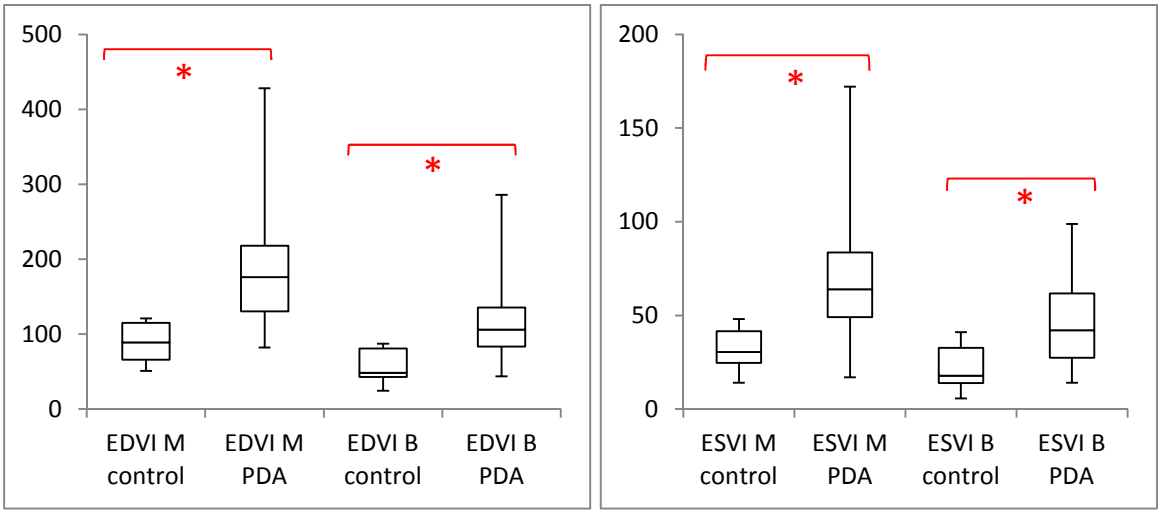

Fig 15.Box and whisker plot of EDVI and ESVI, measured by M- or B-mode. A statistical difference was found in all categories between healthy dogs and dogs with PDA.

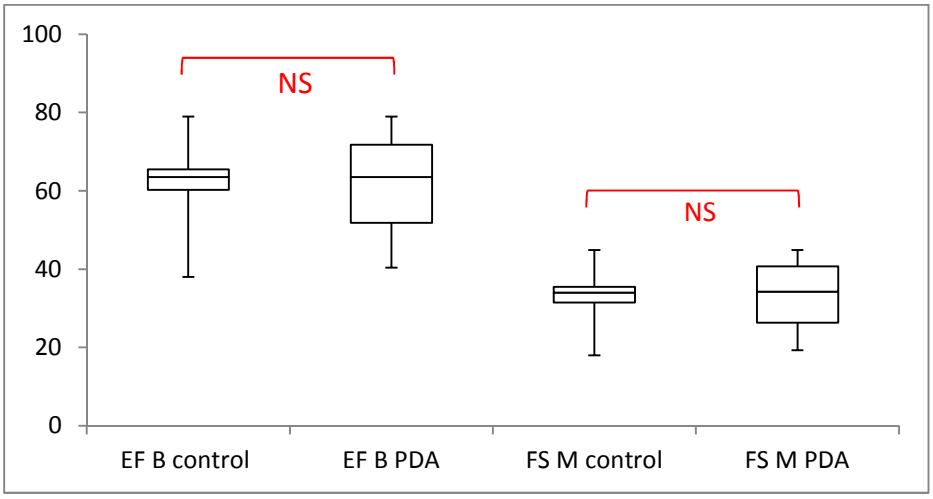

Fig 16. Box and whisker plot of FS and EF in the two groups of dogs. No statistical difference was found between healthy dogs and dogs with PDA.

STE-derived echocardiographic parameters

Concerning advanced echocardiographic techniques, a marked difference was observed in all STE derived values, with higher absolute values in dogs affected by PDA as compared to normal dogs (Tab9).

STE	Healthy young dogs	PDA dogs	P value
Circumferential strain	-22.34 ± 4.2 %	-26.22 ± 4.7 %	<0.001
Circumferential strain rate	-2.58 ± 0.7 s^{-1}	-3.32 ± 0.7 s^{-1}	<0.001
Radial strain	35.16 ± 14.3 %	51.39 ± 20.3 %	<0.002
Radial strain rate	2.89± 0.5 s^{-1}	4.79 ± 1.3 s^{-1}	<0.003
Longitudinal strain	-16.74 ± 3.9 %	-22.83 ± 4.5 %	<0.001
Longitudinal strain rate	-1.70 ± 0.4 s^{-1}	-2.65 ± 0.5 s^{-1}	<0.002

Tab 9. STE derived values.

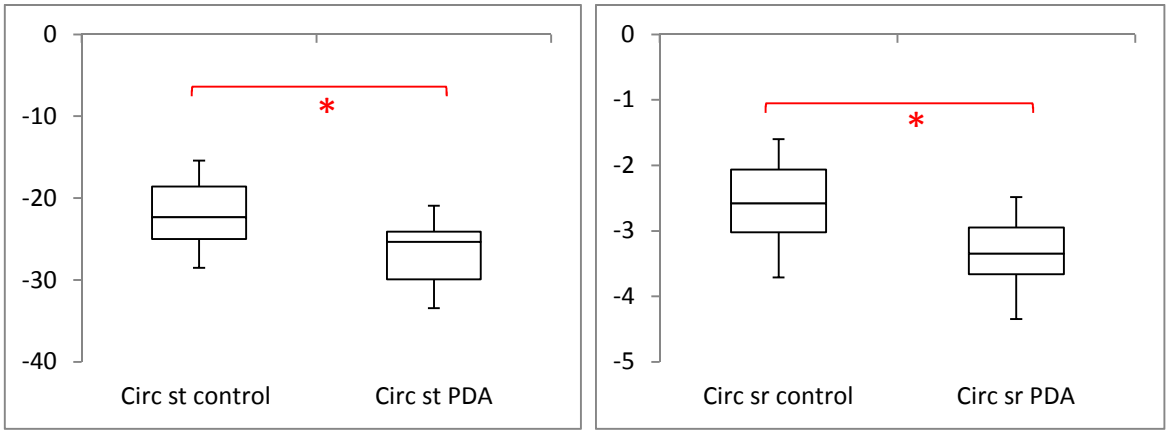

Fig 17. Box and whisker plot of circumferential strain and strain rate: a statistical difference is seen between control dogs and dogs with PDA, with the latter group showing more negative values. P <0.001

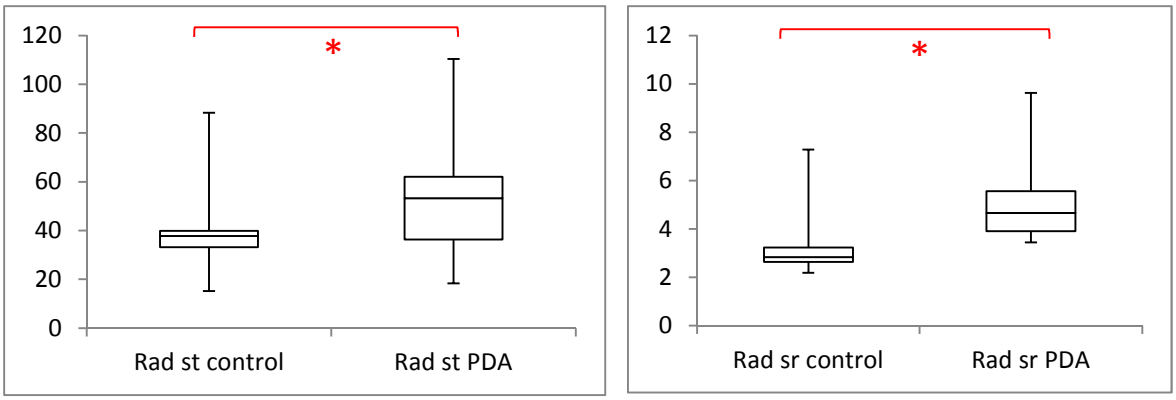

Fig 18.Box and whisker plot of radial strain and strainrate: a statistical difference is seen between control dogs and dogs with PDA, with the latter group showing higher positive results. P <0.002

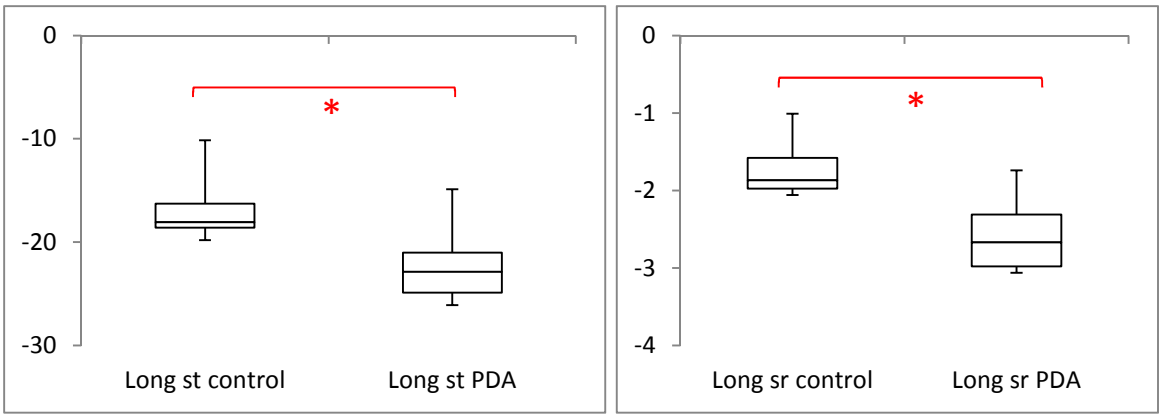

Fig 19.Box and whisker plot of longitudinal strain and strain rate: a statistical difference is seen between dogs with control dogs and dogs with PDA, with the latter group showing more negative results. P <0.002

Correlation between standard and advanced echocardiographic techniques

All the possible correlations between standard echocardiographic techniques and advanced ones were tested with a Pearson's correlation test. The graph below shows all the correlation in a color scale. The deeper the color, the stronger the correlation (see bar chart)

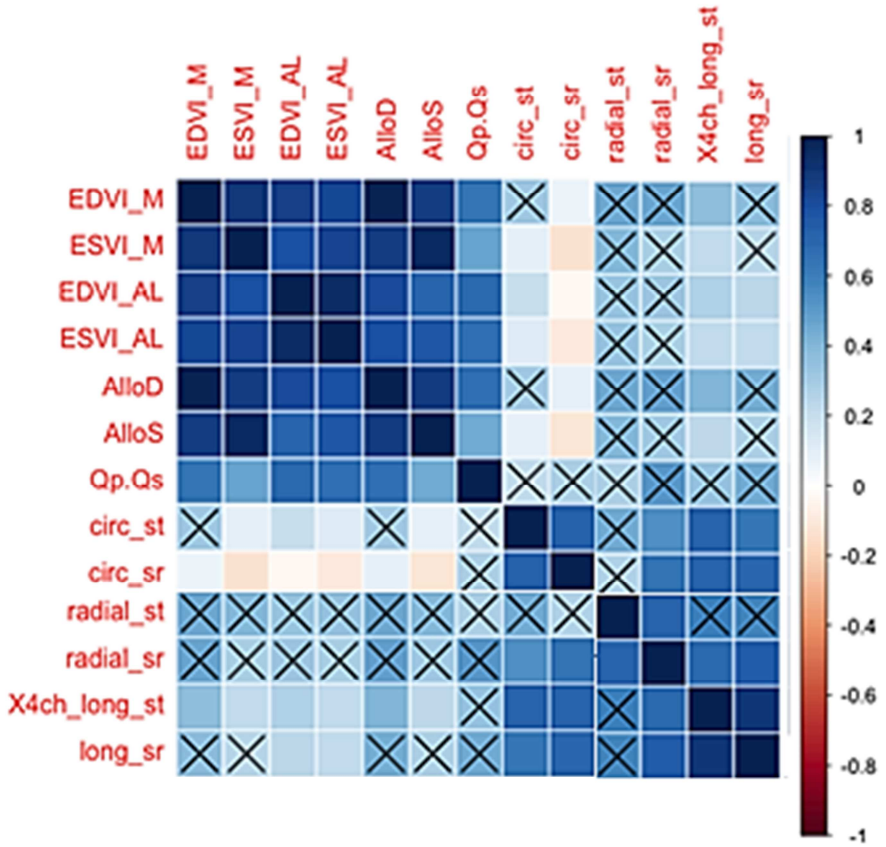

Fig 20. Correlation table. Crossed cells represent correlations not significantly different from 0, basing on a Pearson test on the correlation coefficient with significance level 0.05. Standard and advanced echocardiographic techniques do not correlate well.

As suggested from the image, an excellent correlation was found if different standard echocardiographic techniques are compared between themselves, while a good correlation can be observed if advanced echocardiographic techniques are compared, however no correlation can be found between standard and advanced echocardiographic techniques.

Part II : Cardiac mechanics in dogs undergoing PDA closure

Dogs taking part into this study were evaluated before and 24hours after PDA closure.

The population comprises 24 dogs with a mean age of 17.7 ± 18.4 months and mean weight is 15.1±8.9 Kg.

Female dogs outnumber male dogs, with a 4:1 ratio.

Dog breeds include mongrel dogs (5), German Shepherd (3), Dobermann (3), Dachshund (2), Setter (2), Breton(2),Bolognese, Bichon Frisé, Poodle, Corgi, Cocker, Newfoundland, Labrador.

	Mean ± SD	Min-Max
Age	17.7 ±18 mo	2-76 mo
Sex	19♀ / 5♂	
Weight	15.1±8.9Kg	4.2-35Kg

Tab 10. Population characteristics of part II study.

Standard echocardiographic parameters

Statistical analysis identified a marked difference between pre-closure and post-closure standard echocardiographic values. (Tab 11 and Fig 21)

Pre-closure evaluation identified an increase in all left ventricular volume indexes, both in M- and B-mode (tab). Qp/Qs values were all above normal values (>1), with a mean Qp/Qs value of 1.7±0.57.

EF was normal, while FS was in most cases normal, with few dogs (3) showing pre-closure FS < 25%, but never lower than 21%. Sphericity index identified an increase sphericity of the left ventricle (reference value, >1.65) in all case series. Immediately after PDA closure, a statistically significant decrease in LV end diastolic dimensions can be appreciated, with a slight decrease in end systolic dimensions. After 24 hours, the sphericity of the LV is decreased, but does not return to normal reference ranges.

Mean EF shows a slight decrease after closure, however is within normal ranges. A selected number of patients show a decrease in EF below normal values(depending on M- or B-mode derived, a total of 6/8 patients presented an EF <40%).

FS shows a general decrease, with mean FS within normal values, however 37% of the patients show FS values below the reference range(<23%).

Standard echocardiographic evaluation	Before PDA closure	After PDA closure	P value
$EDVI_M$	188.2±82 ml/m^2 (86.4-423.4)	138.2±43.5 ml/m^2 (81.6-264.5)	<0.001
$ESVI_M$	80.5±40.4 ml/m^2 (32-188)	62.5±30 ml/m^2 (10.3-135.9)	<0.01
$EDVI_B$	129.4±69 ml/m^2 (47.8-305.9)	96.6±36.5 ml/m^2 (39.5-167.3)	<0.004
$ESVI_B$	54.4±29 ml/m^2 (18.4-143.3)	50.1±26.6 ml/m^2 (11.2-102.2)	NS
AlloD	2.20±0.38 (1.64-3.18)	1.94±0.24 (1.57-2.58)	<0.001
AlloS	1.45±0.28 (1.04-2.08)	1.29±0.27 (0.65-1.81)	<0.02
Qp/Qs	1.73±0.57 (1.1-3.1)		-
SI	1.25±0.17 (0.92-1.57)	1.34±0.15 (1.1-1.6)	<0.03
EF_M	57.9±7.5% (43.3-73.5)	51.6±15.32% (28.25-82.5)	<0.02
EF_B	57.4±8.4% (29.9-68.3)	50.5±13.2% (23.9-73.3)	<0.04
FS_M	30.5±5.1% (21.6-41.5)	26.9±10.3% (13.6-49.6)	<0.03

Tab 11. Standard echocardiographic parameters before and after PDA closure

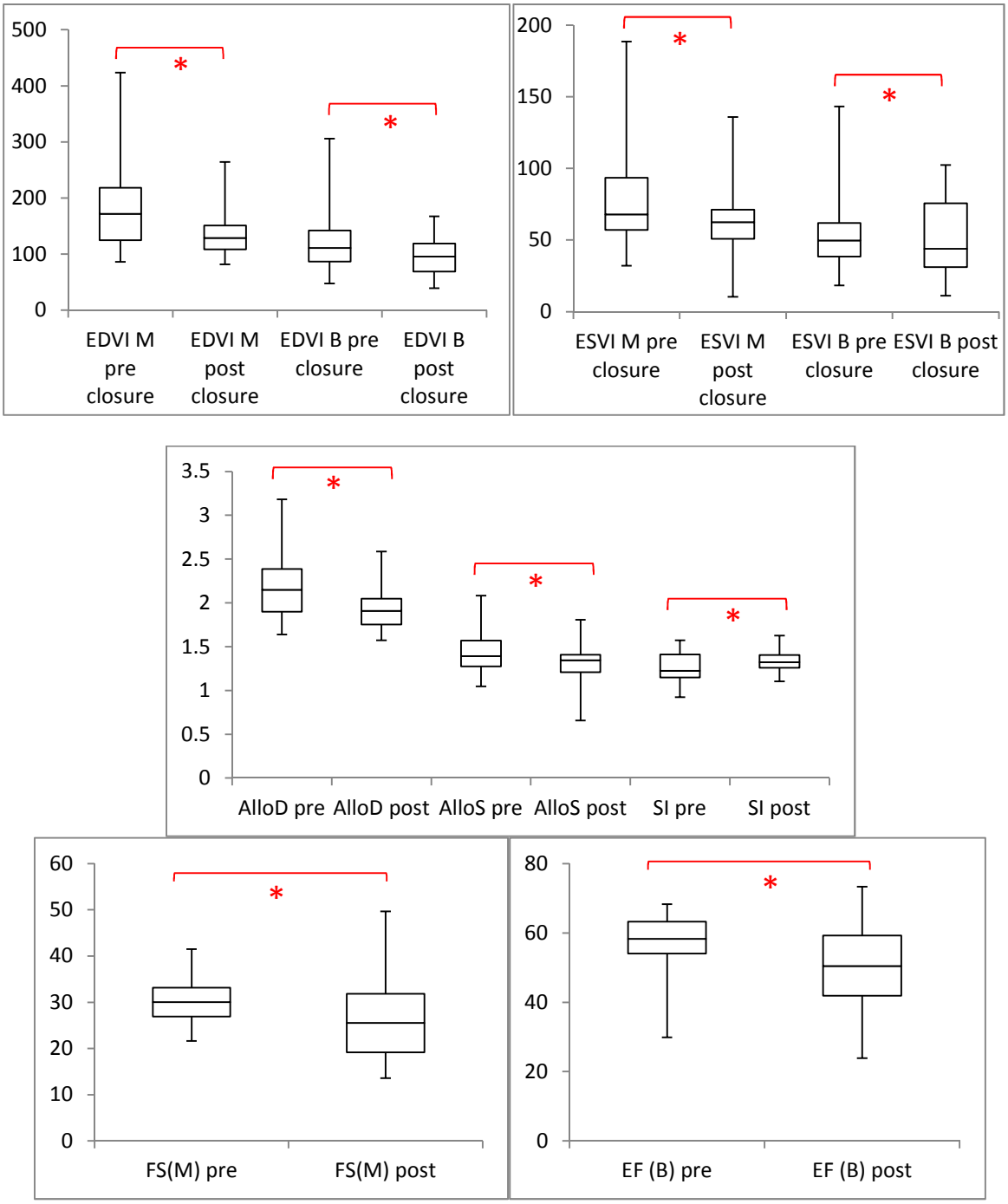

Fig 21. Box and whisker plot of selected standard echocardiographic parameters. A statistically significant change was detected in all parameters

STE-derived echocardiographic parameters

STE was performed in order to evaluate peak systolic circumferential, radial, transversal and longitudinal strain and strain rate. Endocardial and epicardial circumferential and longitudinal strain and strainrate were also performed in order to evaluate endo to epicardial gradient.

Pre-closure circumferential, radial and transversal strain and strainrate were statistically higher as compared to post-closure values. Longitudinal endocardial strain and strainrate showed a basal to apical gradient with increasing values of strain and strainrate from the base to the apex both pre- and post-closure. Although a decrease in longitudinal strain and strainrate was found after the PDA was closed as compared to preoperative evaluation, it did not reach statistical significance.

STE	Before PDA closure	After PDA closure	P value
Circumferential strain	-26.7±5.4 %	-21.2±5.5 %	<0.001
Circumferential strain rate	-3.12±0.8 s^{-1}	-2.47±1.0 s^{-1}	=0.005
Radial strain	51.5±18.2 %	38.1±10.5 %	=0.008
Radial strain rate	4.2±1.25 s^{-1}	3.08±0.97 s^{-1}	=0.002
Longitudinal strain	-19.1±6.1 %	-18.2±4.3 %	NS
Longitudinal strain rate	-2.15±0.78 s^{-1}	-2.13±0.55 s^{-1}	NS
Transversal strain	49.3±17.9 %	35.3±13.1 %	=0.002
Transversal strain rate	4.39±0.92 s^{-1}	3.63±0.93 s^{-1}	<0.001

Tab 12. STE parameters in dogs with PDA, before and after ductal closure.

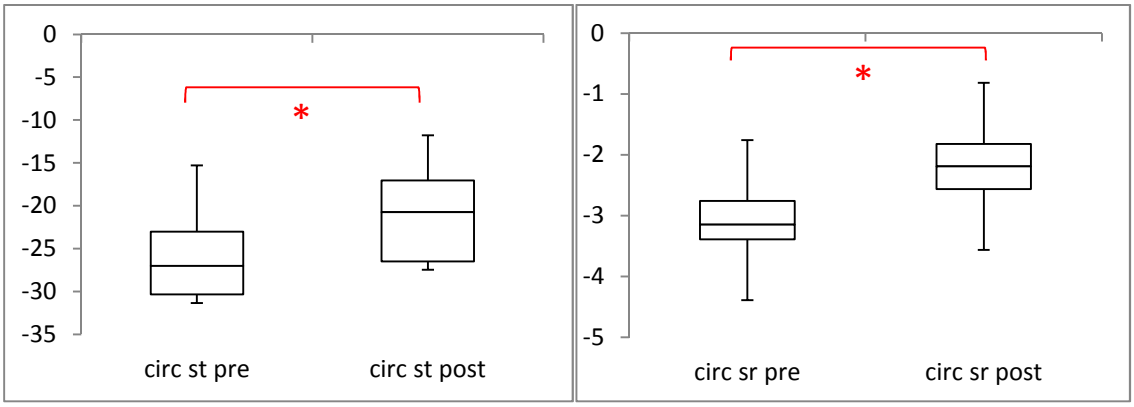

Fig 22. Box and whisker plot of circumferential strain (circ st) and strain rate (circ sr). A marked difference was found before and after PDA closure.

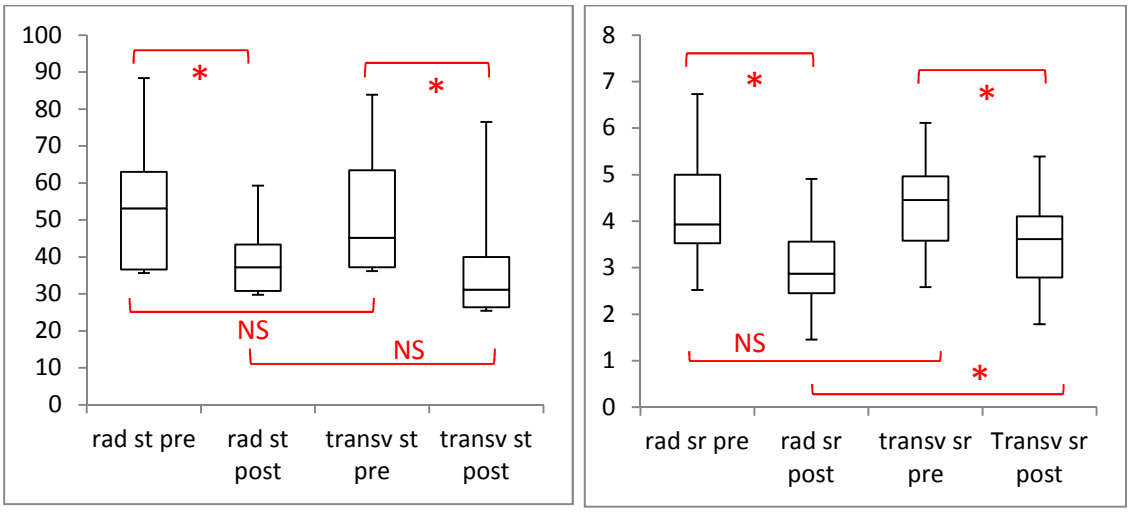

Fig 23. Box and whisker plot of radial and transversal strain (rad, transv st) and strain rate (rad, transv sr). A marked difference was found before and after PDA closure. No difference was found between radial and transversal strain and strainrate except for postoperative radial and transversal strain rate.

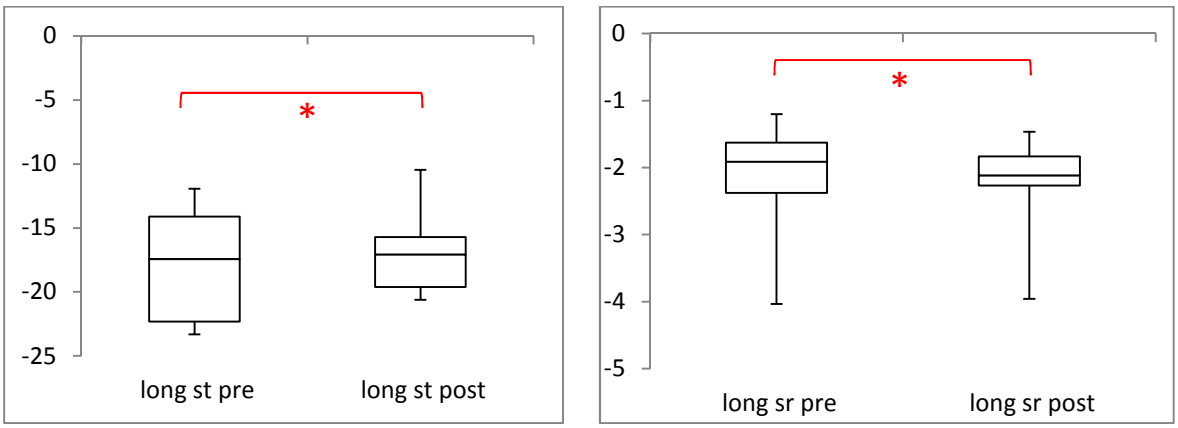

Fig 24. Box-whisker plot of STE-derived strain (st) and strain rate (sr). A marked difference was found in dogs undergoing PDA closure.

A base to apex gradient was found in longitudinal motion, with more negative values at the cardiac apex. PDA closure determined a reduction in absolute values, but maintained the normal base to apex gradient (Tab 13).

	Basal Septal	Mid Septal	Apical Septal	Apical Lateral	Mid Lateral	Basal Lateral
Longitudinal strain pre	-14.8 %	-17.7 %	-21.1 %	-21.3 %	-20.2 %	-19.3 %
Longitudinal strain post	-14.2 %	-17.4 %	-21.1 %	-22.7 %	-17.8 %	-16.6 %
Longitudinal strain rate pre	-1.90 s^{-1}	-2.10 s^{-1}	-2.39 s^{-1}	-2.13 s^{-1}	-2.14 s^{-1}	-2.2 s^{-1}
Longitudinal strain rate post	-1.69 s^{-1}	-1.80 s^{-1}	-2.20 s^{-1}	-2.42 s^{-1}	-2.13 s^{-1}	-2.16 s^{-1}

Tab 13. Mean myocardial wall segments strain and strain rate values before and after PDA closure

Epicardial and endocardial circumferential and longitudinal strain and strain rate differed markedly, with epicardial values being always more positive than the endocardial ones, both before and after PDA closure (Tab 14 & 15)

	Before closure	After closure	P value
Endocardial circumferential strain	-27.7±4.0 %	-24.1±8.4 %	<0.01
Endocardial circumferential strain rate	-3.26±0.83 s^{-1}	-2.82±1.31 s^{-1}	<0.04
Epicardial circumferential strain	-12.17±2.39 %	-8.8±3.21 %	<0.001
Epicardial circumferential strain rate	-1.20±0.35 s^{-1}	-0.85±0.29 s^{-1}	<0.001

Tab 14. Endocardial and epicardial circumferential strain and strain rate before and after PDA closure

	Before closure	After closure	P value
Endocardial longitudinal strain	-19.1±6.1 %	-18.2±4.3 %	<0.01
Endocardial longitudinal strain rate	-2.77±0.78 s^{-1}	-2.27±0.84 s^{-1}	<0.01
Epicardial longitudinal strain	-18.2±4.6 %	-15.7±5.7 %	<0.02
Epicardial longitudinal strain rate	-2.31±0.67 s^{-1}	-1.83±0.65 s^{-1}	<0.01

Tab 15.Endocardial and epicardial longitudinal strain and strain rate before and after PDA closure

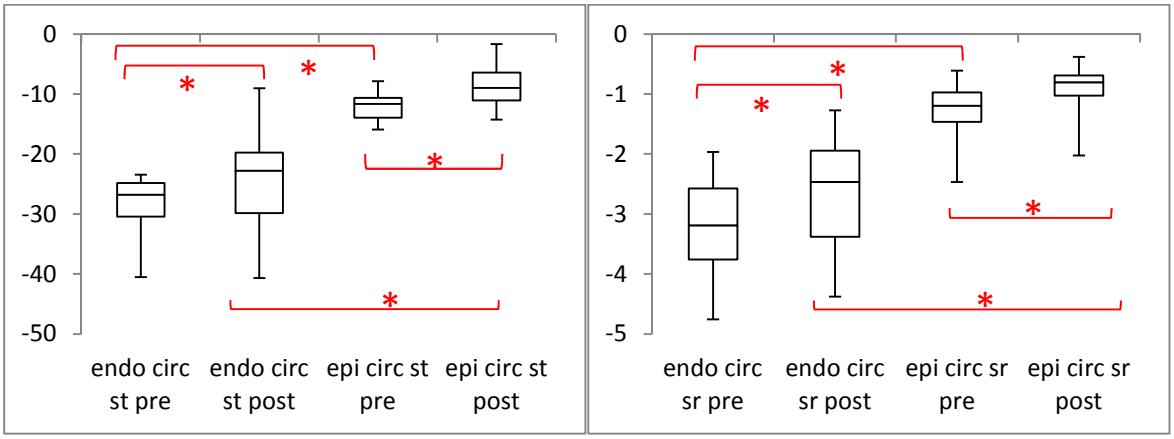

Fig 25. Box and whisker plot of endocardial and epicardial circumferential strain (st) and strain rate (sr) before and after PDA closure.

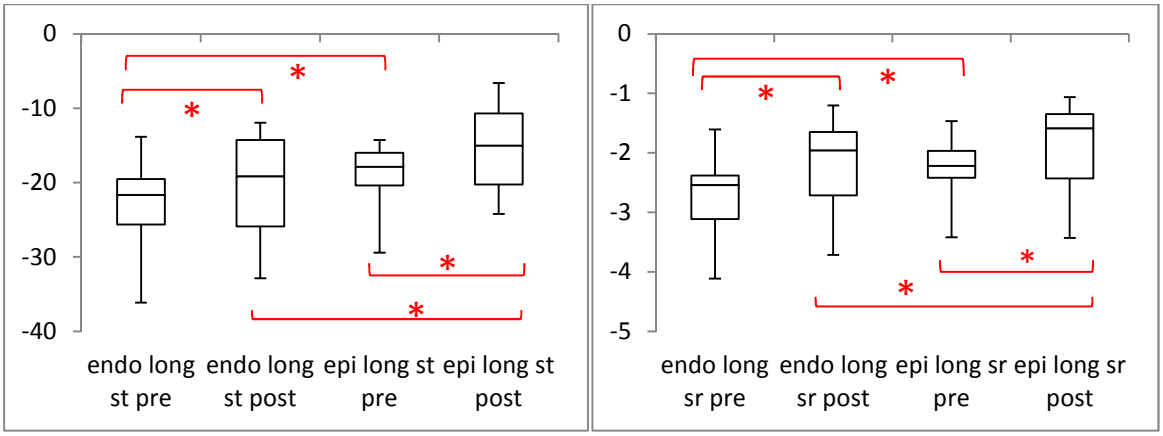

Fig 26. Box and whisker plot of endocardial and epicardial longitudinal strain (st) and strain rate (sr) before and after PDA closure.

Part III Cardiac torsional mechanics in dogs undergoing PDA closure

The dogs undergoing this study were evaluated before and after PDA closure. A total of 24 dogs were included in the study, the same included in the part II study.

Mean age was 17.7±18.4 months, mean weight was 15.1±8.9 Kg. Female dogs outnumbered males and were 79% of total patients.

	Mean ± SD	Min-Max
Age	17.7 ±18 months	2-76 months
Sex	19♀ / 5♂	
Weight	15.1±8.9Kg	4.2-35Kg

Tab 16. Population characteristics

Standard echocardiographic parameters were the same as shown in part II study (Tab 11).

STE-derived torsional mechanics

Torsion was evaluated as global torsion (apical rotation displacement- basal rotation displacement) and as basal and apical rotation.

A slight increase in global torsion was found if pre- and postoperative value was compared, however it did not reach a statistical significance. Basal rotation increased, while apical rotation was similar.

However, both these findings were not statistically significant. (Tab 17) Heart rate did not show any influence on apical and basal rotation.

	Before closure	After closure	P value
Global torsion	11.9±3.6 °	12.9±4.9 °	NS
Basal rotation	-4.27±1.43 °	-5.07±2.5 °	NS
Apical rotation	7.68±2.8 °	7.78±3.17 °	NS
Heart rate	122.1±35.5 bpm	123.8±32.7 bpm	NS

Tab 17. Torsion and basal and apical rotation before and after PDA closure

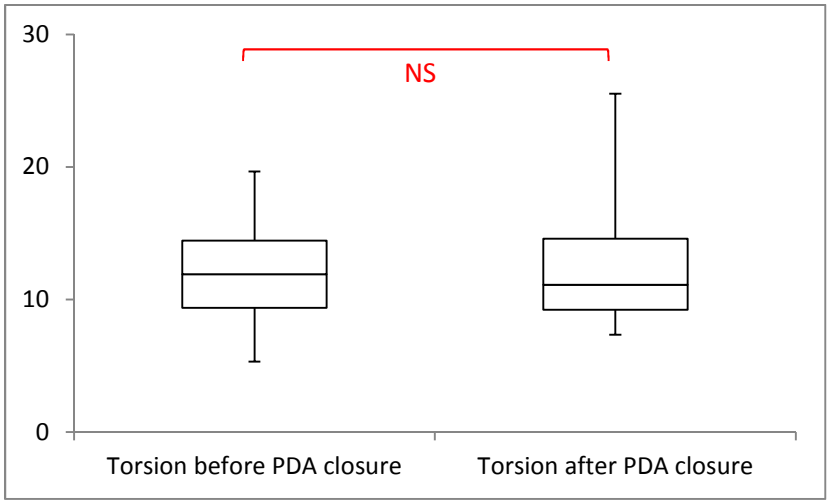

Fig 27. Global torsion before and after percutaneous PDA closure: although a mild increase in mean torsion can be observed, no statistical significance was seen.

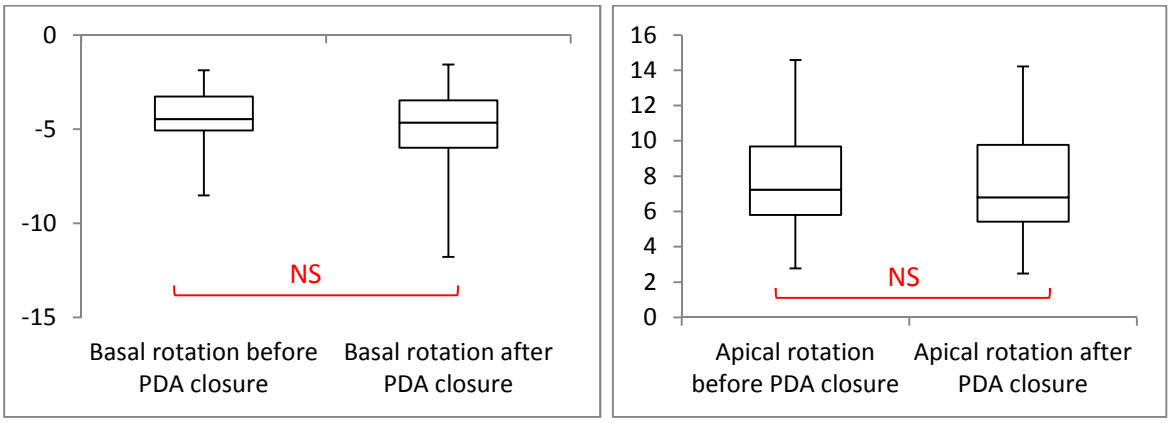

Fig 28. Basal and apical rotation in dogs with PDA: pre and postclosure values are not statistically significant, although a slight difference in basal rotation can be observed.

Discussion

Discussion

Part I: Cardiac mechanics in dogs with PDA VS healthy dogs

Technical issues of STE and comparison with literature

One of the aim of our study was to assess normality ranges and thereafter to compare this population of healthy subjects to the diseased group.

Most of the studies in both human and veterinary study refer to different software, as every ultrasound factory developed a singular algorithm for the analysis of speckle motion around the cardiac cycle. Thus, the reference values provided in literature are mainly dependent not only on strictly technical and repeatability issues, but also on software issues, as the English-speaking scientific world mostly perform echocardiography using machines of a single factory, which is not the one used in this study.

One of the main differences between the most widespread software used and the one used in our study is related to the tracking system used: speckle-tracking echocardiography for its intrinsic definition analyses speckles in the myocardial wall, thus a larger sample is generally used as compared to the software developed by Esaote©, which can be defined as “border tracking” echocardiography; the speckles analyzed in this case are in the epicardial or endocardial border, which means a smaller sample.

Apart from this main difference, another, greater issue has to be underlined: from the new anatomical insight of Torrent Guasp’s ventricular myocardial band, the left ventricular wall is the result of two opposite twisted helices, the right- and left handed helices, which are in opposite contraction during the isovolumic contraction and ejection phase, as they are responsible for the twisting motion of the LV.

This counter directed contraction might be also evident while evaluating, for example, the longitudinal motion of the LV, as the interventricular septum is the anatomical sum of these two components.

It is then clear that if we analyze the speckles in the myocardial wall, we might take some of these from the left-handed helix and some others from the right-handed helix, thus results are the sum of these two opposite motions, while if we keep our area of interest in the epicardial or endocardial wall, we might be more confident to analyse the right or left handed helix and thus provide a "true" endocardial and epicardial strain and strain rate. This should be considered when evaluating STE-derived values from different software.

Normal STE reference ranges in the population of healthy dogs

A small body of literature has been published in veterinary medicine in the latest years concerning the evaluation of STE-derived strain and strain rate in selected categories of patients.

Most of these studies included only one breed of dogs and this is of interest if a standard breed reference range has to be developed, but for general population aim this inevitably lacks the generalized approach required, making extrapolation an option, but with limitations.

If we compare the results from our population of young dogs, we can affirm that on a general basis the results obtained are similar to the ones provided in veterinary literature, even if with slight differences.

Concerning circumferential strain and strain rate, our tracking software provided a more negative mean systolic strain and strain rate with similar standard deviations if we compare the data published in veterinary literature(Tab18). However, if we compare these results with a similar study[62] conducted in people with the same software as the one used in our study, we might appreciate two similarities with our case series: first, the values of global circumferential strain at mitral level in human patients are similar to our results (-21±6%), and furthermore a statistically significant difference was appreciated with age, as younger patients had more negative strain and strain rate values as compared to adults.

The population prospectively recruited in our study was aimed at studying dogs with PDA, which is a congenital heart disease and thus dogs with PDA are generally young; it might be said that the higher absolute circumferential values might be related to younger age as compared to data in veterinary literature. Another possible explanation is related to software issue as well.

Circumferential	Smith 2012	Zois 2013	Carnabuci 2013	Kusunose 2013	Suzuki 2013	Present study
Circ strain (%)	-20.9±3.15	-19.5 (-17.2to-21.2)	-20.4±4.5	-17±7	-19.4±4.4	-22.34 ± 4.2
Circ strain rate (s^{-1})		-2.1 (-1.8to -2.4)	-2.1±0.7		-2.3±0.4	-2.58 ± 0.7

Tab 18. Circumferential strain and strain rate in veterinary literature as compared to our study

Concerning radial deformation, a higher variation of reference ranges can be appreciated in veterinary literature, with higher standard deviation as compared to circumferential strain (Tab19).

Our mean data lie in between the minimum and maximum values provided by different studies, with a slightly higher standard deviation for strain values as compared to other studies.

Carnabuci et al performed deformation imaging analysis by the same software as us and found lower radial strain values than our population, but the dogs included in the study were all Labrador Retrievers older than our population and of greater mean weight. It has been shown by Suzuki et al that age influenced radial deformation, with higher radial strain values in young dogs. It is not yet clear if radial deformation is influenced by weight as identified by Takano for circumferential strain and strain rate values (more negative circumferential strain values in small breed dogs as compared to large breed dogs). Based on these data it might be said that probably weight and age play a role, thus a sensible approach when comparing different studies would be to consider also these parameters.

Radial	Chetboul 2007	Culwell 2011	Smith 2012	Zois 2012	Carnabuci 2013	Suzuki 2013	Present study
Radial strain(%)	46.7±12.2	28.73±7.1	43.9±8.5	53.1±10	27.5±10	52.4±11.1	35.1 ± 14.3
Radial strain rate (s^{-1})	2.69±0.76	2.18±0.44	3.04±0.47	2.9±0.6	2.2±0.8	3.2±0.6	2.89± 0.5

Tab 19. Radial strain and strain rate in veterinary literature as compared to our study

Longitudinal strain and strain rate values in our population are similar to the ones reported in literature, as shown in table 20. Age seem not to influence peak systolic longitudinal strain and strain rate in veterinary case-series, as demonstrated in suzuki et al paper, in contrast with human medicine, where children have higher global peak systolic strain and strain rate. If we compare the only veterinary study where the same software was used, a difference in mean value can be found, with the present study having more negative strain and strain rate values as compared with Carnabuci et al paper[48], with the dogs of our study being also younger and lighter. Whether this is a true difference of values due to age and breed, it cannot be proved with such small body of literature and different populations.

Longitudinal	Culwell 2011	Wess 2012	Zois 2012	Carnabuci 2013	Suzuki 2013	Present study
Longitudinal strain (%)	-16.61±2.21	-15.9 ±5.2	-18.8±1.1	-14.8±1.6	-14.8±3.1	-16.74 ± 3.9
Longitudinal strain rate (s^{-1})	-1.53±0.28	-1.9±0.95	-2.0±0.3	-1.3±0.2	-1.7±0.3	-1.70 ± 0.4

Tab 20. Longitudinal strain and strain rate values in veterinary literature as compared to our study

Echocardiographic evaluation of cardiac function in dogs with PDA vs healthy dogs

As previously and extensively shown by both veterinary and human cardiology studies, PDA determines a modification of cardiovascular hemodynamics, which is supported by a global, homogeneous increase in all markers of LV dimensions (EDVI, ESVI and AlloD/S). The difference in these parameters between normal dogs and dogs with PDA is striking. The increase in all end diastolic and systolic dimensions is a well-known marker of LV overload and is due to the increase in preload and afterload secondary to L-R PDA shunt. It should also be emphasized that the correlation analysis carried out showed an excellent correlation between different methods of evaluating LV dimensions, even if all the techniques used different equations.

Unsurprisingly, shunting quantification as expressed by Qp/Qs ratio showed a marked difference between the two group of dogs.

Following pathophysiology definition, PDA determines an increase of pulmonary flow, but the main increase of blood is seen in the aortic flow due to the presence of the shunt, thus the ratio between aortic flow to pulmonary flow does not equal 1, but it is supposed to increase with increasing LV overload and PDA severity. This explains the statistical significance of Qp/Qs value as shown by our study. Qp/Qs also correlates well with the markers of LV overload and thus might be regarded as another parameter of PDA shunting severity.

Interestingly, all the standard echocardiographic parameters investigated showed statistical significance except two, EF and FS. This finding is an important issue, as EF is the cornerstone of the diagnosis of systolic failure in human cardiology, while FS is one of the most important parameters taken into account by veterinary cardiology guidelines in the identification of systolic dysfunction.

One of the main concerns in pediatric cardiology regarding pre-term infants with PDA is the reduced cardiac reserve of a young heart and thus the increased risk of cardiac impairment and systolic dysfunction by a volume overloading shunt.

It has been however shown by Barlow et al that, although PDA determines an increase in end diastolic and systolic dimensions and that pre-term infants have actually a reduced cardiac reserve, ventricular contractility is preserved and unimpaired in premature neonates with PDA[22].

Furthermore, both EF and FS are load dependent parameters and as a consequence of this they are not reliable markers of decreased contractility and systolic function in dogs with PDA.

STE in dogs with PDA

Advanced echocardiographic techniques represent an important field of study and investigation and help understand better cardiac mechanics. These techniques are furthermore directly related to cardiac contractility and thus provide a direct assessment of cardiac function.
An important finding identified by our study is that strain and strain rate values are increased in dogs with PDA (more negative results for longitudinal and circumferential strain and strain rate values, more positive results for radial strain and strain rate) and thus are not decreased.
Lower than normal strain and strain rate values are regarded as markers of impaired cardiac function in a wide variety of diseases in human cardiology (coronary artery disease, dilated cardiomyopathy, cardiac resynchronization therapy) and the finding that in our cohort of patients with PDA global strain and strain rate are increased rules out the presence of systolic dysfunction in pre-operative assessment.

PDA thus determines an increased contractility response to counteract increasing preload and afterload responsible for LV overload, maintaining effective pump function for long periods of time.

The fact that PDA is a congenital heart defect could be a protective factor in this group of patients, as the immature heart reaches maturity already adapting itself to the presence of an increased load from birth on. The great regenerative capacity of infant myocytes has been shown to decrease with age[63], as 15% of LV resection to 1 day old mice lead to cardiac function restoration and wound healing without scar tissue formation, while the same injury provoked to a 7 day old mice failed to elicit a regenerative response. Cardiomyocytes regeneration is also linked to proliferative capacity of the same myocardial cells[63].

This findings could be applied to the hearts of dogs with PDA and it could be argued that dogs with PDA are able to cope with volume loading better than other disease conditions arising in adult dogs (for example mitral regurgitation secondary to degenerative mitral valve disease) due to an adaptative response and increased myocytes proliferation (eccentric hypertrophy), in order to decrease wall stress by subdividing the load between an increased number of myocytes and gaining a spherical shape of the LV (Laplace's law).

Correlation between standard and advanced echocardiographic parameters

The correlation between standard echocardiographic parameters and STE-derived is poor.

From this point of view, it is clear that it is not possible to extrapolate data from both strain and strain rate and apply them to standard methods, while it would be more sensible to use both in the preoperative evaluation of dogs with PDA.

The reason for this poor relationship is probably linked to the fact that values are obtained by two different methods, on one hand indirect assessment of cardiac contractility and function (standard echocardiographic criterias), while others directly assess cardiac contractility by tracking myocardial speckles (STE derived).

Standard echocardiographic measurements generally assess cardiac dimensions and extrapolate function starting from mono- or bidimensional images. End diastolic and systolic dimensions provide indirect assessment of LV filling and ejection, and thus indirectly can provide information about cardiac contractility. However they are the result of a sum of different factors like preload, afterload, contractility and heart rate and the effect of each single factor cannot be separated from the others. The same principle can be applied to FS and EF so that on particular, selected cardiac disease, a normal FS or EF does not indicate a real acceptable contractility, because preload, afterload or heart rate may mask systolic dysfunction.

On the other hand, STE-derived values are directly assessed based on myocardial motion, and thus, contractility. A decrease in STE-derived values in a patient with heart failure with normal ejection fraction indicates systolic dysfunction, even if standard echocardiographic parameters are normal. Deformation imaging has been proven specific and accurate in evaluating a wide spectrum of cardiac diseases, being able to detect abnormalities before the onset of clinical signs or the detection of abnormal standard echocardiographic parameters[40-43].

The specificity and accuracy of STE has been compared with gaudolinium-enhanced MRI and an excellent correlation was found, indicating high sensitivity of STE in identifying small and localized infarctual areas, which is superior to 2D quantification of regional wall abnormalities[3,4].

Based on our results standard and advanced echocardiographic parameters are complementary tools and can provide a precise evaluation of LV morphology and mechanics.

Part II : Cardiac mechanics in dogs undergoing PDA closure

Standard echocardiographic parameters before and after PDA closure

PDA closure determines an abrupt change in cardiovascular dynamics, as preload is reduced and afterload is increased by shunt closure. This leads to an immediate decrease in end-diastolic dimensions ($EDVI_{M-B}$, AlloD), but not such a rapid decrease in end-systolic dimensions ($ESVI_{M-B}$, AlloS). This findings are well described in both human and veterinary medicine. It has been reported in a human case series that 6 month after successful PDA closure standard echocardiographic parameters were similar to healthy patients, thus all the cardiovascular changes seemed reversible once treated[64].

Veterinary literature shows more conflictual results, but it should be noted that veterinary PDAs are bigger than the ones seen in pediatric cardiology and that before the advent of ACDO device (2007) the presence of residual shunting was frequent and thus it could be difficult to evaluate the significance of persistently abnormal LV dimensions in the subset of studies before 2007. Stauthammer et al identified a general reverse remodeling (ie, normal LV dimensions) in dogs undergoing PDA closure one year after percutaneous closure[34].

If we compare the results of our study with the latest veterinary studies, where the same standard echocardiographic parameters have been used to assess LV dimensions, we can say that the PDA seen in our case series determined greater LV overload preoperatively, however on a general basis the results are in accordance with previous studies (Tab 21).

24 hours after PDA closure two studies identified diastolic dimensions to be within normal ranges[13,65], while Stauthammer et al reported greater than normal diastolic dimensions immediately after PDA closure, with normal diastolic and systolic dimensions one year after PDA closure.

	Campbell	Saunders	Stauthammer	Present study
AlloD				
before	2.0±0.3	2.15 (IQR 1.85-2.37)	2.12±0.05	2.20±0.38
24hr after	1.7±0.3	1.50 (IQR 1.4-1.7)	1.88±0.05	1.94±0.24
12mo after			1.64±0.05	
AlloS				
before	1.3±0.3	1.33(IQR 1.02-1.59)	1.36±0.04	1.45±0.28
24hr after	1.3±0.3	1 (IQR 0.87-1.20)	1.34±0.04	1.29±0.27
12mo after			1.17±0.04	

Tab 21. A comparison between allometric scaling-derived indices of diastolic and systolic dimensions in different studies

Our case series showed a significant decrease in all standard echocardiographic parameters pre- and postoperatively, however none of the parameters after PDA closure was within normal ranges, indicating that cardiac reverse remodeling is not an immediate process and requires time.

A parameter that was not taken into account previously in dogs with PDA was the sphericity index (SI), which was shown to decrease significantly after PDA closure, but even postoperatively was far from being within normal ranges, indicating that the LV still had a more spherical shape as compared to normal hearts.

One of the major concerns in very low birth weight infants with hemodynamically significant PDA is to induce a transient significant decrease in cardiac output postclosure due to the fact that infants' hearts have smaller cardiac functional reserve and are thus more prone not to adapt quickly to a sudden change in loading conditions. Some of the patients need to receive inotropic agents after PDA closure. This need for inotropic agent has never been investigated in dogs with PDA, probably because our population of patients, even if of young age, is not as young and has not such immature hearts as compared to very low birth weight infants. The need for inotropic agents is evaluated by an important decrease in cardiac output, which is not the standard parameter evaluated in veterinary patients.

The standard parameters to evaluate systolic function in veterinary cardiology are FS and EF; there are more than anedoctical reports that identify lower than normal FS in a variable percentage of patients after PDA closure, however the clinical relevance of this has yet to be clearly determined.
FS and EF are expected to decrease after PDA closure due to the combined change in preload and afterload: it is well known that any condition that determines a decrease in preload decreases FS as well as an increase in afterload determines a decrease in FS too[35]. PDA closure determines both these hemodynamic changes, thus it is clear that FS and less significantly EF will decrease.

This decrease is therefore not a marker of decreased contractility itself, but is just the effect of a change in loading condition; this is supported also by one of the latest studies in veterinary medicine, which showed optimal long-term survival time also in dogs with post-closure FS lower than 23%[34].

If we set the reference range for EF <40% and FS of < 23% in our case series only one each of EF_B and FS_M values were below the reference ranges before PDA closure.

After PDA closure, applying the same normality ranges, 4 dogs showed a lower than normal EF_B (23.9-39.2 range), 6 dogs abnormal EF_M (28.25-37.89 range of values) and 9 dogs had FS_M below the normal reference range (13.6-22 range of values). No dog with pre-closure lower than normal values had lower than normal post-closure FS or EF values.
Post-closure lower-than normal EF or FS values were detected in 2 dogs only to be all decreased (EF_M, EF_B and FS_M), while 2 of these 3 parameters were below the reference range in 5 patients and only 3 patients had lower-than normal FS_M with normal EF values.

Circumferential, Radial and Longitudinal Strain and Strain rate before and after PDA closure

Our study was the first in veterinary medicine to analyze deformation imaging by means of STE in dogs with PDA. This condition is unique in veterinary cardiology, because it is one of the most common congenital heart disease in dogs and PDA closure in most, if not all, patients increases survival time markedly, adding a median of 12 years to life expectancy[13]. Furthermore, PDA closure by percutaneous approach has very few complications and a very low mortality rate, which warrants interventional procedure to be taken seriously into account.

Nonetheless, few studies have unraveled the pathophysiologic mechanisms of PDA and the consequences of PDA closure on the myocardium. For its pathophysiology, PDA can be defined as a left to right shunt determining an increase in both preload and afterload, with the latter playing a relative role in the pre-closure evaluation but gaining importance in the post-closure assessment of LV function.

The combined increase in preload and afterload will, as expected, increase cardiac contractility too, due to the fact that the myocardium needs to increase cardiac output and stroke volume in order to counterbalance the increase in blood flow in the LV at every cardiac cycle.
As we already demonstrated in part I study, all strain and strain rate parameters were increased in dogs with PDA without detecting any sign of systolic dysfunction as a consequence of LV overload.

Following PDA closure, a statistically significant decrease in circumferential and radial strain and strain rate values were observed, while longitudinal strain and strain rate decreased slightly, although not reaching statistical significance.

For its definition and anatomical distribution, radial and circumferential deformation parameters analyze the motion of the cardiac fibers from a short axis view, whose anatomical constituents are the circumferential fibers belonging to the basal loop, while the longitudinal motion of the heart is mostly dependent on the longitudinal fibers, which are located in the subendocardial walls of the LV (Fig 29).

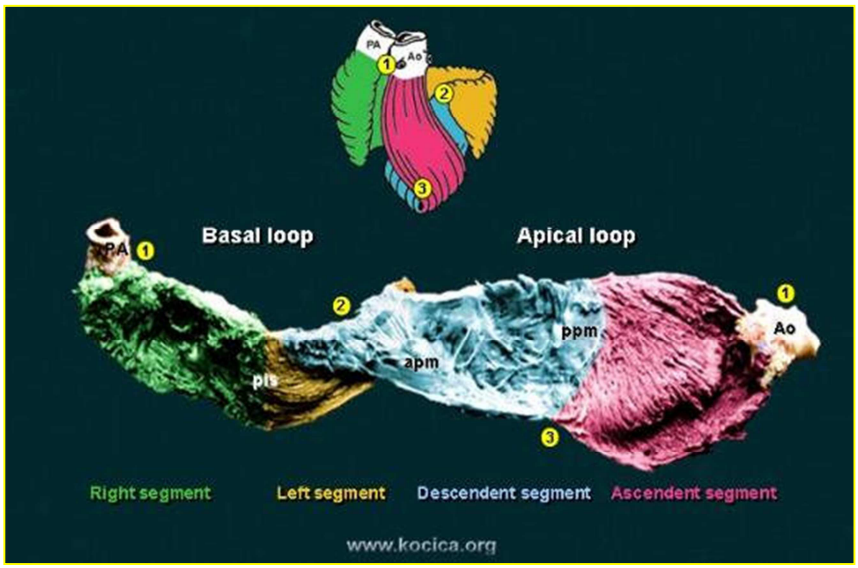

Fig 29: the helical ventricular myocardial band and the reconstruction of the segments related to the figure of eight drawing.

The sudden decrease of both circumferential and radial deformation parameters 24 hours after PDA closure might simply reflect the decrease in preload obtained by PDA closure. The values obtained postoperatively are similar to those obtained in healthy control dogs in part I study, thus it could be argued that PDA closure restores immediately the contractility pattern of circumferential fibers of the LV.

Laplace's law states that tension on the myocardial wall is directly dependent on LV pressures and the radius of the LV divided by left ventricular free wall thickness[10].

If preload decreases following PDA closure, the tension on the myocardial walls decreases as well as the radius of the LV, both of which will lead to a decrease in left ventricular wall thickness and thus in the strength of contraction of the circumferential and radial fibers.

On the other hand, the slight decrease of the longitudinal deformation parameters after PDA closure has a two-fold meaning: first of all, longitudinal fibers are predominant in the subendocardium, which is the main structure responsible for long axis function and is particularly susceptible to ischemia due to the fact that the myocardium is perfused from the epicardium to the endocardium. A fall in long axis velocities is related to an early decrease in systolic function, which is generally accompanied in early stages by an increase in radial velocities and an overall preservation of global ejection fraction.

In our case series, none of these two latter conditions have been reported, as longitudinal strain and strain rate were slightly lower in value as compared to pre-closure, but were higher as compared to the normal population of dogs and radial strain and strain rate instead of increasing, decreased to normal values.

Based on these consideration, systolic dysfunction, considered as a reduction of myocardial contractility, can be ruled out in dogs with PDA both before and, most importantly, after closure.

The second important consideration that has to be made is that longitudinal strain and strain rate could be considered as markers of reverse cardiac remodeling in dogs with PDA, based on the fact that on a 24-hour observational time there is not a statistically significant decrease, thus meaning that longitudinal fibers need greater period of time to reverse PDA-induced remodeling. Global longitudinal strain has already been proven as indicator of effective reverse remodeling (defined as a reduction of <15% of LV inner diameters) in patients undergoing cardiac resynchronization therapy and transcatheter aortic valve implantation[67,68].

Longitudinal strain and strain rate in normal hearts is characterized by a basal to apical gradient, with more negative values at the cardiac apex and less negative values at the basal segments.

Our study identified a basal to apical gradient both pre- and postoperatively, thus this finding is in accordance with normal cardiovascular physiology and literature.
We also compared radial strain and strain rate values with transversal ones, as both evaluate radial motion, but one is measured from short axis view and the second one from long axis view, and found no difference. It can be confirmed that both views are equal to measure radial motion.

Endocardial and epicardial segments of the myocardium belong to two different anatomical structures, the right-handed helix and the left-handed helix[1,5].
In healthy volunteers and in a cohort of Labrador retrievers an epi- to endomyocardial gradient was observed for both longitudinal and circumferential motion, with highest strain and strain rate values to be detected in the endocardium and lower values in the epicardium[48].
We identified the same gradient in dogs with PDA, with statistical differences both between endocardial and epicardial values before and after PDA closure, but also between the same segments (epicardial before vs after, endocardial before vs after). As a consequence of these findings, we can affirm that PDA hemodynamics do not alter the intrinsic cardiac anatomy and physiology of activation and contraction of the myocardial segments.

Part III : cardiac torsional mechanics in dogs with PDA

Cardiac twist seems not to change after PDA closure

STE is a powerful tool to analyze cardiac motion without needing invasive measurements.
Cardiac deformation imaging is based on the quantification of normal strains (longitudinal, circumferential and radial strain and strain rate), but also shear strains (endo to epicardial motion and apex to base rotation).

Cardiac torsion (defined as the net difference between apical and basal rotation normalized for the length of the LV) is probably the most important shear strain identified, because it provided a new insight into cardiac physiology and proved to change under different loading and pathological conditions, thus becoming one of the parameters to take into account in many cardiac disease[5, 56,55,59].

Torsion helps bring a uniform distribution of LV fiber stress and fiber shortening across the wall by enhancing the ejection and suction of blood during the cardiac cycle and thus is vital for the efficiency of LV contraction.

Torsion increase gradually from infancy to adulthood, due to the change in basal rotation: in infants both apex and base rotate counterclockwise, then the base acquires a neutral rotation in early childhood until acquiring the adult torsional pattern of basal clockwise rotation in adolescence. This change is thought to be related to maturation of the helical myofiber architecture[55].
Torsion is load-dependent: cardiac twist is greater with higher preload, while is decreased with afterload[57,58]. The effect of preload on twist is about two-thirds as great as that of afterload, thus afterload plays a greater role in cardiac twist as compared to preload[58].

One of the greatest problems related to loading conditions in clinical fields is that not all disease conditions can mimic experimental conditions, where only preload or afterload can be manipulated while the other is kept stable[57,58]

In our case series, no difference was found in net torsion or basal/ apical twist in our population of patients. Two great considerations have to be made, while evaluating torsional mechanics of the LV in dogs with PDA.

First of all, the population comprised young dogs of variable age and breeds (clinical population), thus the effect of age or breed on torsion cannot be precisely assessed. It has been proven that children present a different twist of the base as compared to adults, however in veterinary medicine no study has ever proven a change in the twisting mechanics of the LV in growing dogs and thus we cannot say at what age they reach adult basal twist.

Dogs with PDA do not show a change in just one loading condition with the second being a fixed parameter, because both before and after closure, preload and afterload change in a different way but simultaneously. It has been stated that an increase in preload increases torsion, while an increase in afterload decreases torsion and this is the case in dogs with PDA before ductal occlusion.

It is true that the increase in preload is more marked and can be defined as preponderant in cardiac mechanics in dogs with PDA, as the increase in afterload is the consequence of LV overload secondary to preload, however by the regression analysis performed by Dong et al, afterload plays a greater role in determining global torsion. It could therefore be stated that although preload increased markedly torsion, the increase in afterload counteracted the effect of it.

After PDA closure, the influence of preload on cardiac mechanics is reduced, with an increase in afterload, which is secondary to the increased systemic resistance determined by the absence of a leak in blood flow through the ascending aorta.

Based on these factors, a decrease in torsion was expected, however was not identified in our population of dogs. A possible explanation of this could be that the increase in afterload is not as big as to determine a decrease in torsion, because, although end diastolic volumes decrease more markedly as compared to end systolic volumes, the latter decrease significantly after PDA

closure too, thus it could be argued that the mixed effect of the changing preload and afterload account for the unchanged magnitude of torsion in the intact ventricle.

Based on these findings, we can affirm that PDA closure is a condition well tolerated by the myocardium in a way that does not need to increase or decrease torsion secondary to the changes in the hemodynamics induced by ductal closure.

Limitations

This study has some limitations. The prospective nature of the study enabled homogeneous data to be acquired, although some other data, which could have been proven interesting to compare (TDI values, velocity of circumferential fiber shortening or Tei index among others), have not been acquired.

The two population of PDA dogs and the healthy control dogs were weight matched and not age matched; further studies might be needed to assess age matched population with an increasing number of control dogs. A greater population of dogs should be selected in order to provide more exhaustive reference ranges with STE- border tracking.

The variability coefficients were not assessed for every study and an interobserver/ intraobserver variability should be performed in order to determine if the coefficients of variation are similar for standard echocardiographic parameters and advanced echocardiographic imaging.

Conclusion

Conclusion

Echocardiography can be considered as a fundamental tool to assess cardiac morphology, dimensions and kinetics. It is a versatile instrument which can provide both basic information and an advanced insight into cardiac pathophysiology and mechanics.

PDA is one of the most common congenital heart disease in dogs and echocardiography has been proven beneficial in unraveling the pathophysiologic mechanisms behind PDA hemodynamics and its therapeutical closure.

PDA is associated with an homogeneous increase in all deformation parameters (circumferential, radial and longitudinal strain and strain rate) as compared to healthy control dogs and no correlation was found with standard echocardiographic parameters that have been routinely used in the past years. Both techniques highlight different aspects of the same disease, which should be evaluated as a whole, thus taking into account both standard echocardiographic technique and advanced echocardiographic imaging.
The standard parameters of FS and EF are less sensitive to evaluate subtle changes in cardiac contractility in dogs with PDA as many factors influence the final value, while deformation imaging has been proven to identify a significant change in cardiac function in dogs with PDA both before and after ductal closure. Based on the result of our study systolic dysfunction has not been identified in none of the patients evaluated in our case series.

Immediately after PDA closure, the circumferential fibers in the LV return to a normal pattern of contraction, as shown by the normalization of both circumferential and radial strain and strain rate, while longitudinal fibers, although not experiencing damage by the persistent LV overload induced by PDA, do not decrease abruptly their contractility pattern and persist on higher than normal values.

Longitudinal strain and strain rate values can be thus considered as markers of reverse remodeling over time, in order to assess when and if the myocardium of dogs with PDA is able to return totally to normal function or if some persistent alteration in the contractility pattern and LV morphology will be present throughout life.

Torsion of PDA hearts does not show a global change in our population of patients before and after ductal closure, probably because of the confounding effects of simultaneous change in preload and afterload, which influence in an opposite way the torsional mechanics of the LV.

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