Accuracy of Volume Determination by Two-dimensional Echocardiography: Defining Requirements Under Controlled Conditions in the Ejecting Canine Left Ventricle


SUMMARY The accuracy of two-dimensional echocardiographic left ventricular volume measurement in an isolated heart preparation was tested using Simpson's reconstruction of progressively fewer short-axis cross sections of known location. Echocardiographic images from five ejecting hearts submerged in a special tank were obtained under conditions designed for maximal accuracy of echocardiographic volume assessment. Echocardiographic determinations of 52 volumes at various times throughout the cardiac cycle were compared, by least-squares linear regression, with simultaneous direct-volume measurements by volumetric chamber (range 9.4-44.8 ml). Echocardiographic and direct measurements correlated well for all numbers of cross sections from 1-19 (r = 0.84-0.97); however, variability of direct volume predicted from a given echocardiographic measurement increased nonlinearly as the number of cross sections per heart decreased, and was especially large when three or fewer cross sections were used (SEE = 4.6-7.1 ml). The accuracy of echocardiographic measures was compared for each number of cross sections per heart, varying from one to 19; accuracy was defined as the mean absolute difference between echocardiographic and direct measurements of volume, ejection fraction, and maximal rate of ejection. The accuracy of echocardiographic measurements was significantly reduced with fewer than four cross sections per heart for ventricular volume, three cross sections for ejection fraction, and five cross sections for maximal rate of ejection. In light of what appears to be required for accurate echocardiographic volume measurement in this controlled, ejecting, noninfarcted, in vitro preparation, additional cross sections may be required in intact animals and human subjects, especially in those with diseases that cause ventricular asymmetry or regional dysfunction.

The Introduction of echocardiography opened the possibility of repeated noninvasive measurements of cardiac volumes. Although two-dimensional echocardiography (2-D echo) can provide clinically useful estimates of left ventricular ejection fraction (EF) as compared to cineangiographic and radionuclide determinations, use of 2-D echo to predict absolute volume has been hindered by broad variability in results, even though 2-D echo and angiographic volume determinations have correlated well. Most studies have relied on the correlation coefficient to indicate the accuracy of predicting the true volume from the 2-D echo data. Unfortunately, the correlation coefficient may not clearly reflect the variability in measurement errors, and can therefore be misleading in assessing the accuracy of a single measurement in a given patient in predicting the true volume.

Additionally, there are errors inherent in the use of single-plane and biplane cineangiographic volume measurements, the standard against which 2-D echo has been judged. First, cineangiographic volume measurement is a nonanatomic standard requiring major geometric assumptions. Cineangiography, as a shadow technique, tends to overestimate left ventricular volume because it cannot compensate for the effect of endocardial trabeculations and papillary muscle entrapment on the angiographic image. Minor errors in cineangiographic calibration may result in large discrepancies in cineangiographic volume estimates. Finally, in most biplane cine systems, the anteroposterior and lateral radiographs are obtained 180° out of phase, introducing a variable error depending on ejection rate and camera speed. In addition, cineangiograms and echocardiograms have only recently been obtained simultaneously in patients.

We previously used isolated, ejecting dog hearts to compare directly measured ventricular volume (without reliance on geometric assumptions) with echocardiographic volume obtained by reconstructing multiple cross-sectional images obtained at 3-mm intervals along the vertical axis of the heart. Comparison of simultaneous data obtained throughout the cardiac cycle revealed not only a high correlation of echo to direct volume, but also a high predictive value of direct volume from any echocardiographic determination owing to low variability (r = 0.972, SEE = 2.93 ml, direct volume range 9.4-54.7 ml). These measurements were obtained under highly controlled conditions and it was not expected that the accuracy of echocardiographic volume determination could be matched in clinical use or in research applications in intact animals. At the very least, one could not obtain the large number of slices used in that study, and we did not assess the accuracy of volume changes with decreasing numbers of slices.

Using the same preparation in the present study, but analyzing the data using progressively fewer slices, we found that at least four cross-sectional images of known location are required to predict ventricular volume without significant loss in accuracy.
Methods

The techniques of ultrasonic imaging by simultaneous direct volume measurement in the isolated canine heart preparation and measurement of ventricular cavity area from echocardiographic cross-sectional images were the same as reported previously. The echocardiographic data for this study were drawn from data included in the earlier study, but the method of analysis differed markedly.

Ultrasonic Imaging

Echocardiographic images were recorded at 60 fields/sec on 1-inch videotape using a wide-angle, phased-array, two-dimensional echocardiograph (Varian V-3000) with a 2.25-MHz transducer approximately 5 cm from the front of the heart. All ultrasonic imaging was performed using a special tank, designed to optimize quality of ultrasonic images with a baffle containing mineral oil and an echo “window” of ultrasound-lucent Mylar. The exact position of the ultrasound beam relative to the target imaged was measured to the nearest millimeter with a calibrated, adjustable echo transducer mount.

Isolated, Ejecting Hearts

The isolated, ejecting heart preparation consisted of a cross-circulated canine heart containing a balloon in the left ventricle attached to a volumetric chamber for continuous direct volume measurement throughout the cardiac cycle. Support dogs for the five isolated heart preparations were anesthetized and mechanically ventilated; coronary perfusion of the isolated hearts was by retrograde flow through the proximal aorta. Hearts were paced at a constant rate and placed in the blood-filled reservoir of the ultrasonic tank where venous flow was collected for return to the support dogs. In two of the preparations, loading conditions were altered by increasing the pressure of the air column in the volumetric chamber to make the hearts eject over a wider volume range. Instantaneous direct volume, measured with a nonlinearity error of 0.5%, or 0.5 ml, over a 100-ml range, was obtained throughout the cardiac cycle, converted on-line to digital form at 5-msec intervals, and stored (Analogic A/D converter, Data General Nova 1220 computer).

Simultaneous echocardiographic data were recorded over 2 or 3 cardiac cycles at 3-mm intervals over the length of the ventricular chamber (fig. 1). Recordings for the entire length of the ventricle were obtained in less than 2 minutes with no more than 1% beat-to-beat variation in simultaneous direct volume. Direct volume measurements recorded during acquisition of all cross sections were averaged for comparison with echocardiographic volume.

Quantification of Echocardiographic Images

Simultaneous echocardiographic and direct volume data were obtained at 50-msec intervals. Selected fields from the echo images of the ejecting heart were digitized using a light-pen computer system (Varian Instruments) programmed to calculate planimetered area. All measurements of ventricular cavity area were made, using the black-white interface, by one observer per dog in duplicate or triplicate and averaged.

Data Analysis

Calculation of Ventricular Volume from Cross-sectional Cavity Area

Ventricular volume at specific points in the cardiac cycle was calculated as the sum of planimetered cavity area times the distance between slices. To test the accuracy of ventricular volume measurement from decreasing numbers of echocardiographic short-axis cross sections, volumes were calculated from the same set of data from seven experimental runs, and progressively fewer cross sections per heart were used in each successive set of calculations. Thus, seven sets of 52 separate volumes were calculated; for each set, fewer equally spaced echocardiographic cross sections were used to determine ventricular volume (fig. 2). For volumes calculated from a single short-axis cross section, the cross section selected was from the midventricular level; here, slice thickness was equal to the echocardiographically measured ventricular vertical length. For volume calculations made from two or more short-axis cross sections, the most basal cross section was always included as the first slice in the reconstructed volume.

For all volume reconstructions where distance between cross-sections exceeded 3 mm, the thickness of the most apical slice was adjusted, if necessary, so that the vertical length of the reconstructed ventricular vol-

![Image of serial reconstruction of left ventricular cavity in the isolated, ejecting heart. Serial tomographic images are recorded every 3 mm along the vertical axis of the stable beating preparation. Images from four different levels are shown. Endocardial and epicardial margins are digitized to give planimetered area within the endocardium and epicardium, respectively. The 3-mm serial slices are summed to reconstruct ventricular volume.](http://circ.ahajournals.org/content/67/4/903)
Volume did not exceed the long-axis dimension of the left ventricle measured directly from a longitudinal view (fig. 3).

In three of the seven sets of volumes measured, there was a timing offset that resulted in a phase shift between the direct volume measurements and echocardiographic determinations (fig. 4). Although this phase shift would not affect the accuracy of end-systolic volume (ESV), end-diastolic volume (EDV), EF or rates of volume change, it would adversely affect the correlation of echocardiographic with direct volume measurements if it were present and not taken into account. To adjust for the contribution of phase shift to the total discrepancy between echocardiographic and direct volume measurements, echocardiographic volume determinations were shifted on the x-axis until the curves were in phase. To compare direct and echocardiographic volumes, interpolated direct volumes were determined on the shifted curves at points in the cardiac cycle corresponding to echocardiographic volumes. Interpolation was done by fitting a third-degree polynomial to each set of shifted direct volumes and calculating predicted volumes at the points corresponding to echocardiographic volumes. All comparisons that follow between direct and echocardiographically determined volumes were performed using values for direct volumes that were interpolated in this manner.

**Statistical Methods**

To examine the correlation between directly measured volume and echocardiographically determined volume, comparisons were made using a bivariate least-squares linear regression analysis. A measure of volume accuracy was calculated for the 52 volumes as the absolute difference between the directly measured volume and the echocardiographically determined volume at each point in the cardiac cycle. The absolute value of differences weights each error in proportion to its magnitude, not to the square of its magnitude.

**Figure 2.** Schema for serial reduction in the number of cross-sectional echocardiographic slices as a test for accuracy of ventricular volume measurement. LV = left ventricular.

**Figure 3.** Method of adjusting the thickness of the most apical echocardiographic cross-sectional slice to ensure that the vertical length of the reconstructed ventricular volume did not exceed the long-axis dimension of the left ventricle measured directly from a longitudinal view.

For each of the heart preparations in which echocardiographic and direct volume data were obtained, values were determined for volume throughout the cardiac cycle as well as ESV, EDV, EF, and maximal velocity of ejection normalized to cycle length (MVE, ml/% cardiac cycle). To obtain these values derived from the direct volume measurements, the third-degree polynomial fitted to the volumes across the cardiac cycle was used. ESV and EDV were defined as the maximum and minimum volume, respectively, on this

**Figure 4.** The timing offset found in four of the seven sets of volumes measured. This offset resulted in a phase shift between the direct volume measurements ($V_{direct}$) and the echocardiographic volume determinations ($V_{echo}$). Data illustrated represent a single beat from one dog. (B) Correction for the timing offset in panel A. $V_{echo}$ was shifted on the x-axis until the curves were in phase.
fitted curve. MVE was estimated by the slope of the fitted curve at its inflection point.

For echocardiographically determined volumes, these indexes were evaluated at each number of cross sections for each heart preparation. MVE was defined as the most negative slope between points on the ejecting portion of the plot of echocardiographic volume by percentage of the cardiac cycle. Accuracy for ESV, EDV, EF and MVE was then calculated as the absolute difference between the direct and the echocardiographic values.

The relationship between the number of cross sections and the accuracy of each of these variables was analyzed by two-way analysis of variance testing for overall differences in accuracy between different numbers of cross sections. The Waller-Duncan multiple-comparisons procedure\(^{13}\) and the analysis of variance procedure were used to test whether the mean of the accuracy variable in each of the progressively fewer number of cross sections differs significantly from that in the maximal number of cross sections, 15–19. This calculation indicates whether other numbers of cross sections show statistically decreased accuracy. For nonhomogeneous variability across the number of slices, a logarithmic transformation was used.

**Results**

Direct measurement of 52 separate volumes ranged from 9.4 to 44.8 ml. The left ventricular EF of the hearts was 19.3–47%, and beat-to-beat variation of direct volume at any point in the cardiac cycle was less than 1%.

A least-squares linear regression of echocardiographically determined volumes on directly measured volumes for all 52 volumes is shown in figure 5. Although echocardiographic and directly measured volumes appeared to correlate well, even when only one echo slice per heart was used, a markedly increased variability in the direct volume predicted from any given echocardiographic measurement was present with decreasing numbers of slices, especially when only one or two cross sections were used. Figure 6 shows the correlation coefficients and variability from these regression analyses. Although the correlation coefficient decreased only modestly when four or fewer cross sections were used for volume reconstruction, variability of results expressed as a standard error of the estimate increased nearly threefold, and stepwise increments were most apparent for volumes calculated from three or fewer cross sections.

An additional assessment of echocardiographic accuracy with decreasing slice number is shown in figure 7, which shows the absolute difference between directly measured volume and echocardiographic volume. The mean absolute difference is shown as a measure of echocardiographic accuracy for each number of cross sections used. Agreement between echocardiographic and direct volume measurement deteriorated nonlinearly as the number of slices decreased. Variability in echocardiographic accuracy, shown as standard deviation, increased in similar fashion. By analysis of variance, the progressive fall in accuracy of echocardiographic prediction of direct volume was significant (\(F = 17.45, p < 0.0001\)). However, the effect on accuracy appeared to be minimal, provided the echocardiographic volume calculations were made from four or more cross sections. In such cases, the mean percent error was less than 10%.

Results of the Waller-Duncan multiple-comparisons procedure (with logarithmic transformation as appropriate) support these observations (table 1). Comparisons for the 52 volumes from all points in the cardiac cycle (row 1) showed a significant loss in accuracy for echocardiographic measurements when fewer than four cross sections were used; ESV alone showed a significant loss in accuracy when fewer than three

**Figure 5.** Least-squares linear regression of echocardiographically determined volumes on directly measured volumes for all 52 volumes. Each panel shows the relationship of echocardiographic and direct volume determinations for given number of cross-sectional echocardiographic slices per heart, in order of decreasing number. There are five hearts with seven loading conditions. Correlation coefficients: 15–19 slices/heart, \(r = 0.968; 8–10, r = 0.955; 5–6, r = 0.943; 4, r = 0.942; 3, r = 0.908; 2, r = 0.877; 1, r = 0.855\).

**Figure 6.** Comparison of correlation coefficients and variability from the regression analyses in figure 5. Despite the modest fall in the correlation coefficient when fewer than four cross sections were used for volume reconstruction, variability of the results, expressed as SEE, increased substantially. There are five hearts with seven loading conditions.
cross sections were used. Echocardiographic determinations of EF calculated from fewer than three cross sections (row 4) differed significantly from those calculated from the maximal number of cross sections. MVE (row 5) was significantly less accurate when calculations were made from one, two, or four cross sections per heart. There was no significant loss in accuracy for EDV (row 3) at any number of cross sections, and thus no multiple comparisons procedure was applied.

Discussion

In the isolated canine heart, under closely controlled conditions, highly accurate volumes with low variability in measurement can be obtained, but this accuracy begins to deteriorate significantly when fewer than four cross sections are used.

A test for accuracy of a new volume measurement technique would ideally compare measurements from the new method with the true volume of the object measured. We have used simultaneous direct measurements obtained with a method of known accuracy for the range of volumes studied. A test of accuracy should not only consider the correlation of measurements with the appropriate standard, but should also define the potential variability in measurements. This variability reflects how accurately the measurement predicts the true value. For example, Schiller et al. found a good correlation (r = 0.90) between biplane echocardiographic and angiographic determinations of ESV. Nonetheless, an echocardiographically determined ESV of 40 ml/m² would predict an angiographic volume of 40–90 ml/m², assuming 95% confidence limits (fig. 7 from that study). Thus, in this example, the variation in predicted angiographic volume would be greater than twofold despite a high correlation between echocardiographic and angiographic volumes. However, the potential variability in a measurement can define the ability of a given echocardiographic volume measurement to predict true volume. Least-squares linear regression analysis for comparison of measurements may be limited because although good correlations may exist, as in the example above, broad variability may result in low predictive accuracy of echocardiographic measures. To make allowances for the limitations of linear regression analysis, we compared the accuracy of echocardiographic measurements (i.e., the difference between echocardiographic

![Figure 7. Comparisons of the absolute difference between directly measured volume (V_D) and echocardiographically determined volume (V_E). The mean absolute difference is shown as an index of echocardiographic accuracy for the number of cross sections. Variability is shown by standard deviation. The statistical method (analysis of variance) applies to the absolute differences. Mean percent error = mean absolute difference divided by mean directly measured volume \times 100. There are five hearts with seven loading conditions.](http://circ.ahajournals.org/)

| TABLE 1. Mean Absolute Differences Between Directly Measured and Echocardiographically Measured Variables |
|---|---|---|---|---|---|---|---|
| No. of cross sections | 1 | 2 | 3 | 4 | 5 | 8 | 15–19 |
| All volumes | | | | | | | |
| \( \mid V_D - V_E \mid \) (ml) | 7.54* | 4.89* | 3.19* | 2.43 | 2.67 | 2.29 | 1.86 |
| ESV_D - ESV_E (ml) | 10.63* | 5.95* | 2.52 | 1.67 | 1.74 | 1.87 | 1.58 |
| EDV_D - EDV_E (ml) | 4.52 | 3.30 | 1.27 | 2.00 | 1.59 | 1.54 | 1.50 |
| EF_D - EF_E (%) | 11.9* | 8.2* | 4.8 | 6.7 | 3.8 | 3.9 | 3.4 |
| MVE_D - MVE_E (ml%) of cardiac cycle | 0.330* | 0.190* | 0.141 | 0.159* | 0.124 | 0.074 | 0.038 |

Means are row 1 calculated from 52 absolute differences from all five preparations under seven loading conditions and at all points in the cardiac cycle; in rows 2–5, from seven absolute differences from all five preparations, seven runs.

*Significantly different from mean derived from 15–19 cross sections (Waller-Duncan k ratio = 100).

Abbreviations: D = directly measured; E = echocardiographically measured; ESV = end-systolic volume; EDV = end-diastolic volume; EF = ejection fraction; MVE = maximal velocity of ejection in ml, normalized for cycle length.
volume and "true volume") at each number of cross sections per heart with the accuracy obtained with the maximal number of cross sections at 3-mm intervals.

Other investigators have emphasized the application of techniques requiring only one short-axis view and either one longitudinal view or a long-axis dimension, which may more easily be applied clinically or in the intact animal.\textsuperscript{3, 5} Such techniques are based upon major geometric assumptions that may limit their application in diseases that produce ventricular asymmetry or regional dysfunction. For one of these methods, it has been shown that in the presence of regional dysfunction, knowledge of such ventricular regional wall motion is required for accurate volume assessment by echocardiography.\textsuperscript{5} The easy application of such techniques to clinical and experimental situations may, moreover, be offset by limitations in accuracy and wide variability in volumes predicted by these methods. Even without precise localization of echocardiographic cross sections, several investigators have reported more accurate volume reconstruction from multiple short-axis cross-sections than with other methods.\textsuperscript{4, 5, 8-11} For these reasons, we used a method of echocardiographic volume assessment based on multiple short-axis, cross-sectional images of the left ventricle. By serially reducing the number of cross sections used for volume reconstruction, we sought to determine the effect on accuracy of volume measurement with methods that are potentially suited for clinical use, given accurate localization of cross-sectional slices. The highly controlled conditions provided by the in vitro preparation offer an experimental method most suited to answer this question because factors other than the number and location of cross sections are limited.

In the present study, maximal agreement between echocardiographic and direct measurements of ventricular volume was sought by correcting for a timing offset between echocardiographic and direct measurements, which resulted in the phase shift previously described. This synchronization error presumably arose from delayed display of pacing spikes on the ECG channel of the video display, which were used to synchronize echocardiographic fields and direct volume data. This phase shift would not affect the apparent accuracy of echocardiographic measurements of EDV and ESV judged as the maximum and minimum points, respectively, in the cardiac cycle, but would adversely affect agreement at intermediate points. Therefore, the effects of phase shift on comparisons of volume measurement were minimized to allow comparisons of echocardiographic accuracy only as a function of the number of cross sections.

Our data show a nonlinear decline in regression correlation and an increase in variability as the number of cross sections is reduced (fig. 6). The number of cross sections at which a significant loss in accuracy appears, relative to accuracy with cross sections at 3-mm intervals, was identified using the Waller-Duncan multiple-comparisons procedure. The number of cross sections required for preservation of maximal accuracy depends on the particular volume-related variable tested. Measurements of rates of volume change required the greatest number of cross sections. Maximal ejection rate would require no fewer than five cross sections of known location to maintain maximal accuracy. An exception to this may exist for maximal ejection rates calculated from three cross sections, only because of the inclusion of one cross section at the midventricular level where circumferential shortening may account for large changes in total volume. For examination of volume from all points in the cardiac cycle, four or more cross sections per heart were required for accuracy to remain statistically comparable to that with 15–19 cross sections. Additionally, except for calculations limited to four cross sections, EDV was measured more accurately than ESV. This difference may relate to the greater irregularity of the endocardial surface at end-systole, but the differences are minor when volumes are calculated from five or more cross sections. Although echocardiographic determinations of left ventricular EF showed no significant fall in accuracy when more than two cross sections per heart were used (table 1), the slightly greater accuracy of volume reconstructions from three as opposed to four cross sections also suggests an influence of the midventricular cross section on estimates of EF.

The results of the present study suggest that even under highly controlled conditions, accurate 2-D echo volume measurement in this beating heart preparation requires at least four cross-sectional, short-axis images of known location. This study is intended to define the requirements for accuracy of echocardiographic volume measurement under the most controlled circumstances and does not enable definition of the accuracy of the technique for studies in patients and intact animals, especially those with diseases that alter regional ventricular function and shape.

Further technical developments providing accurate localization of ultrasonic beam position\textsuperscript{15-20} and automated computer processing of echocardiographic images\textsuperscript{21-23} should lead to practical application to man of the methods evaluated in this study. Recent refinements in transducer localization using spark-gap techniques\textsuperscript{15} and mechanical arms\textsuperscript{19} have been particularly encouraging as crucial steps in three-dimensional reconstruction. A number of algorithms have recently been developed for this purpose.\textsuperscript{15, 16, 18, 20} These developing applications might be especially useful in patients with diseases that alter ventricular shape and function regionally, where the utility of models requiring major geometric assumptions would be limited.

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