Original Article

Evaluation of Serum NT-proBNP Levels in Dogs with Respiratory Distress Associated with Heart disease and Respiratory Disease

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Abstract

The association between severity of heart disease and serum N-terminal prohormone B-type natriuretic peptide (NT-proBNP) was determined in dogs presented with respiratory distress. Four groups of dogs were divided into healthy control (n=11), heart failure (n=58), heart disease without heart failure (n=17) and respiratory disease (n=20). Serum NT-proBNP concentrations were measured with an ELISA designed for using in dogs. *Dirofilaria* antigen tests were detected in dogs with heart disease or heart failure. Serum NT-proBNP concentrations of dogs with heart failure (2,977±184 pmol/l) were significantly higher than dogs with heart disease (611±46 pmol/l), respiratory disease (583±52 pmol/l) or physically healthy (426±32 pmol/l). In dogs with heart failure, the serum NT-proBNP concentrations from the female dogs (3,291±266 pmol/l) were slightly higher than the male dogs (2,770±247 pmol/l), but did not reach the statistical significant. *Dirofilaria* infections were also found in dogs with heart disease (n=9) and dogs with heart failure (n=7). Interestingly, NT-proBNP concentration from *Dirofilaria* infected dogs with heart disease (533±53 pmol/l) was significantly lower than *Dirofilaria* infected dogs with heart failure (2,576±504 pmol/l). Different cut-off values (at 300, 600 and 900 pmol/l) were used to discriminate the dogs with heart failure from other groups, high cut off values slightly reduced percent sensitivity (98.3%, 93.1% and 93.1%, respectively) while significantly improved percent specificity (7.7%, 59.0% and 92.3%, respectively). In conclusion, the serum NT-proBNP levels were comparable in dogs with and without heart diseases except including the heart failure dogs. Furthermore, gender and *Dirofilaria* infection did not influence in the serum NT-proBNP concentration. The use of higher cut-off values for serum NT-proBNP significantly improves specificity of the test for the diagnosis of congestive heart failure in dogs with respiratory distress.

Keywords: Dog, heart failure, heartworm, NT-proBNP, respiratory disease

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บทคัดย่อ

การประเมินระดับซีรั่มเอ็นที-โปรบีเอ็นพีในสุนัขที่มีปัญหาหายใจลำบากเนื่องจากปัญหาโรคหัวใจล้มเหลว ปัญหาโรคระบบทางเดินหายใจ และโรคพยาธิหนอนหัวใจ

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การศึกษาความสัมพันธ์ระหว่างความรุนแรงของโรคหัวใจและระดับซีรั่มเอ็นที-โปรบีเอ็นพี จัดทำในสุนัขที่แสดงอาการหายใจลำบาก ทำการจัดข้อมูลสุนัขแบ่งออกเป็น 4 กลุ่ม โดยเป็นกลุ่มควบคุมซึ่งมีสุขภาพปกติ (n=11) กลุ่มภาวะหัวใจล้มเหลว (n=58) กลุ่มโรคหัวใจแต่ไม่เกิดหัวใจล้มเหลว (n=17) และกลุ่มโรคระบบทางเดินหายใจที่ไม่เกี่ยวข้องกับโรคหัวใจ (n=20) ทำการวัดระดับความเข้มข้นซีรั่มเอ็นที-โปรบีเอ็นพีโดยใช้ชุดตรวจสอบอีไลซ่าที่ออกแบบสำหรับสุนัข การตรวจแอนติเจนของพยาธิหนอนหัวใจทำในสุนัขโรคหัวใจและโรคหัวใจล้มเหลว พบระดับซีรั่มเอ็นที-โปรบีเอ็นพีในสุนัขหัวใจล้มเหลว (2,977±184 pmol/l) สูงกว่ากลุ่มสุนัขโรคหัวใจแต่ไม่เกิดหัวใจล้มเหลว (611±46 pmol/l) กลุ่มปัญหาโรคทางเดินหายใจ (583±52 pmol/l) หรือกลุ่มสุขภาพปกติ (426±32 pmol/l) กลุ่มสุนัขหัวใจล้มเหลวพบว่าระดับซีรั่มเอ็นที-โปรบีเอ็นพีจากสุนัขเพศเมีย (3,291±266 pmol/l) สูงกว่าสุนัขเพศผู้ (2,770±247 pmol/l) เล็กน้อยโดยไม่แตกต่างอย่างมีนัยสำคัญทางสถิติ พบสุนัขมีพยาธิหนอนหัวใจในกลุ่มภาวะหัวใจล้มเหลว (n=9) และกลุ่มโรคหัวใจแต่ไม่เกิดหัวใจล้มเหลว (n=7) เป็นที่น่าสนใจที่ระดับเอ็นที-โปรบีเอ็นพีในสุนัขมีพยาธิหนอนหัวใจแต่ไม่เกิดหัวใจล้มเหลว (533±53 pmol/l) มีระดับต่ำกว่าในสุนัขที่มีพยาธิหนอนหัวใจร่วมกับโรคหัวใจล้มเหลว (2,576±504 pmol/l) เมื่อทำการเปลี่ยนระดับค่าตัดสิน (ระดับ 300, 600 และ 900 pmol/l) ที่ใช้แยกระหว่างสุนัขหัวใจล้มเหลวจากกลุ่มอื่นๆ พบว่า การเพิ่มค่าตัดสินสูงขึ้นส่งผลลดค่าความไวของการทดสอบเพียงเล็กน้อย (ร้อยละ 98.3, 93.1 และ 93.1 ตามลำดับ) ขณะที่เพิ่มค่าความจำเพาะอย่างมีนัยสำคัญ (ร้อยละ 7.7, 59.0 และ 92.3 ตามลำดับ) โดยสรุปผลการศึกษาในครั้งนี้บ่งชี้ว่าการประเมินระดับซีรั่มเอ็นที-โปรบีเอ็นพีไม่แตกต่างระหว่างสุนัขโรคหัวใจและสุนัขระบบทางเดินหายใจยกเว้นกรณีภาวะหัวใจล้มเหลว นอกจากนี้พบว่าเพศและการติดเชื้อพยาธิหนอนหัวใจไม่ส่งผลต่อระดับซีรั่มเอ็นที-โปรบีเอ็นพี การเลือกใช้ค่าตัดสินที่สูงขึ้นในการประเมินระดับซีรั่มเอ็นที-โปรบีเอ็นพี ช่วยเพิ่มความจำเพาะของการตรวจวินิจฉัยภาวะหัวใจล้มเหลวในสุนัขที่มีปัญหาหายใจลำบาก

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Introduction

Tachypnea, dyspnea and exercise intolerance are symptoms that commonly identified in dogs with either respiratory problems (Hawkins, 2003) or congestive heart failure (Ware, 2003). However, increased respiratory rate and effort may be manifestations of other causes including dirofilariasis, anemia, metabolic acidosis, hyperthermia and pain (Mowby, 2005; Muir et al., 2007). Diagnosis approaches to identify the cause of presenting dyspnea can be done by interpretation of the overall physical examination, blood examination, urinalysis, radiography, electrocardiography and echocardiography (Kittleson and Kienle, 1998).

Recent development for detection of NT-proBNP (N-ternimal pro-B-type natriuretic peptide) has facilitate the diagnosis of dyspneic patient that caused by heart failure in human (Wright et al., 2003; Clerico and Emdin, 2005) as well as in canine patients (DeFrancesco et al., 2007; Prosek et al., 2007; Boswood et al., 2008). Heartworm disease is a common mosquito-borne disease worldwide. Recent report indicated a high prevalence of heartworm infections in stray dogs (13.9%) in Thailand (Jittapalapong et al., 2005). The residence of the mature heartworms are in right atrium and ventricle and in pulmonary arteries leading to a dilatation of the right ventricle (Miller, 1997). An increase in right ventricular wall tension by heartworm (Miller, 1997) may also influence the release of circulating natriuretic peptide levels, however, the effects of *Dirofilaria* infection on the serum levels of NT-proBNP have not been studied.

NT-proBNP has been proved to be a useful maker for diagnosis of heart disease and failure in human medicine for more than a decade (Morita et al., 1993; Epiner, 1997; Koglin et al., 2001). Recent development of canine NT-proBNP assay stimulates more studies that support the usefulness of NT-proBNP in assessment of heart disease in dogs (DeFrancesco et al., 2007; Boswood et al., 2008; Piantedosi et al., 2009). Nevertheless, amount of water and salt retention in dogs with heart disease may be affected by the severity of heart disease (Masson et al., 2008) and the effects of sex hormonal status (Redfield et al., 2002), thus inevitably in turn influencing the level of serum NT-proBNP. In the present study, the serum NT-proBNP concentrations were investigated in dogs with heart diseases with and without clinical signs of heart failure, dogs with respiratory diseases and healthy dogs. The levels of serum NT-proBNP were compared between dogs with different genders. Furthermore, the influences of *Dirofilaria* infection on serum NT-proBNP levels were compared between dogs with and without clinical signs of heart failure.

Materials and Methods

A survey was performed in 106 dogs that were more than 8 years of age visiting heart clinic at Kasetsart University, Veterinary Teaching hospital, Bang Khen, Bangkok 10900 between June 2008 and July 2009. The dogs with presenting signs of dyspnea, tachypnia or exercise intolerance were included in the study. After physical examination, all dogs underwent further diagnostic tests including chest radiography, electrocardiography and echocardiography. Dogs were divided into 4 groups including control (healthy), heart disease with congestive heart failure, heart disease without heart failure and respiratory disease groups (Table 1). *Dirofilaria* antigens were detected by IDEXX SNAP3-DX (DKSH (Thailand) Ltd., Bangkok).

Table 1 The Number of dogs administered in the present study.

Classification		Group 1 Control	Group 2 Heart failure	Group 3 Heart disease without HF	Group 4 Respiratory disease
N		11	58	17	20
Age	1-5 years	9	8	2	3
	> 5 years	2	50	15	17
Gender	Male	6	35	12	10
	Female	5	23	5	10
Breed	Small (<12 kg)	1	17	6	15
	Medium (12-24 kg)	10	33	7	1
	Large (>25 kg)	0	8	4	4
Heartworm infection		0	7	9	0

Of the 106 dogs, 11 were physically healthy (Group 1), 58 were determined to have heart disease with heart failure (Group 2), 17 had heart disease without clinical signs of congestive heart failure (Group 3), and 20 had respiratory disease (Group 4). The 58 dogs in heart failure group all had either current signs or on medicine controlled signs of right- or left-sided congestive heart failure and 7 were also positive to heartworm disease. Of the 17 dogs with heart disease without clinical signs of congestive heart failure, 6 had endocardiosis, 3 had cardiac masses, 1 had cardiac arrhythmia, 1 was hypertension and total of 9 were positive to heartworm disease. Of the 20 dogs with respiratory problem without cardiac disease, 4 had upper respiratory tract diseases (brachycephalic syndrome, tracheal collapse and tracheitis), 6 had lower respiratory tract disease unrelated to heart disease (pneumonia, pleural effusion) and 10 were overweight dogs with increased respiratory effort due to a narrowing of the upper airway. All control dogs were physically healthy without signs of respiratory and heart diseases and no abnormalities were detected on radiographic and echocardiographic studies.

Blood samples were taken from the dogs as a routine venipuncture for standard diagnostic procedures. One milliliter of blood was placed into a tube contain potassium-EDTA, an anticoagulant, and three milliliter was placed into a tube containing no anticoagulant. Samples were centrifuged and the supernatant separated from the cells. The serum was kept at -20^{o}C until the analysis was processed.

The serum concentration of NT-proBNP was measured by enzyme-linked immunoassays kit (Canine Cardio-SCREEN, Guildhay Ltd., England) designed to measure the NT-proBNP in canine plasma sample. Serum concentrations of NT-proBNP were expressed as mean$\pm$S.E. and data were analyzed using one way analysis of variance (SPSS). The difference between two groups was compared using a Tukey's HSD test. A *p* value <0.05 was considered significant in the present study.

Results

Average serum concentrations of NT-proBNP in control dogs were 426±32 pmol/l (Figure 1). Serum NT-proBNP concentrations in heart failure dogs (2,977±184 pmol/l) were significantly higher than control group (426±32 pmol/l) (Figure 1). Interestingly, an average serum concentrations of NT-proBNP were slightly increased in dogs with heart disease without failure (611±46 pmol/l) or in dogs with respiratory disease (583±52 pmol/l) compared to healthy dogs (426±32 pmol/l), however, it did not reach the statistical significance (Figure 1).

The gender difference may lead to a different serum concentration of NT-proBNP. In the present study, the serum NT-proBNP concentrations of the control dogs were not significantly different between male (380±39 pmol/l) and female (482±44 pmol/l) dogs (Figure 2). In heart failure groups, the NT-proBNP concentrations of female dogs (3,291±266) were slightly higher than in male dogs (2,770±247), but did not reach the statistical significant (Figure 2). In heart disease without failure group, the serum concentrations of NT-proBNP were comparable between male (592±46 pmol/l) and female (564±54 pmol/l) dogs (Figure 2).

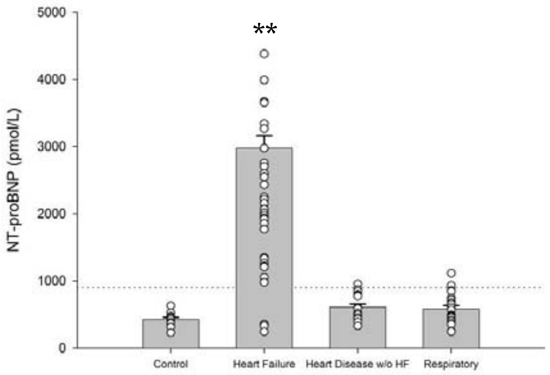

Figure 1 The serum concentration of NT-proBNP in dogs according to disease group. Open circle indicates individual NT-proBNP level. **p<0.01 compared to control.

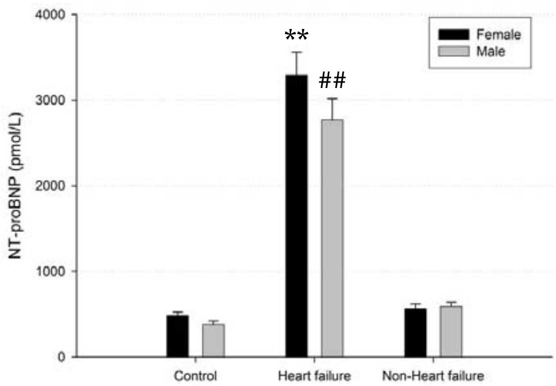

Figure 2 Average NT-proBNP concentration in dogs according to disease group compared between genders. **p<0.01 compared to control (female), ##p<0.01 compared to control (male).

The possibility that heartworm infection may cause an enlargement of right ventricular chamber leading to an elevation of serum NT-proBNP was investigated in the present study. *Dirofilaria* affected dogs had a wide range of serum NT-proBNP concentration (330-4,378 pmol/l). Classification of *Dirofilaria* affected dogs into heart disease with and without heart failure revealed that serum NT-proBNP concentrations from the affected dogs with heart failure (2,576±504 pmol/l) were significantly higher than those without heart failure (533±53 pmol/l) (Figure 3). Moreover, the NT-proBNP concentrations in *Dirofilaria* affected dogs with heart disease were comparable to that of the control group (Figures 1 and 3).

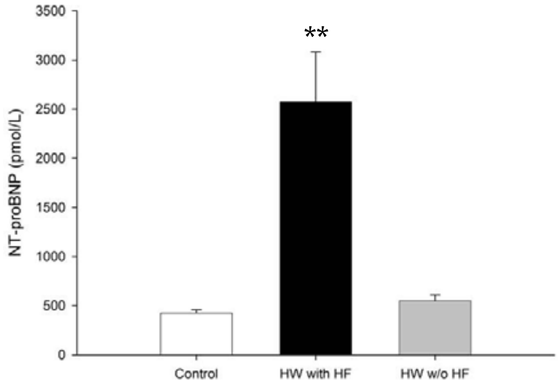

Figure 3 Average NT-proBNP concentration in *Dirofilaria* affected dogs with and without heart failure. **p<0.01 compared to control.

In the present study, we identified an optimum cut-off value for a prediction of heart failure in dogs using serum NT-proBNP levels by varying a cut-off value at 300, 600 and 900 pmol/l, respectively. We found that the sensitivity was slightly decreased with the higher cut off value (98.3%, 93.1% and 93.1%, respectively), while the specificity was increased substantially with the higher cut off value (7.7%, 59.0%, and 92.3%, respectively) (Table 2).

Table 2 Sensitivity and specificity for NT-proBNP concentrations with different cut off values.

Peptide	Cut-off values (pmol/l)	Sensitivity (%)	Specificity (%)
NT-proBNP	300	98.3	7.7
NT-proBNP	600	93.1	59.0
NT-proBNP	900	93.1	92.3

Discussion

In the present study, the serum NT-proBNP concentration measured by ELISA can differentiate accurately between dogs with heart failure and those with respiratory disease or heart disease without failure. The serum concentrations of NT-proBNP were not affected by gender difference. Heartworm infected dogs had a wide range concentrations of NT-proBNP, however, only the dogs with heart failure had elevated serum NT-proBNP concentrations. Furthermore, the use of higher cut off values than that of the recommended value (300 pmol/l) substantially improved specificity of the test while minimally affected the sensitivity.

The compensatory mechanisms in congestive heart failure patients lead to the activation of sympathetic (norepinephrine) and rennin-angiotensin-aldosterone system (Piantedosi et al., 2009) causing a constriction of distal arterioles and induce water and salt retention. The body compensates for the excess water and salt retention by releasing various natriuretic peptides. Atrial natriuretic peptide (ANP) is released from the cardiac atrium when there is an increase in wall tension of upper cardiac chambers, in response to high blood pressure (Boswood et al., 2008; Widmaier et al., 2008).

B-type natriuretic peptide (BNP) is mainly released from atrial and ventricular cardiomyocytes (Collinson, 2008), interestingly recent study indicated a released of BNP from cardiac fibroblasts (Tsuruda et al., 2002). C-type natriuretic peptide (CNP) is expressed in the vascular tissue and central nervous system (Collinson, 2008). Both ANP and BNP play an important role under physiological and pathological conditions (Epiner, 1997); however CNP does not play a significant role in water and salt regulation (Collinson, 2008). The increased concentrations of ANP and BNP were linked to various causes including myocardial infarction, cardiac hypertrophy and heart failure (Morita et al., 1993; Nishikimi et al., 1996; Koglin et al., 2001). Both ANP and BNP are used in human medicine as cardiac biomarkers and are routinely used for predicting the severity of heart failure (Piantedosi et al., 2009), however, the specific assays for canine ANP and BNP are not currently available.

Pro-BNP, a precursor of BNP, released in the blood stream is divided to NT-proBNP and BNP (Collinson, 2008). BNP has a short half life and is removed quickly by endopeptidase and also shares the same 17 amino acids that found in ANP and CNP (Collinson, 2008). NT-proBNP can be used as an indicator for the ventricular problems because it has a long half life and distinct amino acids compared to other natriuretic peptides (Collinson, 2008). In dogs, the half life of canine BNP is shorter than human BNP (90 seconds in dogs vs. 22 minutes in human), suggesting NT-proBNP measurement a preferable assay for veterinary practice (Boswood et al., 2008).

Clinical manifestations of dogs with heart and respiratory diseases are quite similar. The rapid, accurate diagnosis of heart failure facilitates an appropriate treatment especially during a critical period. Development of various tests for cardiac biomarkers was significantly improved in both human and veterinary medicines. In the present study, we tested the usefulness of ELISA assay for canine NT-proBNP. The serum NT-proBNP concentrations were elevated in dogs with heart failure compared to unaffected dogs or dogs with respiratory disease. Interestingly, dogs with heart disease without failure had comparable serum NT-proBNP concentration to unaffected dogs and dogs with respiratory disease. These results suggest that elevated NT-proBNP concentration help confirm the diagnosis of heart failure; nevertheless, the unelevated NT-proBNP concentration does not exclude the possible involvement of cardiac diseases. Thus, the combination of tests including laboratory studies, thoracic radiography, electrocardiography and echocardiography remains a reliable mean for detection of heart disease.

Dirofilariasis is quite commonly diagnosed in Thailand (Jittapalapong et al., 2005). Heartworms can be found in both right atrium and ventricle and pulmonary arteries. The influence of *Dirofilaria* infection on serum concentration of NT-proBNP is unknown. In the present study, a wide range of serum NT-proBNP concentration was found in *Dirofilaria* affected dogs with and without heart failure. Serum NT-proBNP concentrations were elevated in *Dirofilaria* affected dogs with heart failure but were not elevated in *Dirofilaria* affected dogs without heart failure. These results suggest that the ELISA assay for detection of serum NT-proBNP is not affected by *Dirofilaria* infection.

A study evaluated 46 dogs with cough or respiratory distress and determined that the median NT-proBNP concentration from dogs with congestive heart failure (median, 2,554 pmol/l, interquartile range, 1,652-3,476) had significantly higher than those with respiratory disease (median, 357 pmol/l, interquartile range, 193-566) (Fine et al., 2008). These results suggested the clinical usefulness of NT-proBNP to determine the underlying cause of respiratory signs in dogs. Moreover, in a study of 115 dogs with moderate to severe respiratory signs (i.e. cough, wheeze, dyspnea, etc.), serum NT-proBNP above 1,200 pmol/l had a positive predictive value of 85.5% and a negative predictive value of 81.3% (Oyama et al., 2009) for discriminating dogs with congestive heart failure from those with clinical signs from primary respiratory disease. Interestingly, the manufacturer of the canine NT-proBNP assay (VetSign Canine CardioScreen, Guildhay) suggested a lower cut-off values of 210 pmol/l and 300 pmol/l with 95% and 99% probability to detect heart disease in dogs, respectively. In the present study, using a cut off value of 300 pmol/l, leads to a sensitivity of 98.3% and a specificity of 7.7% for differentiating dogs with heart failure from other groups, which cannot be employed as a standard for clinical usage. Elevating the cut-off values to 600 and 900 pmol/l slightly decreased the sensitivity to 93.1% for both values, while improving the specificity to 59.0% and 92.3%, respectively (Table 2). Our study is in agreement with previous study suggesting a need for higher cut-off value for clinical usage of NT-proBNP assay (Oyama et al., 2009; Takemura et al., 2009; Tarnow et al., 2009).

The cardiac troponin T and I (cTnT and cTnI) have been identified to have excellent sensitivity and specificity and are superior to creatine kinase-MB (CK-MB) as indicators of myocardial necrosis in human medicine (Maynard et al., 2000). Nevertheless, cTnT and cTnI are not specific to the underlying cause of myocardial damage. Systemic diseases including end-stage renal failure, sepsis and trauma can lead to elevations of both cTnT and cTnI (Reynolds and Oyama, 2008). The lack of specificity of both cTnT and cTnI make them unlikely to be useful as the stand-alone screening tests for heart disease. The serum concentrations of NT-proBNP are sometimes affected by systemic illnesses. NT-proBNP in dogs with severe pulmonary hypertension or renal azotemia can be falsely elevated and this may compromise the analysis of the test results (Reynolds and Oyama, 2008; Raffan et al., 2009). Based on the currently available research, it is suggested that patients with respiratory signs, an NT-proBNP>1,200 pmol/l is likely involved with congestive heart failure.

In conclusion, the present study revealed that detections of NT-proBNP concentrations were distinctively different between dogs with heart failure and that with respiratory disease. Elevated serum NT-proBNP levels specifically suggest the present of heart failure, however, the unelevated serum NT-proBNP concentration does not rule out the possibility of cardiac diseases in dogs. Furthermore, our study suggests a use of higher cut-off value for clinical application of NT-proBNP.

Conflict of Interest Statement: None of the authors of this paper has a financial relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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References

Boswood, A., Dukes-McEwan, J., Loureiro, J., James, R.A., Martin, M., Stafford-Johnson, M., Smith, P., Little, C. and Attree, S. 2008. The diagnosis accuracy of different natriuretic peptides in the investigation of canine cardiac disease. J. Small Anim. Pract. 49: 26-32.

Clerico, A. and Emdin, M. 2005. Clinical relevance of BNP/NT-proBNP assay in heart failure. Business Briefing: Emergency Medicine Review. 49-51.

Collinson, P.O. 2008. NT-proBNP Biology: How is It Made, What is Circulating and What Exactly Are We Measuring? In: NT-proBNP as a biomarker in Cardiovascular Diseases. A. Bayes-Genis and J.L. Januzzi (eds.). Barcelona: Prous Science. 3-10.

DeFrancesco, T.C., Rush, J.E., Rozanski, E.A., Hansen, B.D., Keene, B.W., Moore, D.T. and Atkins, C.E. 2007. Prospective clinical evaluation of an ELISA B-type natriuretic peptide assay in the diagnosis of congestive heart failure in dogs presenting with cough or dyspnea. J. Vet. Intern. Med. 21: 243-250.

Epiner, E.A. 1997. Physiology of natriuretic peptides: cardiovascular function. In: Natriuretic Peptides in Health and Disease. W.K. Samson and E.R. Levin (eds.). Totowa: Humana Press. 123-146.

Fine, D.M., Declue, A.E. and Reinero, C.R. 2008. Evaluation of circulating amino terminal-pro-B-type natriuretic peptide concentration in dogs with respiratory distress attributable to congestive heart failure or primary pulmonary disease. J. Am. Vet. Med. Assoc. 232(11): 1674-1679.

Hawkins, E.C. 2003. Clinical Manifestrations of Lower Respiratory Tract Disorders. In: Small Animal Internal Medicine 3rd edition. R.W. Nelson and C.G. Couto (eds.). St. Louis: Mosby. 250-254.

Jittapalapong, S., Pinyopanuwat, N., Chimnoi, W., Nimsupan, B., Saengow, S., Simking, P. and Kaewmongkol, G. 2005. Prevalence of heartworm infection of stray dogs and cats in Bangkok metropolitan areas. Proceedings of 43th Kasetsart University Annual Conference: Veterinary Medicine. 97-105.

Kittleson, M.D. 1998. Signalment, history, and physical examination. In: Small Animal Cardiovascular Medicine. M.D. Kittleson and R.D. Kienle (eds). St. Louis: Mosby. 36-46.

Koglin, J., Pehlivanli, S., Schwaiblmair, M., Vogeser, M., Cremer, P. and vonScheidt, W. 2001. Role of brain natriuretic peptide in risk stratification of patients with congestive heart failure. J. Am. Coll. Cardiol. 38(7): 1934-1941.

Masson, S. Latini, R. and Salio, M. 2008. NT-proBNP and Prognosis in Chronic Heart Failure. In: NT-proBNP as a biomarker in Cardiovascular Diseases. A. Bayes-Genis and J.L. Januzzi (eds). Barcelona: Prous Science. 75-81.

Maynard, S.J., Menown, I.B.A. and Adgey, A.A.J. 2000. Troponin T or troponin I as cardiac markers in ischaemic heart disease. Heart. 83: 371-373.

Miller, M.W. 1997. Heartworm disease. In: Handbook of Small Animal Practice 3rd ed. R.V. Morgan (ed). Philadelphia: W.B. Saunders com. 123-132.

Morita, E., Yasue, H., Yoshimura, M., Ogawa, H., Jougasaki, M., Matsumura, T., Mukoyama, M. and Nakao, K. 1993. Increased plasma levels of brain natriuretic peptide in patients with acute myocardial infarction. Circulation. 88(1): 82-91.

Mowby, D.M. 2005. Dyspnea and Tachypnea. In: Textbook of Veterinary Internal Medicine 6th ed. S.J. Ettinger and E.C. Feldman (eds) St. Louis: Elsever Saunders. 192-195.

Muir, W.W., Hubbell, J.A.E., Bednarski, R.M. and Skarda, R.T. 2007. Patient Monitoring during Anesthesia. In: Handbook of Veterinary Anesthesia 4th ed. St. Louis: Mosby. 269-303.

Nishikimi, T., Yoshihara, F., Morimoto, A., Ishikawa, K., Ishimitsu, T., Saito, Y., Kangawa, K., Matsuo, H., Omae, T. and Matsuoka, H. 1996. Relationship between left ventricular geometry and natriuretic peptide levels in essential hypertension. Hypertension. 28(1): 22-30.

Oyama, M.A., Rush, J.E., Rozanski, E.A., Fox, P.R., Reynolds, C.A., Gordon, S.G., Bulmer, B.J., Lefbom, B.K., Brown, B.A., Lehmkuhl, L.B., Prosek, R., Lesser, M.B., Kraus, M.S., Bossbaly, M.J., Rapoport, G.S. and Boileau, J.S. 2008. Assessment of serum N-terminal pro-B-type natriuretic peptide concentration for differentiation of congestive heart failure from primary respiratory tract disease as the cause of respiratory signs in dogs. J. Am. Vet. Med. Assoc. 235(11): 1319-1325.

Piantedosi, D., Cortese, L., Di Loria, A., Brignola, G., Prisco, M., Persechino, A. and Ciaramella, P. 2009. Plasma Atrial Natriuretic Peptide (proANP 31-67), B-type Natriuretic Peptide (Nt-proBNP) and endothelin-1 (ET1) concentrations in dogs with chronic degenerative valvular disease (CDVD). Vet. Res. Comm. 33(suppl. 1):

S197-S200.

Prosek, R., Sisson, D.D., Oyama, M.A. and Solter, P.F. 2007. Distinguishing cardiac and noncardiac dyspnea in 48 dogs using plasma atrial natriuretic factor, B-type natriuretic factor, endothelin and cardiac troponin-I. J. Vet. Intern. Med. 21: 238-242.

Raffan, E., Loureiro, J., Dukes-McEwan, J., Fonfara, S., James, R., Swift, S., Bexfield, N., Herrtage, M.E. and Archer, J. 2009. The cardiac biomarker NT-proBNP is increased in dogs with azotemia. J. Vet. Intern. Med. 23(6): 1184-1189.

Redfield, M.M., Rodeheffer, R.J., Jacobsen, S.J., Mahoney, D.W., Bailey, K.R. and Burnett Jr, J.C. 2002. Plasma brain natriuretic peptide concentration: Impact of age and gender. J. Am. Coll. Cardiol. 40: 976-982.

Reynolds, C. and Oyama, M. 2008. Biomarkers in the diagnosis of canine heart disease. Vet. Focus. 18(3): 1-6.

Takemura, N., Toda, N., Miyagawa, Y., Asano, K., Tejima, K., Kanno, N., Arisawa, K., Kurita, T., Nunokawa, K., Hirakawa, A., Tanaka, S. and Hirose, H. 2009. Evaluation of plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations in dogs with mitral valve insufficiency. J. Vet. Med. Sci. 71(7): 925-929.

Tarnow, I., Olsen, L.H., Kvart, C., Hoglund, K., Moesgaard, S.G., Kamstrup, T.S., Pedersen, H.D. and Häggström, J. 2009. Predictive value of natriuretic peptides in dogs with mitral valve disease. Vet. J. 180(2): 195-201.

Tsuruda, T., Boerrigter, G., Huntley, B.K., Noser, J.A., Cataliotti, A., Costello-Boerrigter, L.C., Chen, H.H. and Burnett, J.C.Jr. 2002. Brain natriuretic peptide is produced in cardiac fibroblasts and induces matrix metalloproteinases. Circ. Res. 91(12): 1127-1134.

Ware ,W.A. 2003. The cardiovascular examination. In: Small Animal Internal Medicine 3rd ed. R.W. Nelson and C.G. Couto (eds). St. Louis: Mosby. 1-11.

Widmaier, E.P., Raff, H. and Strang, K.T. 2008. Vander's Human Physiology 11th ed. New York: McGraw-Hill. 291, 509–510.

Wright, S.P., Doughty, R.N., Pearl, A., Gamble, G.D., Whalley, G.A., Walsh, H.J., Gordon, G., Bagg, W., Oxenham, H., Yandle, T., Richards, M. and Sharpe, N. 2003. Plasma amino-terminal pro-brain natriuretic peptide and accuracy of heart-failure diagnosis in primary care: A randomized, controlled trial. J. Am. Coll. Cardiol. 42: 1793-1800.