# Prediction of unexpected sudden death among healthy dogs by a novel marker of autonomic neural activity

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**BACKGROUND** Unexpected sudden death among apparently healthy individuals remains a daunting problem. We have previously shown that autonomic modulation of cardiac arrhythmias and autonomic markers, such as baroreflex sensitivity (BRS) and heart rate variability (HRV), carry predictive power after myocardial infarction.

**OBJECTIVE** We tested the hypothesis that a parameter combining BRS and HRV could predict risk for ventricular fibrillation (VF) during a first ischemic episode in otherwise healthy dogs.

**METHODS** In 43 fully instrumented dogs, BRS and frequency domain analysis of HRV were determined, as well as the occurrence (n = 10, high-risk) or absence (n = 33, low-risk) of VF during 2 minutes of myocardial ischemia superimposed on submaximal exercise. TARVA (Tonic and Reflex Vagal Activity), expressed in units, is the parameter resulting from the multiplication of BRS by HF/LF (an index of tonic vagal activity).

**RESULTS** High-risk dogs had markedly lower TARVA values, reflecting lower cardiac vagal activity, than low-risk animals (12 ± 5 versus 56 ± 43 units, $P < .001$). The area under the receiver-operator characteristic curve for TARVA was 0.96 (95% confidence interval 0.86 to 0.99); its optimal cutoff had a 100% sensitivity and a 88% specificity with positive and negative predictive values of 71% and 100%, respectively.

**CONCLUSION** Differences in cardiac autonomic activity, present in healthy dogs, allow prediction of arrhythmic risk during a first ischemic episode. Increased risk is associated with reduced vagal activity. If confirmed in humans, this finding would open the way to the identification of those apparently healthy subjects at risk for sudden cardiac death during their first episode of myocardial ischemia.

**KEYWORDS** Sudden death; Autonomic nervous system; Ventricular fibrillation; Baroreflex sensitivity; Heart rate variability

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## Introduction

Arrhythmic and nonarrhythmic cardiac mortality has markedly decreased over the last 20 years.[1,2] This decline is mostly caused by the availability of effective pharmacologic and nonpharmacologic interventions for patients with overt ischemic heart disease.[3] At variance with this positive trend, the incidence of sudden arrhythmic death in subjects without a known cardiac disease has remained unaltered over the same period.[4,5] Every year more than 100,000 Americans die of sudden death[6] that is often the first, and last, manifestation of their ischemic heart disease. This major public health issue has not been tackled because of the widespread belief that high-risk individuals cannot be identified with enough predictive accuracy to warrant specific preventive interventions.[7]

Beginning in the early 1980s, we and others have provided compelling experimental[8,9] and clinical evidence[10–14] that autonomic imbalance, manifested by depressed baroreflex sensitivity (BRS) and/or heart rate variability (HRV) and revealing a decrease in vagal activity and an increase in sympathetic activity, is an independent risk factor for cardiac and arrhythmic mortality after myocardial infarction (MI). Our experimental studies also showed a wide range of autonomic responses in dogs pre-MI,[9] thus raising the possibility that these responses are controlled genetically.[15] This was followed by clinical reports indeed suggesting genetic influences over the neural control of the heart[16,17] and by the evidence for genetic factors in the risk for sudden cardiac death in the general population.[18] Furthermore, it was shown recently that heart rate response to exercise, a marker of autonomic reflex activity, is strongly predictive for the subsequent risk of sudden cardiac death even when collected in young healthy individuals, thus pointing to a genetic predisposition toward greater or lower vagal or sympathetic reflexes.[19]

In the present study, we tested the hypothesis that an analysis incorporating the autonomic markers already proven effective post-MI could identify, even among healthy conscious dogs, those at risk of developing ventricular fibrillation (VF) during a first and brief ischemic episode.

## Methods

A total of 43 healthy adult mongrel dogs (20 to 25 kg body weight) were used for the study. The protocol was approved by the Institutional Animal Care and Use Committee at the University of Oklahoma Health Sciences Center and also followed federal standards pertaining to the appropriate use of laboratory animals.

### Surgical preparation

Adult healthy mongrel dogs (age range 2 to 5 years by dentition) were purchased from US Department of Agriculture–approved dealers and were preconditioned (2 to 3 weeks) for the study. The animals were surgically instrumented and studied according to previously described protocols.[20] Briefly, under general anesthesia the chest was opened, the heart was suspended in a pericardial cradle, and the left circumflex coronary artery was dissected free of connective tissue. A 20-mHz Doppler flow probe and distal pneumatic vascular occluder were both placed around the artery to allow reversible occlusion of it as verified by the blood flow signal. The chest was closed and evacuated, and instrumentation leads were exteriorized at the neck dorsum. Analgesics (pentazocine 0.3 mg/kg, nalbuphine 0.6 mg/kg) were administered in the postoperative 24-hour period and as necessary. Three to four weeks were allowed for surgical recovery and for environmental adaptation.

### Autonomic assessments

Markers of reflex (BRS) and tonic (HRV) cardiac autonomic activity were determined before evaluation of the risk for VF. Studies were performed in a quiet room with the dog conscious, but not sleeping, with neither sedation nor restraint while resting on a padded table.

BRS was measured using the phenylephrine method.[8,9,21,22] Electrocardiographic and phasic blood pressure were recorded while a bolus intravenous injection of phenylephrine (2 to 3 µg/kg) raised systolic arterial pressure by $41 \pm 15$ mm Hg starting from baseline values of $122 \pm 12$ mm Hg in low-risk dogs and of $109 \pm 15$ mm Hg in high-risk dogs (not significant). BRS (msec/mm Hg) was defined by slope of the regression line correlating the blood pressure changes to the consequent lengthening in RR interval. The linear portion of the blood pressure/RR interval relationship was used to calculate the BRS slope. Four BRS measurements were made in each dog, and the mean value of the 2 values with the highest correlation coefficient was calculated as previously described.[8,9]

On another day, HRV was derived from 25 minutes of continuous digitized (400 Hz) transthoracic electrocardiographic recordings collected while the dog was quiet and resting. Frequency domain analysis of the RR time interval series was performed using fast Fourier transformation with Hanning windowing technique[23] to express total spectral power of detrended data (0.0 to 0.50 Hz). Percentage of the HRV power in the low-frequency band (LF; 0.03 to 0.15 Hz), and high-frequency band (HF; 0.15 to 0.50 Hz) were determined. The sympathovagal balance was expressed by the HF/LF ratio. This was at variance from the standard LF/HF and was meant to use a ratio best reflecting the vagal cardiac control.

These 2 autonomic markers (BRS and the HF/LF ratio) were combined into a single parameter by mathematically merging the 2 variables. The new autonomic parameter TARVA (Tonic and Reflex Vagal Activity) was calculated by multiplying HF/LF by BRS. The formula TARVA = HF/LF $\times$ BRS gives equal weight to each marker. By multiplying these 2 parameters, we obtained a robust index (because of the possibility that within single individuals markers of tonic versus reflex vagal activity could have variable predictive power) with a positive correlation with overall vagal activity.

### Arrhythmic risk identification

After all autonomic measurements had been completed, each dog was acclimated to the motorized treadmill and subsequently evaluated for presence or absence of lethal arrhythmias during a submaximal exercise and myocardial ischemia test. Copper plates were taped to the shaved chest for transthoracic electrocardiographic measurement and for immediate cardioversion in the event of VF. Dogs ran for 12 to 15 minutes, with increasing speed and elevation from 4.8 km/h at 0% incline up to 6.4 km/h at 12% to 16% incline, incremented at 3-minute intervals. When heart rate reached a target range of approximately 200 to 210 beats/min, representing 70% to 75% of the highest heart rate expected in a dog, 2 minutes of acute myocardial ischemia followed; after 1 minute of coronary occlusion, the treadmill was stopped. The occurrence of VF with this protocol identifies dogs at high risk for sudden death while low-risk dogs are able to survive the ischemic event without major arrhythmias.

### Statistical analysis

Continuous data are presented as the mean $\pm$ 1 standard deviation of the mean and are compared by the Mann-Whitney $U$ test. Categorical data are presented as counts and percentages and are compared by the Fisher exact test. The receiver-operator characteristic (ROC) curve analysis was used to identify the optimal cutoffs of BRS, HF/LF, and TARVA (maximizing the sensitivity and the specificity) for recognizing dogs with lethal arrhythmia. The area under the ROC curve, sensitivities, and specificities are reported together with their 95% confidence interval (CI). Stata 9 (2005, StataCorp, College Station, TX) and MedCalc 8 (Mariakerke, Belgium) were used for computation. All tests were 2-sided. A value of $P <$ .05 was considered significant.

## Results

Forty-three dogs underwent the exercise and ischemia test for arrhythmic risk assessment. Of these, 10 (23%) devel-