Overestimation of infarct size by quantitative two-dimensional echocardiography: the role of tethering and of analytic procedures

THOMAS FORCE, M.D., ANDREW KEMPER, M.D., LORRI PERKINS, B.A., MICHELE GILFOIL, B.S., CAROL COHEN, B.S., AND ALFRED F. PARISI, M.D.

ABSTRACT Analyses of regional left ventricular systolic wall motion or thickening overestimate infarct size. We used quantitative two-dimensional echocardiographic analysis of systolic thickening and contrast two-dimensional echocardiography to evaluate causes for that overestimation. The following possibilities were considered: (1) "tethering," defined as dysfunction of contrast-enhancing myocardium adjacent to ischemic or contrast-negative regions, and (2) the role of standard center of mass analysis algorithms, which may overestimate wall motion abnormalities because of the axis shift produced by simultaneous systolic expansion of the ischemic segment and systolic contraction of the nonischemic segment. In the short-axis view in 12 animals, the echo contrast defect (ECD) occupied 32 ± 7% of the left ventricular circumference. Extent of dysfunction by the center of mass analysis was 39 ± 5% of the left ventricular circumference and correlation with ECD size was .68 (SEE = 5.2%). Thus 8 ± 6% of the circumference of the left ventricle was assessed to be dysfunctional yet enhanced with contrast. Tethering accounted for only half of this (4 ± 4% of left ventricular circumference) and involved less than 1 cm on either side of the ECD. The remaining overestimation by the center of mass analysis correlated significantly (r = .89, p < .01) with the amount of systolic expansion of the ECD. This expansion of the ECD (increase in angle subtended by the ECD of 11 ± 8%) was produced by the systolic shift in the center of mass toward the dysfunctional segment from contraction of the opposite, nonischemic segment, since true systolic lengthening of the ischemic (contrast-negative) segment was minimal (increase of only 3 ± 5%; p < .01 vs increase in angle subtended by ECD). When systolic function was analyzed independent of a center of mass with the ECD as an internal reference, the correlation between extent of dysfunction and ECD size improved to .84 (SEE = 3.8%). In conclusion, two-dimensional echocardiography has exaggerated the importance of tethering because of flaws in standard analysis algorithms. Tethering does lead to an unavoidable overestimation of infarct size, but the amount of myocardium involved is small and relatively predictable. The remainder of the overestimation of infarct size by two-dimensional echocardiography is critically dependent on systolic function of the opposite, nonischemic wall. Since this is variable, it accounts in large part for the suboptimal correlation between infarct size and extent of dysfunction by standard two-dimensional echocardiographic analyses.

Circulation 73, No. 6, 1360–1368, 1986.
dimensional echocardiographic data. First, the studies done to date have all required precise retrospective matching and aligning of the two-dimensional echocardiographic section with the pathologic section, a process that relies on internal landmarks that are distorted by the postmortem processing. Second, Armstrong et al., in a preliminary report, found that at least some portion of this overestimation of ischemic region size was caused by inherent limitations of the two-dimensional echocardiographic analysis procedures used to evaluate regional left ventricular function. These procedures analyze thickening on a radial coordinate plot emanating from a floating center of mass and express extent of dysfunction as a percentage of the diastolic left ventricular circumference. However, extent of dysfunction is actually determined by the percentage of the circumference of the systolic image that thickens abnormally (figure 1). Thus the center of mass analyses may overestimate the size of the pathologic ischemic region if the percent circumference of the ischemic segment in systole is greater than it is in diastole. This can occur by two mechanisms. One is true lengthening of the ischemic segment and the other is shortening of the nonischemic segments in systole, both of which are believed to occur.

Although neither of these problems pertain to sonomicrometry, this technique is limited by its inability to examine more than a few isolated regions of any ventricle. Therefore, we designed this study to examine the determinants of the overestimation by two-dimensional echocardiographic analysis of the size of the ischemic region to determine how important tethering is in relation to overall left ventricular function and to examine some of the factors that may contribute to tethering. We took advantage of contrast echocardiography, which allowed us to delineate in vivo the border between ischemic and nonischemic myocardium and to analyze systolic thickening with an internal reference, thus avoiding the potential limitations of the percent circumference analysis. Tethering was then defined as systolic dysfunction of border myocardium that enhanced with contrast.

Methods

Experimental animal preparation. Twelve dogs were premedicated with 1 mg/kg intramuscular morphine sulfate, anesthetized with 5 mg/kg pentobarbital, intubated, and placed on a Harvard respirator. Arterial blood gas determinations ensured adequate oxygenation and ventilation. The heart was exposed through a left fifth intercostal space thoracotomy and a pericardial sling was constructed. The right femoral artery and left atrial appendage were cannulated for pressure monitoring and the electrocardiogram, arterial pressure, and left atrial pressure were continuously recorded on a Grass polygraph. The left circumflex coronary artery distal to the first obtuse marginal artery was dissected free. Lidocaine was given in a dose of 1.5 mg/kg and the pericardial sling was released. The pericardium was not closed. Five minutes later the circumflex artery was occluded with a suture and the chest was closed. An additional dose of lidocaine (1 mg/kg) was given 7 min after occlusion. All animals were killed at 6 hr after occlusion.

Contrast echocardiography. The technique of supra-aortic hydrogen peroxide (H2O2) injection for delineation of region at risk of infarction has been outlined in previous communications. Briefly, a mixture of 2 ml of 0.3% H2O2, mixed with 1 ml of autologous blood was flushed into the aortic root through a modified No. 8F pigtail catheter. The echocardiographically defined region at risk, or the echo contrast defect (ECD), is that myocardium which fails to enhance with ultrasonic contrast. In our laboratory, the size of the ECD has been shown to be highly correlated to region at risk determined by monastral blue (r = .93, SEE = 7.7%) or autoradiographic techniques (r = .89, SEE = 4.5%).

We have shown previously that amount of echo contrast enhancement, assessed qualitatively or quantitatively, accurately reflects the amount of regional myocardial blood flow measured by radioactive microspheres. In the Appendix we have expanded these observations. These data are a topographic plot of microsphere-determined transmural regional myocardial function.
blood flow measured on either side of the ECD border. These and previous data confirm that the contrast two-dimensional echocardiographic border accurately delineates the border between ischemic and nonischemic myocardium whether assessed with monastral blue, autoradiography, or radioactive microsphere–determined blood flows.

**Image acquisition.** Animals were imaged from the right parasternal transducer position with a 90 degree mechanical sector scanner (ATL-ADR, Advanced Technology Labs, Inc.). A Sony Betamax videotape recorder was used to record all images for later analysis.

Imaging was performed at the short-axis level that included mitral chordae and was just superior to the papillary muscles. After preoclusion two-dimensional echocardiographic studies in all animals, the circumflex coronary artery was ligated as outlined above. At 120 min after occlusion, the animals were imaged for analysis of systolic thickening. To avoid any potential depression of regional function resulting from H$_2$O$_2$, the supra-aortic injection was performed at the end of the imaging period. The imaging period from the time of systolic thickening analysis to the delineation of the ECD was no longer than 10 sec, during which the transducer position was constant.

**Delineation analysis.** Endocardial and epicardial outlines of the left ventricle at end-diastole (defined as peak of R wave) and end-systole (defined as the smallest cavity area) were traced onto acetate overlays. After each tracing was completed, the video tape was advanced to the supra-aortic H$_2$O$_2$ injection and ECD was marked directly onto the tracings for both end-diastole and end-systole.

Systolic thickening analysis was performed by two methods. The first method, hereafter referred to as the center of mass analysis (figure 1), was a typical computer-generated short-axis analysis system with a radial coordinate plot emanating from a floating center of mass (Franklin Quantic 1200, Franklin Industries, Inc.). Epicardium and endocardium for both diastole and systole were traced onto a digitizing tablet with a light-pen system. The computer generated the center of mass for the diastolic image by averaging the epicardial and endocardial centers of mass. It then constructed a radial coordinate plot and determined end-diastolic thickness along 64 radii. Similarly, a center of mass was generated for the systolic image and end-systolic thickness was determined along 64 radii. Percent systolic thickening was calculated for each radius from the formula: (thickness at end-systole – thickness at end-diastole)/(thickness at end-diastole × 100%). Thus, as illustrated in figure 1, with this system analogous portions of the ventricle (for determination of percent thickening) are matched by their positions on the diastolic and systolic radial coordinate plots (e.g., radius 1 in diastole is matched with radius 1 in systole by their equivalent positions relative to the horizontal). As illustrated in figure 1, with asymmetric contraction similarly numbered radii may not intersect the same segment of myocardium in diastole and systole.

We have found systolic rotation to be a minor phenomenon at the mitral chordae level in the infarcted canine heart, as Schnittger et al. found in the human heart. In this series, systolic rotation (using the ECD borders as references) averaged only 3.5 ± 4 degrees. Consequently, for the center of mass analysis we did not realign the systolic tracing with the diastolic one.

The second method, hereafter referred to as the internal reference analysis, has been described previously and is illustrated in figure 2. With the same tracings employed in the center of mass analysis, radii are placed every 4.5 degrees on both the diastolic and systolic outlines. Analogous radii are determined by their positions relative to the border of the ECD (e.g., radius 1 is the first radius outside the contrast defect border in both diastole and systole). Percent systolic thickening is again determined as indicated above. Amount of myocardium tethered as determined by this method is the extent (percent circumference) of dysfunction outside of the ECD.

To examine severity of dysfunction in border regions after coronary occlusion, a preocclusion baseline value was needed. Precise matching of identical segments of myocardium before and after occlusion is difficult because the internal reference of the ECD border is absent before occlusion. Consequently, preocclusion systolic thickening (control, figure 3) was taken to be the mean systolic thickening of six radii within a region of myocardium matched to the postocclusion border region by its similar position on the diastolic tracing using other available landmarks (insertion of the right ventricular free wall and the position of the mitral chordae).

For this series of animals, less than 10% systolic thickening...
was selected as the cutoff value to identify abnormal segments, since baseline (preocclusion) examinations showed fewer than 5% of radii with less than 10% systolic thickening. However, since a 0% systolic thickening cutoff has also been used in studies of infarct size determination,4, 5, 26 data reflecting a 0% cutoff are also presented.

The determination of changes in length of the ischemic and nonischemic segments in systole was performed with a digitizing tablet and light-pen system (Microsonics CAD 888). The average of the endocardial and epicardial lengths of the ECD were determined for both diastole and systole, and then a percent expansion was generated from the formula (length at end systole − length at end diastole)/length at end diastole × 100%. Increase in percent circumference of the region at risk was determined by measuring the angle subtended by the ECD in diastole (relative to the diastolic center of mass) and in systole (relative to the systolic center of mass).

Reproducibility in the determination of region at risk by the supra-aortic H2O2 method has been examined previously in our laboratory. Correlation coefficients for both intraobserver and interobserver variability have been greater than .94 and SEEs have been 3.2%.21 In this study, reproducibility of the percent circumference for both the end-diastolic and end-systolic ECD was examined. For the end-diastolic ECD, r = .89 and SEE = 4%. For the end-systolic ECD, r = .94 and SEE = 3.6%. For absolute length of the diastolic and systolic ECDs, r = .94 and SEE = 0.4 cm. Reproducibility for the center of mass analysis has been examined previously, and the mean difference in extent of abnormal thickening between paired observations was 4.9 ± 1.4%.9 In this study, the correlation between observers’ estimates of extent of dysfunction (<10% systolic thickening) by the internal reference analysis was r = .91 and SEE = 3.9%. The mean difference between observers’ estimates of the amount by which the center of mass analysis overpredicted extent of dysfunction determined by the internal reference analysis was 1.5 ± 1.2% of the circumference of the ventricle.

Pathologic analysis. Immediately before animals were killed (at 6 hr after occlusion), their chests were reopened. Monastral blue pigment (0.5 ml/kg) was then injected via the left atrial line22 and 20 sec later the animals were killed with potassium chloride. The heart was excised, frozen with liquid refrigerant, and cut into 1 cm thick slices from apex to base. The pathologic slice corresponding to the two-dimensional echocardiographic section imaged was identified as the one immediately superior to the section containing the tips of the papillary muscles. Since the systolic thickening analyses were referenced to the region at risk (ECD) in vivo rather than to a pathologic one, intramyocardial markers that might affect systolic function were avoided. However, we have repeatedly shown this to be an easily identifiable pathologic section and have found high correlation between ECD and pathologic region at risk whether or not markers are used.21–23, 25

The pattern of monastral blue staining on both sides of the interest slice was traced on an acetate overlay and the slices were photographed. The slices were then incubated in triphenyltetrazolium chloride (TTC) for determination of infarct size. Tracings were again made of both sides of the interest slice on acetate overlays. Size of the region at risk was the mean of the percentage of the left ventricular circumference from both sides of the pathologic slice that did not stain with monastral blue. Infarct size was the mean of the areas of myocardium on both sides of the pathologic slice that did not stain with TTC and was expressed as a percentage of the area of myocardium not stained with monastral blue.

Statistical analysis. Significant differences between paired data were determined by Student’s t test for dependent variables. Where multiple comparisons were required, a randomized block-design analysis of variance and Tukey’s formula were used. All data are presented as the mean ± SD. One-variable linear regression techniques were used exclusively.

Results

Accuracy of contrast two-dimensional echocardiography. The correlation between region at risk determined by monastral blue and the diastolic ECD was .89 with an SEE of 3.4%. Since changes in size of the region at risk caused by changes in collateral flow over the course of an experiment could significantly alter conclusions on extent of tethering, the stability of the size of the ECD over time (at 30 min, 2 hr, and 6 hr) was examined. No two observations differed by more than 5%. Correlation coefficients for each examination vs the others ranged from .90 to .95 and SEEs were 2.5% or less (table 1).

Determinants of functional overestimation of the size of the ischemic region

Tethering. Contrast two-dimensional echocardiographic ischemic region size was 32 ± 7% of the diastolic left ventricular circumference. With the ECD-based internal reference analysis, extent of dysfunction was 36 ± 7% of the left ventricular circumference. Thus 4 ± 4% of the left ventricular circum-

<table>
<thead>
<tr>
<th>Temporal stability of ECD</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>30 min vs 2 hr</td>
</tr>
<tr>
<td>30 min vs 6 hr</td>
</tr>
<tr>
<td>2 hr vs 6 hr</td>
</tr>
</tbody>
</table>

TABLE 1
ference functioned abnormally (systolic thickening < 10%) but was enhanced with contrast (i.e., tethered). An alternative means of examining extent of tethering is illustrated in figure 3, which compares control (preocclusion) systolic thickening to mean percent systolic thickening after occlusion for the first six radii within normally contrast-enhancing myocardium on either side of the contrast defect. Systolic thickening for the fourth radius (5% of the left ventricular circumference) from the ECD border anteriorly and third radius posteriorly was not significantly different than control. Since the average midmyocardial circumference was 15.7 ± 1.5 cm, systolic thickening was not significantly different than that before occlusion, 0.8 cm outside of the ECD border.

Using the internal reference analysis, we examined several aspects of the size and function of the ischemic zone to determine whether they were related to the size of the tethered region or severity of the dysfunction within that region. No significant correlations were found between extent of tethering (number of radii with < 10% systolic thickening with the internal reference analysis) and size of the region at risk (r = .50), percentage of the region at risk that was infarcted (r = .16), or degree of systolic dysfunction of the adjacent ischemic region (first six radii inside the contrast border) (r = .09; p > .05 for all). Similarly, no significant correlations were found between mean percent thickening of the first six radii outside the contrast border and size of the region at risk (r = −.20), percentage of the region at risk that was infarcted (r = −.30), or degree of systolic dysfunction of the adjacent ischemic region (r = .28; p > .05 for all). There was, however, a weak relationship between systolic function before and after occlusion in the tethered region. Mean percent thickening after occlusion of the six radii outside the contrast border correlated significantly with the baseline (preocclusion) mean percent thickening in that region (r = .70, p < .05).

Analytic procedures. With the center of mass analysis, 39 ± 5% of the left ventricular circumference was dysfunctional. ECD size (32 ± 7%) was overestimated by 27 ± 22%, and 8 ± 6% of the left ventricular circumference was assessed to function abnormally but was enhanced with contrast (tethered). This was significantly more than the 4 ± 4% of the left ventricular circumference that was tethered by the internal reference analysis of the same tracings (p < .01, figure 4). The relationship between extent of dysfunction by the internal reference analysis and ECD size (r = .84, SEE = 3.8%, p < .01) was better than that between the center of mass analysis and ECD size (r = .68, SEE = 5.4%, p < .05). The difference between apparent extent of dysfunction by the center of mass analysis and extent of dysfunction by the internal reference analysis was 4 ± 3%. Figure 5 compares this difference to the estimate by center of mass analysis of the amount of systolic expansion of the ECD. The correlation between the systolic expansion of the ECD by the center of mass analysis and the overestimation of dysfunctional region size by that analysis (with a 10% systolic thickening cutoff) was .89 (p < .01). The correlation with a 0% systolic thickening cutoff (r = .66) was also significant (p < .05). Thus, independent of the lower limit of normal thickening chosen, the greater the systolic expansion of the ECD, the greater was the overestimation of dysfunctional region size by the center of mass analysis.

Figure 6 examines the mechanism of the systolic expansion of the ECD seen with the center of mass analysis. As a percentage of the circumference, the ECD increased by 11 ± 8% from diastole to systole. However, percent increase in absolute length was minimal (3 ± 5%; p < .01 vs increase in percent circumference). The majority of increase in the percent
circumference of the ECD was caused by a contraction in the length of nonischemic (contrast-enhancing) segments, which averaged 13 ± 5% (figure 7).

Discussion

Quantitative analysis of regional wall motion from two-dimensional echocardiograms consistently overestimates the extent of ischemia or infarction at pathologic examination. That is, myocardium adjacent to an area of ischemia appears to be dysfunctional even though there is no evidence of ischemia. Falsetti et al. have reviewed some possible causes for this. One relates to the temporal relationship between ischemia and mechanical dysfunction. After a period of transient ischemia insufficient to produce infarction, mechanical abnormalities may persist for prolonged periods. In the canine preparation of acute infarction there may be recruitment of collaterals leading to increases in flow into the ischemic region. Thus myocardium that is ischemic early after coronary occlusion may have recovery of flow to normal or near-normal levels, yet recovery of function often takes much longer. This could lead to dyssynergy that is apparently outside the region at risk at time of death and thus to overestimation of the size of the ischemic region by wall motion analyses. Contrast two-dimensional echocardiography can separate those animals with improving flow in the ischemic region from those without such increases. All animals in this series had stable contrast-determined regions at risk throughout the experiment.

Tethering or dysfunction of nonischemic myocardium adjacent to ischemic or infarcted myocardium is the second reason wall motion analyses may overestimate infarct size. Homans et al. found that segment shortening of subendocardial microcrystal pairs placed 1 cm or more from the border of TTC staining was equal to preocclusion levels. Similar results were found by Gallagher et al. As noted, however, sonomicrometer data such as these do not allow characterization of the dimensions of the hypofunctioning zone. In attempting to quantify this with two-dimensional echocardiography, Lima et al. found that systolic dysfunction involved an extensive portion of the ventricle: extent of dysfunction exceeded the boundaries of the ischemic myocardium by an average of 2.49 cm. Other echocardiographic studies have also found dysfunction outside the ischemic region to be an extremely important phenomenon.

Our data may help to reconcile the differences between studies using two-dimensional echocar-
graphic techniques and those using sonomicrometers, which are independent of reference systems. Our data, which were obtained with contrast echocardiographic landmarks for the border between ischemic and normally perfused myocardium, agree closely with the sonomicrometer data of Homans et al.\textsuperscript{16} We found that tethering involved only about 5\% of the circumference of the ventricle (or approximately 0.8 cm) on either side of the ischemic region. Yet when we analyzed the same tracings with a typical center of mass system, we, like Lima et al.,\textsuperscript{8} found far greater overestimation of ischemic region size. Our contrast two-dimensional echocardiographic data suggest that the discrepancies are caused by an inherent limitation of two-dimensional echocardiographic analysis systems. These systems employ a floating center of mass (epicardial, endocardial, or an average of the two) so that the radii generated will intersect the myocardial wall perpendicularly to the tangent. The extent of dysfunction is therefore defined by the percent circumference of the systolic rather than diastolic image that has failed to thicken. We found that in systole, the percentage of the left ventricular circumference occupied by the ischemic segment (or ECD) increased significantly and that the amount of this increase was directly proportional to the amount by which the center of mass analysis overestimated extent of dysfunction.

Of note, we found that the systolic increase in percent circumference of the ischemic segment was due more to shortening (or decrease in percent circumference) of the nonischemic segment than to lengthening of the ischemic segment. This implies that the more hypercontractile normal myocardium is, the more will the center of mass analysis overestimate extent of dysfunction. Similarly, the less hypercontractile normal myocardium is, the less will extent of dysfunction be overestimated. Thus the finding by Lima et al.\textsuperscript{8} of a decrease in tethering after propranolol may be due to a decrease in contractile function of the normal regions rather than to improved function of the tethered zone.

**Mechanisms of tethering.** We examined several variables of ischemic zone size and function to determine whether they had any influence on tethering. We found that only baseline (preocclusion) systolic thickening had a significant relationship to postocclusion systolic function of the region bordering the ischemic zone. None of the variables pertaining to the ischemic zone (size, percent infarcted, or degree of systolic dysfunction) appeared to have any influence on function of the border region. Homans et al.\textsuperscript{16} also found no relationship between the size of the ischemic zone and severity of tethering. The mathematical model of the acutely infarcted ventricle described by Bogen et al.\textsuperscript{30} may partially explain this, since it predicts that the increased wall stress in myocardium adjacent to the ischemic zone would not depend on the size of the ischemic zone. In any case, we have found tethering to involve such a small and minimally variable amount of myocardium that it would be difficult to show a significant relationship to any variable.

In summary, we found that center of mass analyses of regional wall motion or thickening overestimate ischemic region size for two reasons. Tethering accounts for about half of the overestimation but involves a quantitatively small amount of myocardium and is relatively predictable. The extent of true systolic dys-
function closely reflects extent of ischemia. An inherent limitation of the floating center of mass analysis procedures commonly employed results in the remainder of the overestimation of ischemic region size and exaggerates the significance of tethering. The degree of overestimation is primarily dependent on the systolic function of the nonischemic segment. Because this varies in an unpredictable fashion, it is responsible to a large extent for the suboptimal relationship between two-dimensional echocardiographic estimation of extent of dysfunction and infarct size.

Appendix

Figure 8 shows a topographic plot of microsphere-determined transmural regional myocardial blood flow (mean ± SD) on either side of the border of the echocardiographic contrast defect (ECD). Data are derived from 20 simultaneous microsphere and echocardiographic contrast injections in animals with stable ECDs over time by methods described previously. Each echocardiographic segment is 4.2% of the circumference of the ventricle. Echocardiographic segments to the left of the vertical line delineating the ECD border are the first three contrast-enhancing segments outside this border. Those to the right are the first two noncontrast-enhancing segments inside the ECD border and the segment at the center of the contrast-negative region (CI).

Flow for each segment within the ECD is significantly less than flow for each of the contrast-enhancing segments (*p < .05 by analysis of variance and Tukey’s formula). Flow at the center of the ischemic zone (CI) is not significantly different than flow for other segments within the ECD.

References


2. Weiss JL, Bulkley BH, Hutchins GM, Mason SJ: Two-dimension-
gen peroxide contrast echocardiography: in vivo quantification of risk during coronary occlusion and salvage following myocardial reperfusion. Circulation 70: 309, 1984


Overestimation of infarct size by quantitative two-dimensional echocardiography: the role of tethering and of analytic procedures.

T Force, A Kemper, L Perkins, M Gilfoil, C Cohen and A F Parisi

_Circulation_. 1986;73:1360-1368
doi: 10.1161/01.CIR.73.6.1360

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/73/6/1360

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/