

University of Prince Edward Island

Faculty of Veterinary Medicine

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DEGREE OF MASTER OF SCIENCE

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Clinical utility of exercise testing and high-sensitivity cardiac troponin I in early diagnosis of arrhythmogenic right ventricular cardiomyopathy in apparently healthy Boxer dogs

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a primary myocardial disease recognized in dogs and humans. It is characterized by fibrofatty replacement of right ventricular myocardium, and less frequently, of the interventricular septum, left ventricular and atrial myocardium. Boxer dogs have a genetic predisposition for ARVC. Family history of the disease, documentation of ventricular arrhythmias (premature ventricular complexes, PVCs), history of syncope, or exercise intolerance are important clinical data to guide the diagnosis in affected dogs. In dogs with a lack of clinical signs (apparently healthy dogs), the electrocardiographic finding of ventricular arrhythmias is a major means of suspecting a diagnosis of ARVC antemortem. Of concern, however, is that the earliest latent stage of ARVC is characterized by normal electrocardiographic findings with undetectable structural heart disease but a risk for sudden cardiac death. The identification of this subclinical population of Boxer dogs having latent disease is challenging but important for prognosis and monitoring recommendations and in selection of breeding animals. Therefore, testing that can unmask ventricular arrhythmias or increase blood levels of biomarkers of myocardial injury in subclinical Boxer dogs may be beneficial in guiding monitoring and treatment recommendations. In the present study, we 1) investigated the effects of exercise testing (ExT) on the occurrence of ventricular arrhythmias in apparently healthy Boxer dogs; 2) performed an analytical and clinical comparison between point-of-care (POC) cardiac troponin I (cTnI) and high-sensitivity cTnI (hs-cTnI) assays; and 3) investigated whether an association could be demonstrated between ventricular arrhythmias, cTnI concentration ([cTnI]), and genetic mutation status in these Boxer dogs. One hundred and fifty-eight client-owned Boxer dogs were screened to select a population of apparently healthy dogs. Of these, 30 dogs completed the study.

This study showed that the effects of ExT on number and complexity of ventricular arrhythmias are inconsistent in apparently healthy Boxer dogs. However, in many dogs, PVCs were clustered around exercising (median, 33.3% of total daily PVCs occurring in the peri-exercise period). The results of this study demonstrated that the two cTnl assays cannot be used interchangeably, the hs-cTnl assay may be a more sensitive and specific test than the POC assay, and [cTnl] measured by either assay does not differ in dogs with and without the striatin mutation that has been associated with ARVC in some Boxer dogs. Furthermore, the results of this study suggest that the exercise-associated increase in [cTnl] and number of PVCs may be a manifestation of increased propensity to the development of the ARVC phenotype in Boxer dogs. Dogs' recent exercise history should be considered when measuring [cTnl] and when quantitating PVCs via 24 h at-home electrocardiogram (Holter monitor). Long-term follow up of these Boxer dogs may provide further evidence in support of our hypotheses and overall conclusion, which could be a focus for future studies.

Publications

Peer-reviewed Publications (in chronological order)

1. LeBlanc NL, Agarwal D, Menzen E, Nomi K, Sisson DD, Scollan KF. Prevalence of major complications and procedural mortality in 336 dogs undergoing interventional cardiology procedures in a single academic center. *Journal of Veterinary Cardiology* 2019;23:45-57.
2. Ranganathan B, LeBlanc NL, Scollan KF, Townsend K, Agarwal D and Milovancev M. Comparison of short-term complications and rate of survival to hospital discharge between surgical ligation and an amplatz canine ductal occluder for treatment of left to right patent ductus arteriosus in dogs – a retrospective cohort study. *Journal of American Veterinary Medical Association* 2018;253(8):1046-52.
3. Dange RB, Agarwal D, Teruyama R, Francis J. Toll-like receptor 4 inhibition within the paraventricular nucleus

attenuates blood pressure and inflammatory response in a genetic model of hypertension. *Journal of Neuroinflammation* 2015;18:12:31.

4. Dange RB, Agarwal D, Masson GS, Vila J, Wilson B, Nair A, Francis J. Central blockade of TLR4 improves cardiac function and attenuates myocardial inflammation in angiotensin II-induced hypertension. *Cardiovascular Research* 2014;103(1):17-27. (featured in editorial, *Cardiovascular Research* 2014;103(1):3-4)
5. Agarwal D, Dange RB, Raizada MK, Francis J. Angiotensin II causes imbalance between pro- and anti-inflammatory cytokines by modulating GSK3 β in rat neuronal cells. *British Journal of Pharmacology* 2013;169(4):860-74.
6. Shenoy V, Gjymishka A, Jarajapu YP, Qi Y, Afzal A, Rigatto K, Ferreira AJ, Fraga-Silva RA, Kearns P, Douglas JY, Agarwal D, Mubarak KK, Bradford C, Kennedy WR, Jun JY, Rathinasabapathy A, Bruce E, Gupta D, Cardounel AJ, Mocco J, Patel JM, Francis J, Grant MB, Katovich MJ, Raizada MK. Diminazene attenuates pulmonary hypertension and improves angiogenic progenitor cell functions in experimental models. *American Journal of Respiratory and Critical Care Medicine* 2013;187(6):648-57. (featured in editorial, *American Journal of Respiratory and Critical Care Medicine* 2013;187(6):569-71)
7. Agarwal D, Dange RB, Vila J, Otamendi AJ, Francis J. Detraining differentially preserved beneficial effects of exercise on hypertension: effects on blood pressure, cardiac function, brain inflammatory cytokines and oxidative stress. *PLoS ONE* 2012;7(12): e52569. doi:10.1371/journal.pone.0052569.
8. Agarwal D, Elks CM, Reed SD, Mariappan N, Majid DS, Francis J. Chronic exercise preserves renal structure and hemodynamics in spontaneously hypertensive rats. *Antioxidants and Redox Signaling* 2012;16(2):139-52.
9. Cardinale JP, Sriramula S, Mariappan N, Agarwal D, Francis J. Angiotensin II-induced hypertension is modulated by nuclear

factor-kappa β in the paraventricular nucleus. Hypertension 2012;59(1):113-21.

10. Agarwal D, Welsch MA, Keller JN, Francis J. Chronic exercise modulates RAS components and improves balance between pro-and anti-inflammatory cytokines in the brain of SHR. Basic Research in Cardiology 2011;106(6):1069-85.

11. Agarwal D, Haque M, Sriramula S, Mariappan N, Pariaut R, Francis J. Role of proinflammatory cytokines and redox homeostasis in exercise-induced delayed progression of hypertension in spontaneously hypertensive rats. Hypertension. 2009 Dec;54(6):1393-400. (featured in editorial, Hypertension 2009: 54(6):1206-8)

Manuscripts in review

1. Côté E, O'Sullivan ML, Agarwal D, Santilli R. Left atrial tachycardia and third-degree atrioventricular block in a dog. CASE: Cardiovascular Imaging Case Reports. In review.

2. Domenegato BM, Côté E, Agarwal D, O'Sullivan ML, Reveler ED, Dobbin E, McMahan E. Pseudohyperkalemia due to in vitro clotting of feline blood samples collected in plain tubes 2 Journal of the American Veterinary Medical Association. In review.

3. Agarwal D, Côté E, Lakhdhir S, Reveler ED, O'Sullivan ML. Common Atrium or Atrioventricular Septal Defect: What is in a Name? CASE: Cardiovascular Imaging Case Reports. In review.

Presentations

1. Agarwal D, Côté E, O'Sullivan ML, Reveler ED, Steiner JM. High-Sensitivity and Point-of-Care Cardiac Troponin I in Apparently Healthy Boxers with and without Ventricular Arrhythmia. Research abstract-oral presentation. ACVIM forum 2021.

2. Dange RB, Agarwal D and Francis J. Inhibition of TLR4 within the PVN attenuates hypertension and inflammatory signaling cascade in spontaneously hypertensive rats. American College of Veterinary Pathologists Meeting 2014, Atlanta, GA. Poster presentation.

3. Agarwal D, Dange RB, and Francis J. Role of central GSK-3 β in mediating exercise-induced improvement in inflammatory cytokines and reduction in blood pressure in hypertensive rats. High Blood Pressure Research 2013 Scientific Sessions, New Orleans, LA. Poster presentation.

4. Agarwal D, Cardinale J, Sriramula S, Dange RB, Nair AR, Francis J. Glycogen synthase kinase-3 β inhibition in the PVN attenuates hypertensive response: role of inflammation. High Blood Pressure Research 2013 Scientific Sessions, New Orleans, LA. Poster presentation.

5. Masson GS, Nair AR, White ME, Agarwal D, Michelini LC, and Francis J. Angiotensin II induced ER stress in the heart is attenuated by exercise. High Blood Pressure Research 2013 Scientific Sessions, New Orleans, LA. Poster presentation.

6. Dange RB, Agarwal D, Wilson B, and Francis J. Central blockade of TLR4 improves cardiac function and attenuates pro-inflammatory cytokines and oxidative stress in hypertensive rats. Phi-Zeta Research Emphasis Day 2012, Louisiana State University. Poster presentation.

7. Otamendi AJ, Agarwal D, Dange RB, Vila J, and Francis J. Moderate intensity exercise training attenuates angiotensin II-induced hypertension by modulating MAPK-I κ B-NF κ B Pathway. Annual Meeting of the American College of Veterinary Pathologists 2012, Nashville, Tennessee. Podium presentation.

8. Islam MT, Agarwal D, Francis J, and Majid DS. Superoxide production in macrophage cell in response to increases in sodium concentration in the culture media is enhanced by nitric oxide synthase inhibition. Hypertension 2011;58(5):e33-e183. Poster presentation.

9. Islam MT, Agarwal D, Francis J, and Majid DS. Production of tumor necrosis factor- α induced by nitric oxide inhibition in macrophage cell is dependent on superoxide production by NADPH oxidase. *Hypertension* 2011;58(5):e33-e183. Poster presentation.
10. Islam MT, Agarwal D, Francis J, and Majid DS. Increase in salt concentration in the culture media induces redox imbalance in macrophage cells. *Experimental Biology Meeting 2011, Washington DC. FASEB Journal* 2011;25:643.27. Poster presentation.
11. Islam MT, Agarwal D, Francis J, and Majid DS. Inhibition of nitric oxide synthase enhances the production of tumor necrosis factor- α in macrophage cells. *Experimental Biology Meeting 2011, Washington DC. FASEB Journal* 2011;25:1030.7. Poster presentation.
12. Whiting C, Castillo A, Agarwal D, Francis J, and Majid DS. Treatment with tumor necrosis factor- α blocker, etanercept, attenuates the Angiotensin II induced hypertensive response in eNOS knockout mice. *Experimental Biology Meeting 2011, Washington DC. FASEB Journal* 2011;25:640.112. Poster presentation.
13. Agarwal D and Francis J. Chronic exercise training reduces blood pressure by modulating central RAS and improving balance between pro-and anti-inflammatory cytokines in SHR Rats. *The 23Scientific Meeting of International Society of Hypertension (ISH) 2010, Vancouver, Canada. Podium Presentation.*
14. Agarwal D, Raizada MK, and Francis J. Angiotensin II causes imbalance between pro- and anti- inflammatory cytokines by modulating GSK3 β in rat neuronal cells. *Hypertension* 2010;56(5):e153. Poster presentation.
15. Agarwal D, Pelych L, Cardinale J, Raizada MK, and Francis J. Exercise training activates GSK3 and increases CREB-mediated gene expression in the paraventricular nucleus of angiotensin-II- induced hypertensive rats. *Hypertension* 2010;56(5):e152. Poster presentation.
16. Mariappan N, Agarwal D, Raizada MK, Changaram VS, and Francis J. Phosphorylation of glycogen synthase kinase 3 β (GSK3 β) contributes to the development of pulmonary hypertension. *Hypertension* 2010;56(5):e111. Poster presentation.
17. Mariappan N, Elks CM, Agarwal D, Haque M, and Francis J. Exercise training improves renal function in obese Zucker rats. *Hypertension* 2010;56(5):e98. Poster presentation.
18. Agarwal D, Elks CM, Haque M, and Francis J. Chronic exercise training improves renal function by modulating RAS components and decreasing pro-inflammatory cytokines in the kidney of young SHR rats. *Hypertension* 2009;54:e26-e127;P254. Poster presentation.
19. Cardinale J, Sriramula S, Agarwal D, Pariat R, and Francis J. Nuclear factor kappa-B in the paraventricular nucleus contributes to angiotensin-induced high blood pressure in rats. *Hypertension* 2009;54:e26-e127;019. Podium presentation.
20. Agarwal D, Haque M, Sriramula S, Mariappan M, and Francis J. Pressure lowering effect of chronic exercise mediated by reduced myocardial pro-inflammatory cytokines and oxidative stress in hypertensive rats. *FASEB Journal* 2009;23:1017.9. Poster presentation.
21. Agarwal D, Haque M, and Francis J. Tumor necrosis factor and angiotensin II interaction modulates class I HDAC expression in neuronal cell culture. *FASEB Journal* 2009;23:805.17. Poster presentation.
22. Agarwal D, Haque M, Sriramula S, Mariappan N, and Francis J. Chronic exercise training reduces proinflammatory cytokines and oxidative stress in young spontaneously hypertensive rats. *Phi-Zeta Research Emphasis Day 2008, Louisiana State University. Poster presentation.*

23. Agarwal D, Kirubaharan JK, and Srinivasan SR. Plasma concentration of proANP 31-67 in relation to severity of heart failure in dogs. International conference 2006 held at Madras Veterinary College, India, jointly conducted by Madras Veterinary College and Michigan State University. Poster presentation.

24. Agarwal D, Ayyappan S, Prathaban S. Nephrotic syndrome and hepatitis associated with leptospirosis in a dog – a case report. National symposium 2006 (XXIV Annual Convention of Indian society for Veterinary Medicine), Bangalore, India. Podium Presentation.

Awards

2020 3 Minute Thesis Competition (First prize and People's Choice Award), UPEI

2019 Alice Peake Residency in Companion Animal Scholarship, UPEI

2019 Class of 2006 Mentorship Award, University of Prince Edward Island

2014 Best Poster Presentation Award, Annual Meeting of the American College of Veterinary Pathologists, Atlanta, GA

2014 Best Poster Presentation Award at the Phi Zeta Research Emphasis Day, Louisiana State University, Baton Rouge, LA

2012 Best Poster Presentation Award, Phi Zeta Research Emphasis Day, Louisiana State University, Baton Rouge, LA

2011 Best Poster Presentation Award, Annual Meeting of the American College of Veterinary Pathologists, Nashville, TN

2011-2012 Graduate School Dissertation Fellowship, Louisiana State University, Baton Rouge, LA

2007 Young Scientist Award, VIII Annual Convention & National Symposium of Indian Association of Lady Veterinarians, India

2006 Gold Medal for the best master's student, Madras Veterinary College, India

2006 Field Veterinarian Best Presentation Award, XXIV Annual Convention of Indian Society for Veterinary Medicine, India

2004-2006 Merit scholarship (Pattukkottai Azhagiri Endowment Award), Madras Veterinary College, India

2004 Academic excellence award in Veterinary Pathology, Veterinary Science and Animal Husbandry, India

2001-2004 Merit scholarship, College of Veterinary Science and Animal Husbandry, India