Pulmonary Flow as an Improved Method for Determining Cardiac Output in Mice after Myocardial Infarction

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Background: Echocardiography is a valuable noninvasive technique to estimate cardiac output (CO) from the left ventricle (LV) not only in clinical practice but also in small-animal experiments. CO is used to grade cardiac function and is especially important when investigating cardiac injury (e.g., myocardial infarction [MI]). Critically, MI deforms the LV, invalidating the assumptions fundamental to calculating of cardiac volumes directly from the LV. Thus, the purpose of this study was to determine if Doppler-derived blood flow through the pulmonary trunk (pulmonary flow [PF]) was an improved method over conventional LV–dependent echocardiography to accurately determine CO after MI.

Methods: Variations in CO were induced either by transverse aortic constriction or MI. Echocardiography was performed in healthy ($n = 27$), transverse aortic constriction ($n = 25$), and MI ($n = 41$) mice. CO calculated from PF (pulsed-wave Doppler) was internally compared with CO calculated from left ventricular images using M-mode (Teichholz formula) and the single-plane ellipsoid two-dimensional (2D) formula and externally compared with the gold standard, flow probe CO.

Results: In healthy mice, all three echocardiographic methods (M-mode, 2D, and PF) correlated well with flow probe–derived CO. In MI mice, only PF CO values correlated well with flow probe values. Bland-Altman analysis confirmed that PF was improved over M-mode and 2D echocardiography. Inter- and intrauser variability of PF CO was reduced, and both inter- and intraclass correlation coefficients were improved compared with either M-mode or 2D CO calculations.

Conclusions: PF CO calculated from pulsed-wave Doppler through the pulmonary trunk was an improved method of estimating CO over LV–dependent formulas after MI. (J Am Soc Echocardiogr 2017;30:612-23.)

Keywords: Stroke volume, Diastolic dysfunction, MRI, Regurgitation, Ejection fraction

Cardiac output (CO), the product of heart rate and stroke volume, is an important measure of cardiac performance. Although various methods exist for estimating CO (e.g., thermodilution, pulse pressure, magnetic resonance imaging, etc.) these techniques are generally invasive, are impractical in experimental models, or require highly specialized and expensive equipment. Both clinically and experimentally, echocardiography remains a frequently used method to rapidly and noninvasively estimate CO with low overhead cost for equipment and training.

The ability to accurately determine CO is critical for assessing and comparing myocardial function. Using echocardiography to determine CO is commonly accomplished by calculating left ventricular (LV) volumes directly from one-dimensional (1D) and two-dimensional (2D) images of the LV. Yet these calculations are limited when the shape of the LV no longer adheres to the assumptions defined by the formulas. Two common formulas are the 1D "M-mode" formula described by Teichholz *et al.*[1] and the 2D single-plane ellipsoid formula.[2] These formulas were derived under the assumption that the LV resembles an approximated "ellipsoid" shape with uniform wall movement through each plane (vertical plane; M-mode, long-axis plane; 2D). The accuracy with which these 1D and 2D methods estimate stroke volume (diastolic volume − systolic volume) is thus contingent on the LV's maintaining both this ellipsoid shape and uniformity of contraction.

Directly determining CO using these formulas is robust and comparable in models without perturbations in cardiac symmetry—healthy or hypertensive hearts (e.g., transverse aortic constriction [TAC])—in which the LV is symmetric, approximates an ellipsoid shape, and exhibits uniform contraction.[3-5] These formulas are

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Abbreviations

1D = One-dimensional

2D = Two-dimensional

CO = Cardiac output

ICC = Intra- and interclass correlation coefficient

LV = Left ventricle, ventricular

MI = Myocardial infarction

MR = Mitral regurgitation

PF = Pulmonary flow

TAC = Transverse aortic constriction

VTI = Velocity-time integral

fallible when the LV no longer adheres to these assumptions, as is the case after myocardial infarction (MI) (see Figure 1). Here, (1) the chamber is dilated and the LV shape distorted, (2) the LV wall is nonuniform with both hyper- and hypocontractile myocardial tissue and/or akinetic scar, and (3) pathology interferes with proper landmarking (e.g., nonvisible papillary muscles). Although these limitations are recognized,[6,7] and although caution is suggested when using 1D and 2D approximations of LV volumes in ischemic models (e.g., MI),[4] LV–dependent echocardiographic methods for determining CO continue to be relied on. Accordingly, the purpose of this study was to identify an improved echocardiographic method to grade CO after MI that is more precise and accurate.

In large-animal MI models (e.g., porcine, ovine, and canine), the complications associated with single-image formulas can be avoided by calculating the volume of multiple cross-sectional images, collectively accounting for differences in chamber shape and contractile function between different ventricular segments. In small-animal models of MI (e.g., murine, cricetine, and leporine), chamber size, heart rate, and poor resolution of the thinly ballooned scar impair the ability to obtain multiple cross-sectional volumes with reproducible accuracy. Indeed, an improved method would determine CO regardless of LV pathology. In 2011, Tournoux *et al.* introduced the use of pulsed-wave Doppler blood flow through the pulmonary trunk (termed pulmonary flow, PF) as an alternative method to approximate CO in healthy mice and following acute endotoxic shock.[8] What has yet to be investigated is whether this methodology can be applied to the most common experimental models of heart failure, for example, pressure overload (i.e., TAC) or volume overload (i.e., MI). In this study, we present data establishing PF as a preferable method over classical LV–dependent echocardiographic formulas for calculating CO, particularly in a mouse MI model, both increasing the accuracy and reducing the variability of CO measurements. This has relevance when investigating treatments for MI.

METHODS

Surgical Model

Briefly, 8- to 9-week-old male CD-1 mice (~35 g body weight) were anesthetized with isoflurane/oxygen (2%:100%), intubated, and ventilated (Harvard Apparatus, Holliston, MA) at 150 breaths/min at a tidal volume of about 300 μL. Animals were randomly assigned to receive either MI or TAC. In the MI group, a thoracotomy was performed on the left side of each animal to expose the left anterior descending coronary artery, which was ligated with 7-0 Surgipro II polypropylene suture (Covidien, Dublin, Ireland) directly inferior to the left atrium. MI was confirmed by myocardial blanching. In the TAC group, the cartilaginous connection between the sternum and ribs 2 and 3 was separated to expose the upper mediastinum. The transverse aorta was isolated and subsequently set with 7-0 Sofsilk thread (Covidien) to the diameter of a blunted 26-gauge needle. For both surgical models, the ribs and skin were closed using 5-0 Sofsilk suture. Mice were taken off anesthetic and allowed to recover on 100% oxygen. Housing and experimental procedures were approved by the animal care committee at the University of Guelph and were in accordance with the guidelines for laboratory animal welfare set forth by the University of Guelph.

Echocardiographic Analysis

Images were obtained between 9 AM and 3 PM using the Vevo2100 system (VisualSonics, Toronto, ON, Canada). All TAC mice were evaluated 18 weeks after surgery. All MI mice were evaluated 4 to 8 weeks after surgery. Mice were anesthetized with isoflurane/oxygen (1%–1.5%:100%) just below the level of a pedal reflex to ensure comparable anesthesia and maintained at 37°C throughout procedures. All LV images were acquired within 10 min of induction with the MS550D ultrasound transducer set to 40 MHz. All measurements were done using the Cardiac Package (VisualSonics). M-mode (1D) images were analyzed using the LV-trace function, and long-axis B-mode (2D) images were analyzed using the 2D area calculation. M-mode images were obtained from either long- or short-axis views of the heart at the midpapillary level. Two-dimensional images were acquired from the long-axis view of the LV, where probe placement was such that the LV chamber was visualized from the apex to the outflow tract.

PF was measured from a parasternal short-axis view of the pulmonary trunk just distal of the pulmonary semilunar valves with probe placement arranged to obtain an image as depicted in Figure 2D, similar to the long-axis view of the LV but with the probe moved 1 to 2 mm superiorly. Aortic flow was measured from a parasternal long-axis view of the ascending aorta (Supplemental Figure 1, available at www.onlinejase.com). Pulmonary and aortic flows were calculated as the product of the vessel area ($2\pi r^2$) and the velocity-time integral (VTI) of the pulsed-wave Doppler of flow at that level (Figure 2C, Supplemental Figure 1D, available at www.onlinejase.com). Vessel diameter was measured just distal to the valve leaflets where VTI was obtained. Doppler gain was consistently set between 32 and 35 dB at a frequency of 32 MHz to limit color Doppler variability.[9,10] Beam steering, a novel function of the Vevo2100 system, was used to ensure that the 2D angle between the direction of the pulmonary trunk visualized in vivo and the sound beam(s) was <45°.

All echocardiographic images (M-mode, 2D, and flows) were obtained in triplicate. All volumes were calculated as the mean of three images sets; the mean of each set was five consecutive heartbeats. All images were reanalyzed in a blinded manner to identify any subjectivity, which was defined as any mean exceeding 10% between repeated measures. In all cases of discrepancy, image quality was poor in part or in entirety, and values were excluded from the analysis (see Table 1). All images were acquired within 10 min of induction, and calculations were done after acquisition.

Flow Probe

Similar to surgical intervention, mice were intubated and ventilated (Harvard Apparatus) at 150 breaths/min at a tidal volume of about 300 μL with an isoflurane/oxygen mix (2%:100%). Mice were maintained at 37°C with a rectal probe and heating lamp throughout data collection. Ribs were separated at the cartilaginous connections of ribs 2, 3, and 4 to expose the upper mediastinum. A piece of 7-0 silk thread was looped around the ascending aorta used and to delicately