2.7. Echocardiography in Rodents

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A. OBJECTIVE

Obtain non-invasive evaluation of cardiac structure and function in anesthetized rodents including endpoints that are consistent with echocardiographic evaluation of clinical patients.

B. CAUTIONS

- Cost: Use of research based echocardiography platform described in this SOP costs approximately $250,000. While this is a significant investment, the platform offers imaging capabilities that far surpass the use of clinical platforms with high frequency probes on rodents.
- Sedation: Use of this research-based echocardiography platform requires the use of sedation which decreases heart rates and may adversely affect cardiac function (see protocol # for more discussion on the impact of anesthetic drugs).
- Time: With an experienced operator, complete echocardiographic evaluations of rodents can take approximately 20 - 30 minutes per rodent for all desired images.

C. MATERIALS

- Vevo 770 (VisualSonics, Toronto, Canada)
- Vevo mouse handling platform with a Physiological Controller Unit.
- Isoflurane.
- Oxygen.
- Anesthesia (isoflurane) blender and tubing with anesthesia scavenging system (activated charcoal absorption filter).
- Heating lamp.
- Depilatory cream.
- Ultrasound gel.
- Ophthalmic ointment.
- Electrode gel.
- Gauze, cotton tip applicators, tape.

D. METHODS

<u>Preparation of mouse:</u>

1. Place the mouse in an induction chamber with constant inflow of 5% isoflurane mixed with 100% oxygen.
2. Once the mouse is unable to right itself, remove it from the induction chamber, weigh it, and place it on a heating platform.
3. Place the nose into a nose cone with 1-2% isoflurane in 100% oxygen. Passively evacuate excess gases using an activated charcoal absorption filter.
4. Cover the eyes with a petroleum-based ophthalmic ointment.
5. Place electrode gel on the paws and tape the paws over the electrocardiogram contact pads on the heating platform.
6. Lubricate a rectal probe with gel, place it in the rectum, and tape it to the platform. Maintain the temperature at 36.5 to 37.5 °C.
7. Continuously monitor the temperature, heart rate (HR), and blood pressure (BP) during the scanning.
8. Apply depilatory cream to the chest of the mouse using a cotton applicator tip and remove the cream after 2 min with a gentle rolling motion of the cotton tips, then clean the chest with distilled water.
9. Place ultrasound gel on the chest of the anesthetized mouse.
10. Place the ultrasound probe in contact with the ultrasound gel and perform the scan.

Scanning procedure:

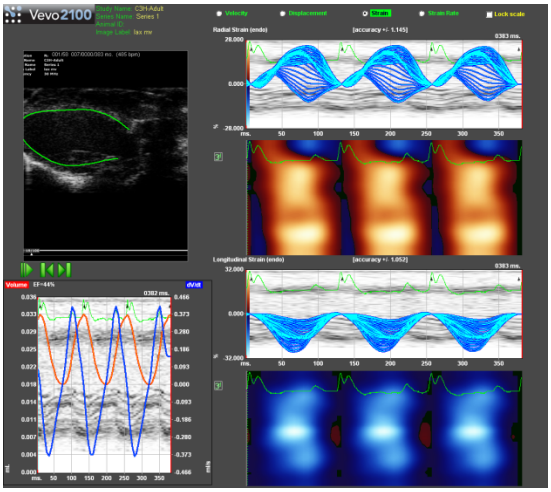
1. Modified parasternal long axis: Orient the scanhead directly in the longitudinal plane, with the notch at approximately 1 o'clock. Locate the ascending aorta and obtain a 2-D cine loop.
2. Modified parasternal long axis: Adjust the mouse platform so that the pulmonary valve and pulmonary artery are imaged. Obtain a 2-D cine loop.
3. Modified parasternal long axis: Change to pulse wave Doppler mode. Place the pulse width gate in the outflow jet of the pulmonary valve. Obtain a spectral Doppler image.
4. Modified parasternal long axis: Obtain a long axis image of the left ventricle with the plane of the mitral valve. Acquire a 2-D cine loop.
5. Turn the transducer 90 degrees so that the notch is at 3 o'clock.
6. Modified parasternal short axis: Obtain an image of the left ventricle at the level of the papillary muscles, and obtain a 2-D cine loop.
7. Modified parasternal short axis: Change to M-mode imaging and obtain an image of the left ventricle, ensuring good endocardial border delineation.
8. Modified parasternal short axis: Adjust the animal platform to image the tricuspid valve. Obtain a 2-D image of the tricuspid valve.

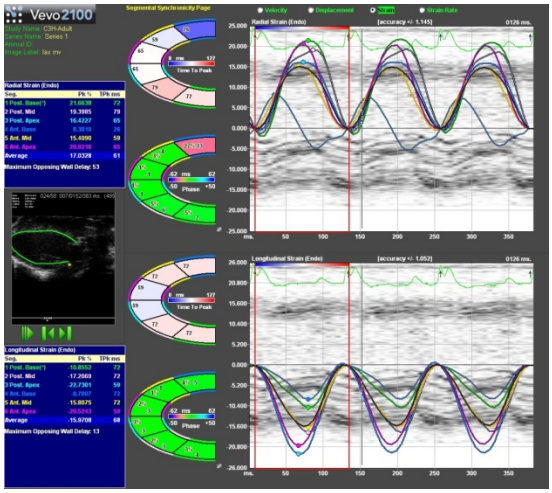
9. Modified parasternal short axis: Change to pulse wave Doppler mode and place the pulse gate just distal to the tricuspid valve, paralleling the inflow jet as closely as possible. Correct the angle as needed, by no greater than 10°. Obtain a spectral Doppler image.
10. Modified suprasternal notch view: Adjust the animal platform and transducer so that the transducer is directed caudally at a 0° angle at the level of the upper sternum. Locate the ascending aorta longitudinally.
11. Modified suprasternal notch view: Change to pulse Doppler mode. Place the pulse width gate in the ascending aorta, paralleling the aortic outflow stream as closely as possible. Correct the angle as needed, by no greater than 55°. Obtain a spectral Doppler image of the aortic outflow.
12. Rotate the transducer approximately 90 degrees leftward and move the mouse platform cranially. Align the scanhead with the lower left chest near the end of the ribcage.
13. Modified apical three-chamber view: Locate the left ventricle at the level of the mitral valve.
14. Modified apical three-chamber view: Obtain a 2-D image of the mitral valve.
15. Modified apical three-chamber view: Change to spectral Doppler mode. Place the pulse width gate in the mitral inflow stream, as close to parallel to the flow as possible. Correct the angle as needed, by no greater than 30°. Obtain an image of the mitral inflow and ascending aortic outflow.
16. Once the imaging is completed, remove all probes and monitors from the mouse.
17. Clean the mouse with water and allow it to recover on the heated platform or in warmed cage. Once the mouse is awake, return it to its cage.

Alternative Methods:

The newly upgraded Vevo2100 ultrasound system has new features including color Doppler, steerable Doppler and Vevostrain when compared to Vevo770 system. VevoStrain software is a new quantification tool available with the Vevo 2100 which uses B-Mode speckle-tracking analysis to track wall motion abnormalities. This tool provides both regional and global wall motion tracking, offering quantification of the velocity of the walls, displacement, strain, strain rate and time to peak analysis. Strain is a valuable tool in cardiovascular disease, cardiac regeneration, cancer therapeutics, and broader drug development areas. It can be used to sensitively monitor cardiac function and ventricular remodeling for early signs of cardiotoxicity in patients or animal models under anti-cancer therapies (Jurcut et al, 2008, Spurney et al, 2011). VevoStrain is a sensitive tool for assessing myocardial performance and showcasing changes in regional and

global heart motion post-infarct or in response to therapeutic agents. Given their ease of use, high reproducibility, and flexibility, strain and strain rate are already being hailed as essential tools in the repertoire of any researcher interested in cardiac function.





E. EVALUATION AND INTERPRETATION OF RESULTS

- Aortic diameter: Measure at the sinotubular junction in the modified suprasternal notch view or the modified parasternal long axis.
- Pulmonic valve diameter: Measure at the level of the pulmonic valve in the modified parasternal long axis.
- Pulmonic outflow peak velocity and velocity time integral: Measure the peak velocity and trace the pulmonic outflow Doppler envelope. Measure the pulmonic outflow accelerate time (PAT) and ejection time (PET).
- M-mode measurement: Using the program calipers, measure the IVS thickness (d), LVID (d), LVPW (d), IVS (sys), LVID (sys), and LVPW (sys) and heart rate.
- Ascending aorta peak velocity and velocity time integral: Measure the peak velocity and trace the aortic outflow Doppler envelope.
- Tricuspid valve E wave, A wave, E at A wave: Measure the peak velocity of the E and A waves of the tricuspid inflow Doppler envelope. At heart rates of around 450 to 500 bpm, the E and A waves fuse, and only one measurement is made, the E at A wave measurement.
- Mitral valve E wave, A wave, E at A wave: Measure the peak velocity of the E and A waves of the mitral inflow Doppler envelope. At heart rates of above 500 bpm, the E and A waves fuse together, and only one measurement is made, the E at A wave measurement.
- Use integrated cardiovascular software package for functional calculations including pulmonary artery (PA)/aortic (AO)/left ventricle (LV) stroke

volume (SV), PA/AO/LV cardiac output (CO), PAT/PET ratio, right ventricular (RV)/LV E/A ratio, LV ejectional fraction (EF), fractional shortening (FS) and myocardial performance index (MPI).

F. REFERENCES

1. Uaesoontrachoon K, Quinn JL, Tatem KS, Van Der Meulen JH, Yu Q, Phadke A, Miller BK, Gordish-Dressman H, Ongini E, Miglietta D, Nagaraju K. Long-term treatment with naproxcinod significantly improves skeletal and cardiac disease phenotype in the mdx mouse model of dystrophy. Hum Mol Genet. 29 (91): 51785(2014)
2. Henriques-Pons A, Yu Q (first co-author), Rayavarapu S, Cohen TV, Ampong B, Cha HJ, Jahnke V, Van der Meulen J, Wang D, Jiang W, Kandimalla ER, Agrawal S, Spurney CF, Nagaraju K. Role of toll-like receptors in the pathogenesis of dystrophin-deficient skeletal and heart muscle. Hum Mol Genet. 23(10):2604-17 (2014).
3. Heier CR, Damsker JM, Yu Q, Dillingham BC, Huynh T, Van der Meulen JH, Sali A, Miller BK, Phadke A, Scheffer L, Quinn J, Tatem K, Jordan S, Dadgar S, Rodriguez OC, Albanese C, Calhoun M, Gordish-Dressman H, Jaiswal JK, Connor EM, McCall JM, Hoffman EP, Reeves EK, Nagaraju K. VBP15, a novel anti-inflammatory and membrane-stabilizer, improves muscular dystrophy without side effects. EMBO Mol Med, 5(10):1569-85 (2013).
4. Quinn JL, Huynh T, Uaesoontrachoon K, Tatem K, Phadke A, Van der Meulen JH, Yu Q, Nagaraju K. Effects of Dantrolene Therapy on Disease Phenotype in Dystrophin Deficient mdx Mice. PLoS Curr. 8(5):1371 (2013).
5. Yu Q, et al. Omigapil Treatment Decreases Fibrosis and Improves Respiratory Rate in dy(2J) Mouse Model of Congenital Muscular Dystrophy. *PLoS One.* **8**(6):e65468 (2013).
6. Sali A, et al. The Proton Pump Inhibitor Lansoprazole Improves the Skeletal Phenotype in Dystrophin Deficient mdx Mice. *PLoS One.* **8**(7): e66617 (2013).
7. Huynh T, et al. Selective modulation through the glucocorticoid receptor ameliorates muscle pathology in mdx mice. *J Pathol.* 231(2): 223-35 (2013)
8. Sali A, et al. Glucocorticoid-treated mice are an inappropriate positive control for long-term preclinical studies in the mdx mouse. *PLoS One*. **7**(4):e34204 (2012).
9. Spurney CF, et al. Losartan decreases cardiac muscle fibrosis and improves cardiac function in dystrophin-deficient mdx mice*. J Cardiovasc Pharmacol Ther.***16**(1):87-95 (2011).
10. Spurney CF, et al. (2011) Membrane sealant Poloxamer P188 protects against isoproterenol induced cardiomyopathy in dystrophin deficient mice. *BMC Cardiovasc Disord.* **11**:20-29 (2011).
11. Spurney C, Yu Q, Nagaraju K. Speckle tracking analysis of the left ventricular anterior wall shows significantly decreased relative radial strain patterns in

dystrophin deficient mice after 9 months of age. *PLoS Curr.***3**:1273 (2011).

12. Guerron AD, et al. Functional and molecular effects of arginine butyrate and prednisone on muscle and heart in the mdx mouse model of Duchenne Muscular Dystrophy. *PLoS One.* **5**(6):e11220 (2010).

13. Spurney CF, et al. Evaluation of skeletal and cardiac muscle function after chronic administration of thymosin beta-4 in the dystrophin deficient mouse. *PLoS One.* **5**(1):e8976 (2010).

14. Spurney CF, et al. Preclinical drug trials in the mdx mouse: assessment of reliable and sensitive outcome measures. *Muscle Nerve.* **39**(5):591-602 (2009).

15. Nie L, Chu H, Cheng Y, Spurney C, Nagaraju K, Chen J. Marginal and conditional approaches to multivariate variables subject to limit of detection. *J Biopharm Stat.* **19**(6):1151-61 (2009).

16. Spurney CF, Knoblach S, Pistilli EE, Nagaraju K, Martin GR, Hoffman EP. Effective rescue of dystrophin improves cardiac function in dystrophin-deficient mice by a modified morpholino oligomer. *Neuromuscul Disord.* **18**(5):371-81 (2008).

17. Spurney CF, Knoblach S, Pistilli EE, Nagaraju K, Martin GR, Hoffman EP. Dystrophin-deficient cardiomyopathy in mouse: expression of Nox4 and Lox are associated with fibrosis and altered functional parameters in the heart. *Neuromuscul Disord.* **18**(5):371-81 (2008).

18. Jurcut R, Wildiers H, Ganame J, D'hooge J, De Backer J, Denys H, Paridaens R, Rademakers F, Voigt JU. Strain rate imaging detects early cardiac effects of pegylated liposomal Doxorubicin as adjuvant therapy in elderly patients with breast cancer. J Am Soc Echocardiogr. 21(12):1283-9 (2008).