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N-Terminal Pro-B-Type Natriuretic Peptide and Phonocardiography in Differentiating Innocent Cardiac Murmurs from Congenital Cardiac Anomalies in Asymptomatic Puppies

S.M. Marinus, H. van Engelen, and V. Szatmári

Background: Differentiating innocent cardiac murmurs from murmurs caused by congenital cardiac anomalies can be challenging with auscultation alone in asymptomatic puppies.

Hypothesis: Plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations and phonocardiograms recorded by an electronic stethoscope can differentiate innocent from pathologic cardiac murmurs.

Animals: A total of 186 client-owned asymptomatic dogs: 135 Cairn Terriers (age: 45–124 days), 20 adult Cairn Terriers (age: 7.5 months to 13.5 years), and 31 puppies of various breeds (age: 29–396 days).

Methods: Study design is a cross-sectional survey. Each dog was auscultated, and when a cardiac murmur was heard, a phonocardiogram was recorded and an echocardiogram was performed. Plasma NT-proBNP concentrations were measured by a single laboratory by an ELISA.

Results: No significant ($P = .41$) difference in plasma NT-proBNP levels was found between puppies without a murmur and puppies with an innocent murmur (median 300 versus 326 pmol/L), and between clinically healthy adult Cairn Terriers and Cairn Terrier puppies. Plasma NT-proBNP levels in puppies with a congenital heart disease were significantly ($P < .001$) higher than those in puppies with innocent murmurs (median 1,102 versus 326 pmol/L). However, some puppies with severe pulmonic stenosis did not have increased plasma NT-proBNP levels. On phonocardiograms, innocent murmurs had a significantly ($P < .001$) shorter "murmur-to-systole duration ratio" than the abnormal ones (median 66 versus 100%). The "murmur-to-S1 (first cardiac sound) amplitude ratio" was significantly ($P < .001$) lower of the innocent murmurs compared with that of the abnormal ones (median 16 versus 58 %).

Conclusions and Clinical Importance: Plasma NT-proBNP concentrations within the reference range do not rule out a congenital cardiac anomaly. Murmurs longer than 80% of the systole are most likely abnormal, whereas murmurs shorter than that could be either innocent or pathologic.

Key words: Auscultation; Biomarker; Dogs; Echocardiography; Functional murmurs; Littmann Electronic Stethoscope; Phonendoscope; Cairn Terrier; Breed.

Detecting a cardiac murmur in clinically healthy puppies during the first veterinary health check is common.[1,2] Innocent murmurs (also known as functional, physiologic, or flow murmurs) in puppies can be caused by low viscosity of blood as a result of low hematocrit.[2] This anemia is physiologic in young dogs and it resolves together with the innocent murmurs with maturation.[2] To determine whether a murmur is innocent or pathologic can be challenging based on cardiac auscultation alone in first-opinion veterinary practices.[1–6] This decision is, however, of major importance, as a pup with a pathologic murmur should ideally be referred to a veterinary cardiologist, whereas innocent murmurs require no further veterinary attention.[1,2] First-line veterinary practitioners lack a simple, in-house diagnostic test that can facilitate differentiating innocent cardiac murmurs from murmurs caused by congenital cardiac anomalies. We aimed to investigate the use of a cardiac biomarker and an electronic stethoscope.

Brain-type natriuretic peptide is synthetized by cardiomyocytes along with the N-terminal pro-B-type natriuretic peptide (NT-proBNP) when a ventricle experiences increased wall stress.[7–9] NT-proBNP can be measured in plasma samples and has become a widespread used test in diagnosing and staging acquired cardiac diseases in dogs.[7–9] However, there are only a few publications about NT-proBNP concentrations in dogs with congenital cardiac anomalies.[10–12]

Phonocardiography gives a visual display of cardiac sounds and murmurs.[13] Several electronic stethoscopes

Abbreviations:

AS	aortic stenosis
CI	confidence interval
DCRV	double-chambered right ventricle
DLVOTO	dynamic left ventricular outflow tract obstruction
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PDA	patent ductus arteriosus
PS	pulmonic stenosis
RI	reference interval
SAM	systolic anterior motion of the septal mitral valve leaflet
VSD	ventricular septal defect

From the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands (Marinus, van Engelen, Szatmári).

Corresponding author: V. Szatmári, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, 3584CM Utrecht, The Netherlands; e-mail: v.szatmari@uu.nl.

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have the possibility to record a phonocardiogram, which can be displayed on a computer screen.[14]

The following null hypotheses were tested in the present study: 1. Plasma NT-proBNP levels of clinically healthy puppies without a cardiac murmur do not differ from NT-proBNP levels of clinically healthy puppies with an innocent murmur. 2. Asymptomatic puppies with a severe, clinically relevant congenital cardiac anomaly have increased NT-proBNP levels. 3. Plasma NT-proBNP levels do not differ in clinically healthy puppies and adult dogs of the same breed. 4. Phonocardiography can differentiate innocent from pathologic cardiac murmurs in asymptomatic puppies.

Materials and Methods

The study was approved by the department's research committee (AVR-16-4).

Animals

A total of 186 client-owned dogs were involved in the study, consisting of 135 clinically healthy Cairn Terrier puppies (median age 52 days [range 45–124 days]), 20 clinically healthy adult Cairn Terriers (median age 7.1 years [range 7.5 months to 13.5 years]), and 31 clinically healthy young dogs of various breeds (median age 119 days [range 29–396 days]).

The breeders of the Cairn Terrier puppies participated in a screening program for congenital portosystemic shunts, and brought their litters to the clinic for individual blood ammonia measurement between September 2014 and September 2015. The asymptomatic adult Cairn Terriers belonged to 3 breeders, who voluntarily offered their dogs for blood sampling, so that breed-specific reference ranges for NT-proBNP could be established. The referred dogs were sent to the cardiology service for evaluation of a murmur; dogs only under 18 months of age were enrolled between September 2014 and December 2015. Only 1 dog was symptomatic with occasional exertional syncope. All owners signed an informed consent. In the group of congenital cardiac anomaly, 31 dogs were included (19 males and 12 females), with a median age of 119 days (range 29–396 days). With echocardiography, the following congenital cardiac anomalies were found: 4 patent ductus arteriosus (PDA), 1 mild pulmonic stenosis (PS), 1 moderate PS, 9 severe PS, 4 mild aortic stenosis (AS), 2 moderate AS, 3 severe AS, 2 small ventricular septal defect (VSD), 3 mitral valve regurgitation caused by dysplasia (2 mild and 1 severe, based on the left atrial and left ventricular internal diastolic diameters), 1 double-chambered right ventricle (DCRV) with severe stenosis and 1 mild dynamic left ventricular outflow tract obstruction (DLVOTO) caused by systolic anterior motion of the septal mitral valve (SAM). Dogs with multiple congenital cardiac anomalies were classified according to the most severe condition. The following breeds were represented in this population: 3 Boxers, 3 French Bulldogs, 3 Rottweilers, 2 English Bulldogs, 2 Golden Retrievers, 2 Yorkshire Terriers, 2 mixed breeds, and 1 of each of the following breeds Boerboel, Border Collie, Cane Corso, Doberman Pinscher, Dutch Schapendoes, English Cocker Spaniel, Flat-Coated Retriever, Jack Russell Terrier, Labrador Retriever, Pit Bull Terrier, Spanish Water Dog, Shi Tzu, Stabyhoun, Shetland Sheepdog, and Swiss Shepherd.

Blood Test

A single experienced veterinary technician (HvE) took blood samples from the Cairn Terriers with jugular venipuncture. About 2 mL of blood was collected in an EDTA tube and was transferred immediately to the clinic's laboratory. After centrifugation, plasma of 155 dogs (ie, all samples of the Cairn Terrier pups and the samples of the referred dogs collected before September 2015) was separated and stored on −70 degrees of Celsius. These samples were shipped at once on dry ice with an overnight courier from Utrecht to a single laboratory in Germany[a] , where all the NT-proBNP measurements took place. The remaining plasma samples were shipped on the day of sampling by an overnight courier to the same laboratory. All the 20 adult Cairn Terriers were sampled on a single day. These 20 Cairn Terriers were selected based on history and physical examination. Inclusion criteria were as follows: clinically healthy animals, the absence of present or previous signs of cardiac diseases, and no abnormalities with physical examination (included cardiac and thoracic auscultation).

Plasma concentrations of NT-proBNP were determined by a species-specific and analytically validated ELISA assay.[b,15] The NT-proBNP levels below 102 pmol/L could not be specified; therefore, these data (n = 3) were set on 102 pmol/L for the statistical analysis.

Auscultation

A single investigator (VSz, ECVIM-CA cardiology specialist) performed the cardiac auscultation on all dogs with a nonelectronic pediatric stethoscope. Each Cairn Terrier puppy was auscultated within an hour after blood sampling. The puppies were placed one by one on a table in a quiet examination room, were identified by chip number, and were auscultated in a standing position. The regions of the heart base and apex were auscultated on the left hemithorax and right hemithorax. If a murmur was detected, the following variables were noted: point of maximal intensity, intensity (scale 1–6), timing (systolic, diastolic, or continuous), and additional characteristics, such as musical character and beat-to-beat variability in murmur intensity.[13] A murmur was defined as intermittent, if a soft (1 or 2 of 6) murmur was heard, which disappeared whereas the auscultation was still performed on the same location, or when the murmur was not audible on each heartbeat.[2]

Phonocardiography

On every dog with an audible murmur, a phonocardiogram was recorded at the point of maximal intensity with an electronic stethoscope[c] by the same investigator, immediately after auscultation. Intermittently audible murmurs were not recorded.

The phonocardiograms were viewed and analyzed on a computer screen by the software of the stethoscope[d]. The display was set on "Digital Filter In Diaphragm Mode", and the speed rate was 370 mm/s (Fig 1). Cardiac cycles with clearly distinguishable murmurs were analyzed. The shape, duration, timing, and amplitude of the murmurs were analyzed by a single investigator (SM). The duration of the systole and the duration of the murmur were measured manually on the computer screen in 3 cardiac cycles per phonocardiogram (Fig 1). The 3 cardiac cycles were chosen based on the clearest visibility of the murmur with as little base line artifacts as possible. If the murmurs of all cardiac cycles were clearly recognizable, 3 of them were randomly chosen. To be able to describe the murmurs uniformly, 2 ratios were calculated from the measured amplitude and duration values: (1) a ratio between the amplitude of the first heart sound (S1) and that of the murmur, and (2) a ratio between the duration of the systole and that of the murmur. The duration of the systole was defined from the beginning of the S1 to the beginning of S2 (Fig 1).[13]

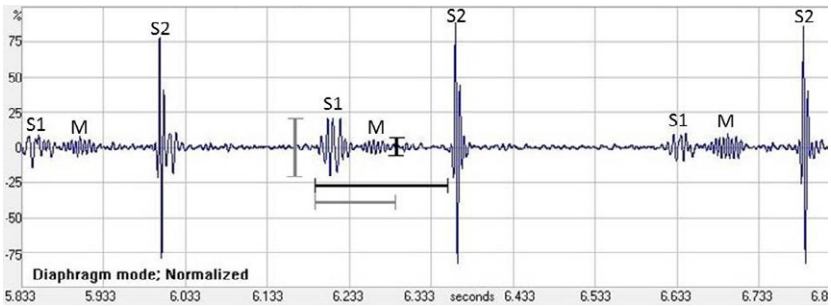

Fig 1. Phonocardiogram of a 2-month-old Cairn Terrier with an innocent murmur recorded by a Littmann 4100WS Electronic Stethoscope. The murmur (M) has a low amplitude (black vertical line) compared with the first heart sound (S1, gray vertical line). The duration of the murmur (M, gray horizontal line) is shorter than 80% of the duration of the systole (black horizontal line). Note the beat-to-beat variation in the S1 amplitude. Duration of the systole: time from the beginning of S1 to the beginning of S2. Speed rate: 370 mm/s.

Echocardiography

Echocardiography was carried out on each dog with a murmur by the same person who performed the auscultation (VSz), with a high-definition ultrasound machine equipped with a 3- to 8-MHz phased-array transducer[e] , within an hour after auscultation. The unsedated Cairn Terrier puppies were held in right lateral recumbency with manual restraint only. Standard 2-dimensional right parasternal long-axis and short-axis views with 2-dimensional, color, and continuous-wave Doppler modes were obtained.[16] A subcostal view was used to measure the peak aortic blood flow velocity. Blood flow velocities in the pulmonic artery and in the aorta below 2.0 m/s were considered to be physiologic, whereas velocities above 2.0 m/s were considered to be the cause of a pathologic murmur.[2]

The puppies that were referred to the cardiology service for the investigation of a murmur had a full echocardiographic examination including right-sided parasternal transthoracic standard views, subcostal view, and left parasternal transthoracic views from the cranial and caudal windows.[16]

Based on the auscultation and the echocardiographic findings, the puppies were classified into 1 of the following groups: no murmur, innocent murmur, and congenital cardiac anomaly. The group of innocent murmurs consisted of puppies with a cardiac murmur and normal echocardiographic findings as well as puppies in which auscultation findings were characteristic for an innocent murmur (ie, systolic murmur with a musical character and a maximal intensity of 2 of 6),[2] but echocardiography detected mild anomalies, such as a trivial mitral or aortic valve regurgitation, which were thought to be unlikely to cause a systolic murmur either because of the timing (diastolic aortic valve regurgitation) or because of their small size (trivial mitral valve regurgitation). Mild tricuspid and mild pulmonic artery valve regurgitations were considered to be physiologic.[2,17,18] The severity of congenital stenotic disorders (ie, AS, PS, DCRV, dynamic left ventricular outflow tract obstruction) was determined by the Doppler-derived pressure gradient as mild (below 40 mmHg), moderate (between 40 and 80 mmHg), and severe (higher than 80 mmHg).[19] The severity of congenital anomalies with left ventricular volume overload (ie, PDA, VSD, or mitral regurgitation due to mitral valve dysplasia) was quantified based on the left ventricular internal diameter in diastole normalized to body weight, calculated as: $LVIDd/BW^{0.294}$ (LVIDd = left ventricular internal diameter in diastole, in cm; BW = body weight, in kg).[20]

Statistics

A commercially available software package[f] was used for data analysis. The Shapiro–Wilk test and Box-Cox transformation were used to test normal distribution of age, NT-proBNP levels, and the phonocardiographic variables. Differences in plasma NT-proBNP levels and phonocardiographic variables were assessed with the Mann–Whitney *U*-test.

Reference intervals (RI) were defined as central 95% intervals bounded by the 2.5th and 97.5th percentiles. The upper and lower limits of the RI with their 90% confidence intervals (CI) were determined.[21] The CIs of the limits of the nonparametric RI were determined by a bootstrap method. Outliers were identified by the Tukey method.

Results

Murmur Etiology

Of the 135 Cairn Terrier puppies, 45 had a cardiac murmur (33%) and 90 did not. Of the 45 Cairn Terrier puppies with a murmur, 42 dogs had an innocent murmur, which is 31% of the total population. The remaining 3 Cairn Terrier puppies had a congenital cardiac anomaly, which is 2% of the population. Of the 31 young dogs of various breeds that were referred to the cardiology service for evaluation of a murmur, 3 dogs had an innocent murmur and 28 had a congenital cardiac anomaly.

In summary, a congenital anomaly was found as the cause of a cardiac murmur in 31 dogs. Innocent cardiac murmurs were identified in 45 puppies (25 males and 20 females): 42 Cairn Terriers, 2 Yorkshire Terriers, and 1 Border Collie. Their median age was 52 days (range 46–94 days). In the group of healthy puppies without a murmur, 90 Cairn Terrier puppies were included (50 males and 40 females), with a median age of 52 days (range 45–59 days).

Phonocardiography

Innocent Murmurs. Of the 45 puppies with an innocent cardiac murmur, 38 phonocardiograms were available for analysis. No phonocardiogram could be recorded in 4 dogs with intermittent murmurs, and the recorded phonocardiograms of 3 other dogs were lost and unavailable for further analysis. Murmurs were recognizable on the phonocardiograms of 30 of the 38 dogs with innocent murmurs.

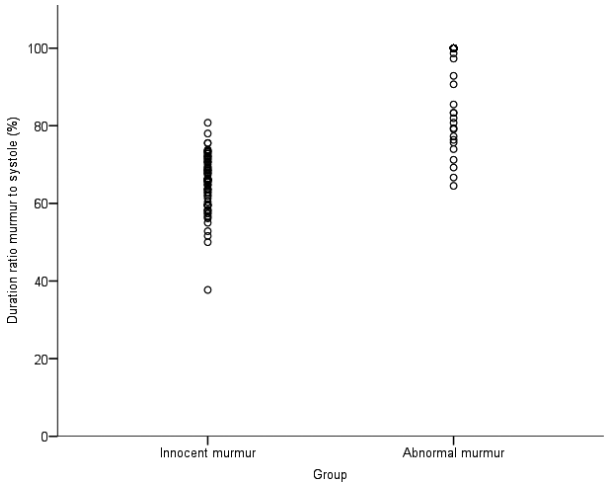

Fig 2. Comparison of the duration of innocent murmurs and murmurs caused by congenital cardiac anomalies on phonocardiograms. A ratio was calculated by dividing the duration of the murmur by the duration of the systole. Innocent murmurs are significantly shorter than murmurs caused by congenital cardiac anomalies.

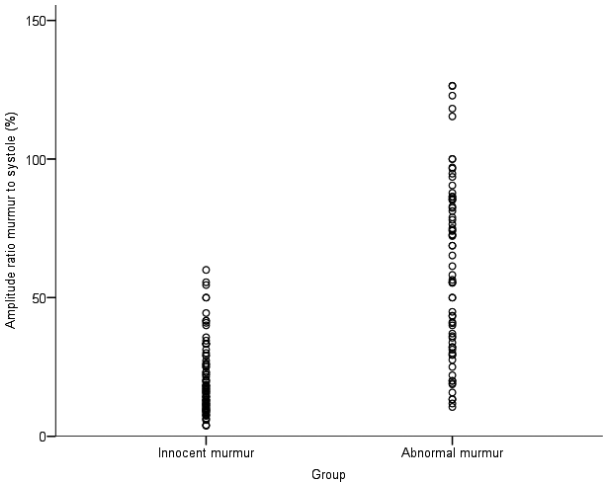

Fig 3. Comparison of the amplitude of innocent murmurs and murmurs caused by congenital cardiac anomalies on phonocardiograms. A ratio was calculated by dividing the amplitude of the murmur by the amplitude of the first heart sound (S1). Innocent murmurs have significantly lower amplitude than murmurs caused by congenital cardiac anomalies.

The shape of the murmur in 25 of these 30 dogs was crescendo-decrescendo (Fig 1), whereas the remaining 5 was plateau-shaped.

The duration of the systole and that of the murmur were measured on 90 heart cycles of the 30 phonocardiograms. The median duration of the systole was 154 (range 122–197) milliseconds and the median duration of the innocent murmurs was 101 (range 62–134) milliseconds. The median murmur-to-systole duration ratio was 66% (range 38–81%) (Fig 2). The calculated RI of the murmur-to-systole duration ratio for innocent murmur was between 51% (90% CI: 48–54%) and 82% (90% CI: 79–85%).

The median amplitude of the first heart sound (S1) was 2.25 (range 0.60–5.30) centimeter and the median maximum amplitude of the murmur was 0.30 (range 0.15–1.00) centimeter of the 90 analyzed cardiac cycles of the 30 puppies with an innocent murmur. The median murmur-to-S1 amplitude ratio of the innocent murmurs was 16% (range 4–60%) (Fig 3). The calculated RI of the murmur-to-S1 amplitude ratio for innocent murmur was between 4% (90% CI: 3.8–6.2%) and 55% (90% CI: 44–60%).

Congenital Anomalies. Of the 31 dogs with a congenital cardiac anomaly, 1 recording (of a severe PS) was lost. Therefore, the murmurs of 90 cardiac cycles of 30 individual phonocardiograms were analyzed. In 71 cardiac cycles of 23 individual phonocardiograms, the S1 and S2 were indistinguishable because of a pansystolic murmur. The systole-to-murmur ratio in these cases was 100%.[13] In the remaining 19 cardiac cycles of 7 phonocardiograms, the median duration of the systole was 176 milliseconds (range 149–211) and the median duration of the murmur was 141 milliseconds (range 108–200). The median ratio between the duration of systole and the duration of murmur caused by congenital cardiac anomalies was 100% (range 64.5–100%) (Fig 2). Of the murmurs of the 90 analyzed heart cycles, 12 murmurs (belonging to 5 dogs) were shorter than 82% of the systole. Of the aforementioned 5 dogs 3 murmurs of each were analyzed, and in 3 of the dogs all 3 murmurs were shorter than 82% of systole (range 64.5–81.9%). The echocardiographic diagnosis in these 5 dogs was: mild AS in 2 dogs, VSD in 1 dog, and mild mitral valve regurgitation because of mitral valve dysplasia in 2 dogs.

The median amplitude of S1 was 3.40 (range 1.00–5.80) centimeter and the median amplitude of the murmur was 1.60 (range 0.20–4.80) centimeter in the 69 cardiac cycles of 23 of the 30 dogs with a congenital cardiac anomaly, in which the amplitude was measurable. The median murmur-to-S1 amplitude ratio was 58% (range 11–126%) (Fig 3).

The median duration ratios of the innocent murmurs were significantly smaller than those of congenital cardiac anomalies ($P < .001$). The mean amplitude ratios of innocent murmurs were significantly smaller than those of congenital cardiac anomalies ($P < .001$).

Plasma NT-proBNP Concentrations

A total of 184 plasma samples were analyzed (166 young and 20 adult dogs), as 2 samples got lost (both of a Cairn Terrier puppy, 1 without and 1 with an innocent murmur).

Only the NT-proBNP levels of the adult Cairn Terriers were normally distributed; therefore, nonparametric tests were used.

The median NT-proBNP concentration of the Cairn Terrier puppies without a murmur was 300 pmol/L (range 102–1,224), which did not differ significantly ($P = .41$) from the median NT-proBNP level of the puppies with an innocent murmur 326 pmol/L (range

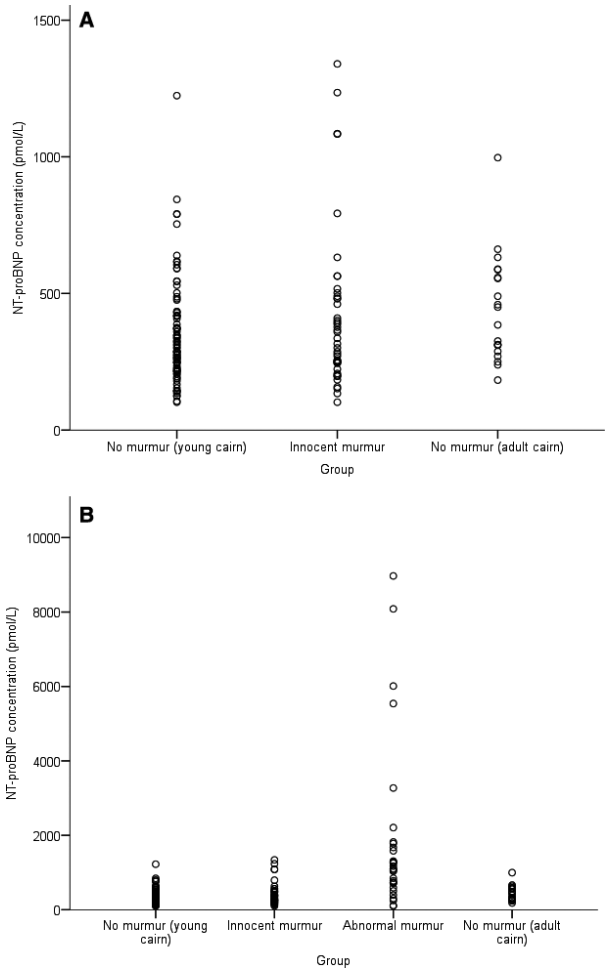

Fig 4. Plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations of Cairn Terrier puppies without a murmur, puppies with an innocent murmur, and adult Cairn Terriers without a cardiac murmur do not differ significantly (**A**). However, NT-proBNP levels of puppies with a congenital cardiac anomaly are significantly higher (**B**).

102–1,340) (Fig 4). The median NT-proBNP concentration of the group with a congenital cardiac anomaly was 1,102 pmol/L (range 102–8,970), which differed significantly from the NT-proBNP concentration of Cairn Terrier puppies with or without a murmur (both $P < .001$) (Figs 4–6). Although the median NT-proBNP concentration of the 10 dogs with severe PS was similar (1,063 pmol/L, range 115–1,778), only 2 of the 10 dogs had an NT-proBNP level that exceeded the highest value measured in the puppies with innocent murmurs (1,340 pmol/L). The mean NT-proBNP concentration of the adult Cairn Terriers was 443 pmol/L (range 183–997), which did not differ significantly ($P = .22$) from the concentrations of the Cairn Terrier puppies without a murmur (Fig 4).

No effect of sex on the NT-proBNP levels was found in the 89 healthy Cairn Terrier puppies without a murmur ($P = .88$) and in the 44 puppies with an innocent murmur ($P = .21$).

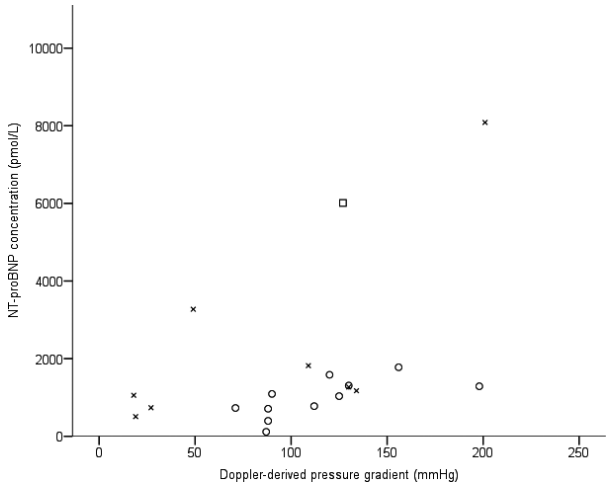

Fig 5. Association between plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels and the severity of congenital stenotic anomalies. The severity of the stenosis is indicated by the Doppler-derived pressure gradient (mmHg). The plasma NT-proBNP level of a dog with a severe double-chambered right ventricle (□) was very high; this dog had exertional syncope. Dogs with pulmonic stenosis (○) have relatively low plasma NT-proBNP concentrations, even with a severe stenosis. Plasma NT-proBNP levels seem to reflect the severity of aortic stenosis (x) more closely.

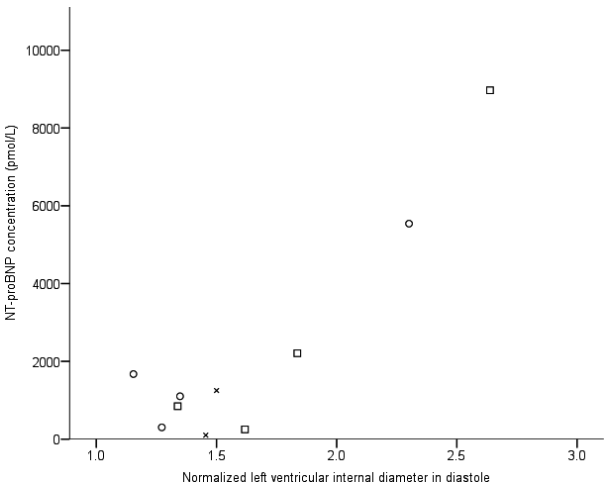

Fig 6. Association between plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels and the severity of congenital cardiac anomalies that lead to left ventricular volume overload (patent ductus arteriosus [□], ventricular septal defect [x], and mitral regurgitation caused by mitral valve dysplasia [○]). In the *x*-axis, the left ventricular internal diameter normalized to body weight is displayed (upper level of reference interval: 1.8). More severe disease, reflected by a larger normalized left ventricular value, results in higher plasma NT-proBNP concentrations.

Calculated NT-proBNP RI for the Cairn Terrier breed was between 120 pmol/L (90% CI: 102–141) and 806 pmol/L (90% CI: 662–997) from the values of the young and adult Cairn Terriers without murmur.

Discussion

The present study showed no difference in NT-proBNP plasma levels of clinically healthy puppies and adult dogs of the same breed. This is similar to another report, however, that study included only dogs between 10 months and 8 years.[22]

The present study identified no sex difference in plasma NT-proBNP levels of Cairn Terrier puppies, in contrast to another study, where significantly higher plasma NT-proBNP levels were found in female dogs.[22] Sexual maturity might have affected these concentrations, as the dogs investigated in that study were adults.[22]

As NT-proBNP levels show significant breed-related differences,[23] the present results can provide NT-proBNP RIs for Cairn Terriers.

As expected, NT-proBNP concentrations of puppies with an innocent cardiac murmur did not differ from those without a murmur.

Plasma NT-proBNP levels of asymptomatic dogs with congenital cardiac anomalies varied from normal to severely increased, depending on the type of disorder and its severity. The low number of dogs per condition did not allow to perform meaningful statistical analysis though. Several dogs with severe congenital PS had NT-proBNP plasma levels within the reference range, similarly to an earlier report.[11] Studies in humans and dogs have shown that left-sided cardiac anomalies and disorders causing volume overload lead to higher plasma NT-proBNP levels than right-sided anomalies and conditions causing pressure overload.[10,11,24,25] Although the number of dogs with PDA was very low in our study, NT-proBNP levels ranged from low to high. The extremely high value was measured in a puppy with cardiogenic pulmonary edema. Although the owner did not notice any symptoms of this puppy, physical examination revealed tachypnea and tachycardia, and thoracic radiographs showed pulmonary edema. The dog with a PDA and low NT-proBNP level had a small PDA, which did not cause left atrial and left ventricular dilatation and the intensity of the continuous murmur was only 2 of 6. Pediatric cardiologists do not advice closing hemodynamically irrelevant PDAs based on NT-proBNP levels, in contrast to their veterinary counterparts.[10,24,26,27] In dogs with PDA, NT-proBNP levels might be used for establishing an indication for PDA closure, and not only for the urgency of the closure as recommended by a previous study.[10]

We conclude that plasma NT-proBNP measurement cannot replace careful characterization of a murmur with auscultation in asymptomatic puppies. Furthermore, interpretation of NT-proBNP levels can be challenging because of large differences between breeds.[22,23]

Innocent murmurs were shorter and of lower amplitude than pathologic murmurs on phonocardiograms. This is similar to the findings of a recent report, where innocent murmurs comprised <75% of the systole[g] . This value is close to our cutoff value (82%), despite the very different population of the 2 studies (puppies versus adult sled dogs). Although phonocardiograms can be analyzed with advanced techniques,[28] our purpose was to look for a simple tool for practicing veterinarians. Phonocardiogram could facilitate differentiation between innocent and pathologic murmurs; however, phonocardiography has a number of limitations. The quality of the recorded phonocardiograms is operator dependent.[14] A good quality phonocardiogram can only be recorded at the point of maximal intensity once the murmur has already been recognized. Moreover, shaking, panting, and moving of the dog might cause so much base line artifacts that the phonocardiogram cannot be interpreted.

Another limitation of the study was that not all dogs (ie, dogs without murmur) underwent an echocardiographic examination. The investigator who performed the auscultation was the same one who performed the echocardiography, so bias cannot be excluded.

We conclude that plasma NT-proBNP is not a suitable screening test for diagnosing congenital cardiac anomalies in asymptomatic puppies with a murmur. However, it might be useful for evaluating the hemodynamic significance of left-to-right shunting congenital cardiac anomalies (such as PDA) similarly to humans.[24–27] Further research is however necessary to investigate whether it is also the case in dogs.

If the duration of murmur is longer than 80% of that of the systole on a phonocardiogram, or the amplitude of the murmur is higher than 60% of that of the S1, the murmur is most likely pathologic, whereas murmurs that are shorter than 80% of systole might be innocent or pathologic.

Footnotes

[a] Vet Med Labor GmbH, division of IDEXX Laboratories, Ludwigsburg, Germany

[b] Cardiopet®, IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME

[c] 3M™ Littmann® Electronic Stethoscope Model 4100WS, 3M Health Care, MN, USA

[d] 3M™ Littmann® Sound Analysis Software Version 2.0.c. for Heart Sounds, 3M Health Care

[e] Philips HD 11 XE, Bothell, WA

[f] IBM Statistics SPSS 22.0, IBM Corp., Chicago, IL

[g] Kvart C. Cardiac murmurs in normal Siberian husky dogs. Poster presentation abstract, congress of the European College of Veterinary Internal Medicine—Companion Animals; Mainz, Germany; September 4–6, 2014

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Côté E, Edwards NJ, Ettinger SJ, et al. Management of incidentally detected heart murmurs in dogs and cats. J Vet Cardiol 2015;17:245–261.

2. Szatmári V, van Leeuwen MW, Teske E. Innocent cardiac murmur in puppies: Prevalence, correlation with hematocrit, and auscultation characteristics. J Vet Intern Med 2015;29:1524–1528.

3. Abbott J. Auscultation: What type of practice makes perfect? J Vet Intern Med 2001;15:505–506.

4. Naylor JM, Yadernuk LM, Pharr JW, Ashburner JS. An assessment of the ability of diplomats, practitioners, and students to describe and interpret recordings of heart murmurs and arrhythmia. J Vet Intern Med 2001;15:507–515.

5. Dennis S. Sound advice for heart murmurs. J Small Anim Pract 2013;54:443–444.

6. Fonfara S. Listen to the sound: What is normal? J Small Anim Pract 2015;56:75–76.

7. Boswood A, Dukes-McEwan J, Loureiro J, et al. The diagnostic accuracy of different natriuretic peptides in the investigation of canine cardiac disease. J Small Anim Pract 2008;49:26–32.

8. Ettinger SJ, Farace G, Forney SD, et al. Evaluation of plasma N-terminal pro-B-type natriuretic peptide concentrations in dogs with and without cardiac disease. J Am Vet Med Assoc 2012;240:171–180.

9. Oyama M, Boswood A, Connolly DJ, et al. Clinical usefulness of an assay for measurement of circulating N-terminal pro-B-type natriuretic peptide concentration in dogs and cats with heart disease. J Am Vet Med Assoc 2013;243:71–82.

10. Hariu C, Saunders A, Gordon S, et al. Utility of N-terminal pro-brain natriuretic peptide for assessing hemodynamic significance of patent ductus arteriosus in dogs undergoing ductal repair. J Vet Cardiol 2013;15:197–204.

11. Kobayashi K, Hori Y, Chimura S. Plasma N-terminal pro B-type natriuretic peptide concentrations in dogs with pulmonic stenosis. J Vet Med Sci 2014;76:827–831.

12. Aramaki Y, Chimura S, Hori Y, Eguchi T. Therapeutic changes of plasma N-terminal pro-brain natriuretic peptide concentrations in 9 dogs with patent ductus arteriosus. J Vet Med Sci 2011;73:83–88.

13. Kvart C, Häggström J. Cardiac Auscultation and Phonocardiography in Dogs, Horses and Cats. TK i Uppsala AB, Uppsala, Sweden: 2002:13–70.

14. Vörös K, Bonnevie A, Reiczigel J. Comparison of conventional and sensor-based electronic stethoscopes in detecting cardiac murmurs of dogs. Tierarztl Prax Ausg K Kleintiere Heimtiere 2012;40:103–111.

15. Cahill RJ, Pigeon K, Strong-Townsend MI, et al. Analytical validation of a second-generation immunoassay for the quantification of N-terminal pro-B-type natriuretic peptide in canine blood. J Vet Diagn Invest 2015;27:61–67.

16. Thomas WP, Gaber CE, Jacobs GJ, et al. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. J Vet Intern Med 1993;7:247–252.

17. Rishniw M, Hollis NB. Prevalence and characterization of pulmonary regurgitation in normal adult dogs. J Vet Cardiol 2000;2:17–21.

18. Adin DB, McCloy K. Physiologic valve regurgitation in normal cats. J Vet Cardiol 2005;7:9–13.

19. Kienle RD, Thomas WP, Pion PD. The natural clinical history of canine congenital subaortic stenosis. J Vet Intern Med 1994;8:423–431.

20. Cornell CC, Kittleson MD, Della Torre P, et al. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 2004;18:311–321.

21. Geffré A. Reference Value Advisor: A new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Pathol 2011;40:107–112.

22. Misbach C, Chetboul V, Concordet D, et al. Basal plasma concentrations of N-terminal pro-B-type natriuretic peptide in clinically healthy adult small size dogs: Effect of body weight, age, gender and breed, and reference intervals. Res Vet Sci 2013;95:879–885.

23. Sjöstrand K, Wess G, Ljungvall I, et al. Breed differences in natriuretic peptides in healthy dogs. J Vet Intern Med 2014;28:451–457.

24. Kunii T, Kamada M, Ohtsuki S, et al. Plasma brain natriuretic peptide and the evaluation of volume overload in infants and children with congenital heart disease. Acta Med Okayama 2003;57:191–197.

25. Cantinotti M, Walters HL III, Crocetti M, et al. BNP in children with congenital cardiac disease: Is there now sufficient evidence for its routine use? Cardiol Young 2015;25:424–437.

26. Farombi-Oghuvbu I, Matthews T, Mayne PD, et al. N-terminal pro-B-type natriuretic peptide: A measure of significant patent ductus arteriosus. Arch Dis Child Fetal Neonatal Ed 2008;93:F257–F260.

27. Cantinotti M, Giovannini S, Murzi B, Clerico A. Diagnostic, prognostic and therapeutic relevance of B-type natriuretic peptide assay in children with congenital heart diseases. Clin Chem Lab Med 2011;49:567–580.

28. Höglund K, Ahlstrom CH, Häggström J, et al. Time-frequency and complexity analyses for differentiation of physiologic murmurs from heart murmurs caused by aortic stenosis in Boxers. Am J Vet Res 2007;68:962–969.