# SUPPLEMENTAL METHODS

## Regional Myocardial Sympathetic Denervation Predicts the Risk of Sudden Cardiac Arrest in Ischemic Cardiomyopathy

**James A. Fallavollita, MD[1,2,3]; Brendan M. Heavey, MPH[2]; Andrew J. Luisi, Jr., MD[2,3,4]; Suzanne M. Michalek, MS[2]; Sunil Baldwa, MBBS[1,2,3]; Terry L. Mashtare, Jr., PhD[5]; Alan D. Hutson, PhD[5]; Robert A. deKemp, PhD[6]; Michael S. Haka, PhD[4]; Munawwar Sajjad, PhD[4]; Thomas R. Cimato, MD[2,3]; Anne B. Curtis, MD[3]; Michael E. Cain, MD[3,7] and John M. Canty, Jr., MD[1,2,3,7,8]**

From the VA Western New York Health Care System[1], the Center for Research in Cardiovascular Medicine[2] and the Departments of Medicine[3], Nuclear Medicine[4], Biostatistics[5], Biomedical Engineering[7], and Physiology&Biophysics[8] at the University at Buffalo;
and the Cardiac PET Centre, University of Ottawa Heart Institute[6].

**Running Head –Denervated Myocardium and Sudden Death**

**Correspondence:**

John M. Canty, Jr., MD
Division of Cardiovascular Medicine
University at Buffalo
Clinical & Translational Research Center, Suite 7030
875 Ellicott Street
Buffalo, NY 14203

Phone: 716-829-2663
Fax: 716-854-1840
Email: canty@buffalo.edu

# SUPPLEMENTAL METHODS

## Study Design

PAREPET was approved by the Institutional Review Boards of the University at Buffalo and the Veterans Affairs Western New York Healthcare System at Buffalo. Recruitment began on September 14, 2004 and ended on April 21, 2009, with follow-up completed on November 30, 2011. Informed consent was obtained from 257 subjects, with 37 subsequently deemed to be ineligible (usually due to non-ischemic etiology) and 16 subjects requested withdrawal before non-invasive testing (usually at the request of the family). Thus, the final study population consisted of 204 subjects with ischemic cardiomyopathy who were considered eligible to receive an ICD for the primary prevention of arrhythmic death (pre-enrollment LVEF ≤35% for New York Heart Association functional Class II or III and ≤30% for New York Heart Association functional Class I). Subjects had stable ischemic heart disease and heart failure on optimal medical therapy and were not considered candidates for coronary revascularization at the time of enrollment. Exclusion criteria included an ICD indication for secondary prevention of cardiac arrest (resuscitated cardiac arrest, sustained VT, or unexplained syncope), previous ICD discharge, recent myocardial infarction (<30 days), percutaneous coronary intervention (<3 months), or coronary artery bypass grafting (<1 year)(1).

## Echocardiography and Positron Emission Tomography

Two-dimensional transthoracic echocardiography was performed on the day of PET imaging. Echocardiograms were obtained with a 2.25 MHz phased-array transducer with harmonic imaging (SONOS 7500, Philips Medical Inc.), as previously described(1,2). An echocardiographer blinded to events quantified cardiac volumes, EF, and mitral regurgitation as recommended by the American Society of Echocardiography(3).

PET imaging was performed on a CTI ECAT EXACT HR+ PET scanner with a 15.5 cm axial field-of-view and a resolution of ~5.4 $mm^3$ full-width-at-half-maximum at 10 cm, as previously described(1,2). Sympathetic nerve norepinephrine uptake was assessed with $^{11}$C-HED [20 mCi (740

MBq)], resting myocardial perfusion was determined with $^{13}$N-ammonia [$^{13}NH_3$, 20 mCi (740 MBq)], and metabolic viability was quantified with $^{18}$F-2-deoxyglucose [$^{18}$FDG ; 6.5 mCi (241 MBq)] during a hyperinsulinemic-euglycemic clamp(2). All isotopes were produced at the University at Buffalo Cyclotron Facility, as previously described(1,4). Attenuation correction of all emission data was performed with a transmission scan using a $^{68}$Ge rod source(1,2).

Some subjects could not complete PET scanning due to claustrophobia/anxiety (n=6), extreme truncal obesity (n=2), or severe back pain (n=1). In the remaining 195 subjects, technical problems (usually failed radiotracer synthesis) precluded 10 scans ($^{11}$C-HED - n=4, $^{13}NH_3$ - n=2, $^{18}$FDG - n=4), and an additional 11 scans were of inadequate quality to be quantified ($^{11}$C-HED - n=3, $^{13}NH_3$ - n=1, $^{18}$FDG - n=7). Thus, 564 of the planned 585 PET images (96%) were completed and quantifiable, with 176 subjects (90%) having complete PET data. The results of the non-invasive studies, including PET imaging, were not provided for patient management.

**Quantitative PET Image Analysis**

PET image analysis was performed with FlowQuant® software (Ottawa Heart Institute)(2,5,6), by a reviewer blinded to all clinical data. Images were reconstructed with a zoom of 2, a Hann filter with cutoff frequency of 0.3 cycles/pixel, and correction for radionuclide decay. Late uptake images were used to define the three-dimensional shape of the LV with combined cylindrical and hemispherical (bottle-brush) sampling(7).

Myocardial tracer uptake was quantified from four frames of each imaging set; from 20-60 minutes after administration of $^{11}$C-HED, 3-19 minutes after administration of $^{13}NH_3$, and 20-40 minutes after administration of $^{18}$FDG. Tracer activity in each of 496 sectors was expressed as a percentage of the highest 5% of sectors per subject(7). Normal $^{11}$C-HED uptake was considered $\geq$75% of peak, and normal $^{13}NH_3$ and $^{18}$FDG uptake were $\geq$80% of peak(1,5,8). All PET parameters were quantified as the sum of the percentages of all sectors, expressed as % of the LV. Denervated myocardium was determined from the sectors with reduced $^{11}$C-HED uptake. Infarcted myocardium and hibernating myocardium were quantified from a mismatch analysis between the $^{13}NH_3$ and $^{18}$FDG, initially described by Beanlands et

al(5). In this analysis, the extent of reduced perfusion within a sector is apportioned between infarcted and hibernating myocardium, as determined by the relative $^{18}$FDG uptake(5). Thus, reduced perfusion with preserved $^{18}$FDG uptake defined hibernating myocardium, and matched reductions in perfusion and $^{18}$FDG denoted infarct. Since we employed a euglycemic-hyperinsulinemic clamp to maximize myocardial $^{18}$FDG uptake(2), $^{18}$FDG uptake was not rescaled to regions with normal perfusion(5). Viable denervated myocardium was determined in an analogous fashion from a mismatch analysis between $^{11}$C-HED uptake and $^{18}$FDG uptake. Since late $^{13}NH_3$ uptake has been shown to be a tracer of myocardial viability(9), $^{13}NH_3$ images were substituted in mismatch analyses in 9 subjects in whom the $^{18}$FDG uptake was unavailable.

**Follow-up and Classification of Events**

After completing the imaging, subjects (or their designee) were contacted by telephone at 3-month intervals to review intervening ICD therapy, hospitalizations, and interval changes in symptoms. The primary end-point was sudden cardiac arrest or equivalent (SCA). This included arrhythmic death as well as ICD discharge for VF or VT>240 bpm(1). Previous studies have demonstrated that the frequency of ICD discharge for these events approximates the absolute reduction in mortality with an ICD(1,10,11). Anti-tachycardia pacing was not included as an end-point. All notes and electrograms associated with ICD discharges were obtained and independently reviewed.

Deaths were classified according to the modified Hinkle-Thaler(12) classification used in the Multicenter Unsustained Tachycardia Trial (MUSTT)(13,14). Consonant with this classification scheme, arrhythmic events and ICD discharge associated with end-stage heart failure(13) or hospice care were classified as cardiac non-sudden death rather than SCA. Cardiac transplantation (n=2) was considered cardiac non-sudden death. All available details (paramedic and medical records, first-hand witnesses, family members, death certificates) including the activity levels and symptoms prior to death were obtained and independently reviewed by two board-certified cardiologists to determine classification as: E, cardiac non-sudden or non-cardiac death. In the event of disagreement, a consensus was reached with a third reviewer.

**Statistical Analyses**

A detailed discussion of the sample size estimate and power calculations has been previously published(1). Although initiation of the study commenced with expansion of clinical guidelines for the utilization of ICDs for the primary prevention of arrhythmic death, subsequent ICD implantation rates at the study site, like those nationally, were considerably lower than anticipated. Since recruitment was below the original target, the NHLBI granted requests for an additional 24 months of follow-up of enrolled subjects to obtain sufficient events.

The primary analytic approach in the PAREPET trial was to assess PET parameters as continuous variables to predict time to SCA (primary end-point)(1). In order to determine the potential independence of these parameters, various demographic, medication, echocardiographic, hemodynamic, and laboratory variables were also tested for their association with SCA. Demographic variables included: age, sex, body mass index, body surface area (BSA), New York Heart Association Heart Failure Class, Canadian Cardiovascular Society Angina Class, clinical diagnosis of diabetes mellitus, history of coronary revascularization, cardiac rhythm other than sinus, and QRS duration. Medications included: beta-blockers, amiodarone, antiplatelet agents (aspirin and/or clopidogrel) or warfarin, angiotensin inhibition therapy (angiotensin converting enzyme inhibitor or angiotensin receptor antagonist), aldosterone antagonists, and digoxin. Echocardiographic parameters included: EF, LV end-diastolic and end-systolic volumes (absolute and indexed for BSA), left atrial volume (absolute and indexed for BSA), LV mass (absolute and indexed for BSA), and severity of mitral regurgitation. Hemodynamic parameters included: systolic blood pressure, heart rate, and rate pressure product (systolic blood pressure • heart rate). Laboratory values included: hematocrit, potassium, sodium, creatinine, and B-type natriuretic peptide (BNP). When both absolute and indexed parameters were significantly associated with time to SCA, only indexed values were reported and used in the multivariate analysis. All statistical analyses were performed with commercial software (Microsoft Excel and SAS version 9.1).

**ACKNOWLEDGEMENTS**

We appreciate the time and patience donated by our PAREPET subjects and their families, without whom this study would not have been possible. We would also like to thank Anne Coe and Marsha Barber for their assistance with trial administration and manuscript preparation. Finally, we would like to acknowledge the technical assistance of Paul Galantowicz (PET), John Gon and Wendy Klein (echocardiography), Ran Klein and Jennifer Renaud (FlowQuant), and Arturo M. Valverde, MD, Donald Switzer, MD, and Chee Kim, MD (clinical cardiac electrophysiology).

This study was supported by the National Heart, Lung and Blood Institute (HL-76252). The sponsor had no role in the study design; in the collection, analysis and interpretation of data; in the writing of the manuscript or in the decision to submit the paper for publication.

REFERENCES

1. Fallavollita JA, Luisi J, A.J., Michalek SM et al. Prediction of ARrhythmic Events with Positron Emission Tomography: PAREPET study design and methods. Contemp Clin Trials 2006;27:374-88.

2. Fallavollita JA, Luisi AJ, Jr., Yun E, Dekemp RA, Canty JM, Jr. An abbreviated hyperinsulinemic-euglycemic clamp results in similar myocardial glucose utilization in both diabetic and non-diabetic patients with ischemic cardiomyopathy. J Nucl Cardiol 2010;17:637-645.

3. Lang RM, Bierig M, Devereux RB et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440-63.

4. Luisi AJ, Jr., Suzuki G, deKemp R et al. Regional $^{11}$C-hydroxyephedrine retention in hibernating myocardium: Chronic inhomogeneity of sympathetic innervation in the absence of infarction. J Nucl Med 2005;46:1368-1374.

5. Beanlands RS, Ruddy TD, deKemp RA et al. Positron emission tomography and recovery following revascularization (PARR-1): the importance of scar and the development of a prediction rule for the degree of recovery of left ventricular function. J Am Coll Cardiol 2002;40:1735-1743.

6. Ananthasubramaniam K, Chow BJ, Ruddy TD et al. Does electrocardiographic Q wave burden predict the extent of scarring or hibernating myocardium as quantified by positron emission tomography? Can J Cardiol 2005;21:51-6.

7. deKemp RA, Ruddy TD, Hewitt T, Dalipaj MM, Beanlands RS. Detection of serial changes in absolute myocardial perfusion with $^{82}$Rb PET. J Nucl Med 2000;41:1426-1435.

8. Allman KC, Wieland DM, Muzik O, DeGrado TR, Wolfe ER, Jr., Schwaiger M. Carbon-11 hydroxyephedrine with positron emission tomography for serial assessment of cardiac adrenergic neuronal function after acute myocardial infarction in humans. J Am Coll Cardiol 1993;22:368-375.

9. Kitsiou AN, Bacharach SL, Bartlett ML et al. 13N-ammonia myocardial blood flow and uptake: relation to functional outcome of asynergic regions after revascularization. J Am Coll Cardiol 1999;33:678-86.

10. Daubert JP, Wilber DJ, Lin A et al. ICD therapy for fast ventricular tachycardia or ventricular fibrillation is a surrogate endpoint for mortality in MADIT II. Circulation 2003;108:IV-385.

11. Moss AJ, Greenberg H, Case RB et al. Long-term clinical course of patients after termination of ventricular tachyarrhythmia by an implanted defibrillator. Circulation 2004;110:3760-5.

12. Hinkle LE, Jr., Thaler HT. Clinical classification of cardiac deaths. Circulation 1982;65:457-464.

13. Buxton AE, Lee KL, DiCarlo L et al. Electrophysiologic testing to identify patients with coronary artery disease who are at risk for sudden death. Multicenter Unsustained Tachycardia Trial Investigators. New England Journal of Medicine 2000;342:1937-1945.

14. Fisher JD, Buxton AE, Lee KL et al. Designation and distribution of events in the Multicenter UnSustained Tachycardia Trial (MUSTT). Am J Cardiol 2007;100:76-83.