Repeatability of Estimates of Left-Ventricular Volume from Blood-Pool Counts: Concise Communication

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Radionuclide ventriculography permits nongeometric calculation of ventricular volume. Accurate and reproducible determination of left-ventricular (LV) blood-pool counts is necessary to perform this calculation. Furthermore, to make serial volume determinations one must know the half-time of in vivo blood-pool activity. We compared five methods of LV count determination in nine patients. Interpatient and intrapatient variability of the in vivo half-time of Tc-99m-labeled red blood cells (RBCs) was measured. Left-ventricular count determinations, derived from temporally and spatially smoothed images using a second-derivative algorithm to identify the LV region of interest (ROI), are less variable than those based on manual ROI determinations. The mean in vivo half-time of Tc-99m RBCs is 4.1 hr, and there is significant interpatient (0.9 ± 0.8 hr) and intrapatient (1.0 ± 0.9 hr) variability. These findings should be considered in the determination of serial, relative ventricular volume by radionuclide ventriculography.

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Hemodynamic data are based on measurements of time, pressure, and volume. The ability to compare these data among patients and different investigators is hindered by the absence of an accurate and universal method for noninvasive determination of ventricular volume.

This volume can be calculated from two-dimensional images obtained by contrast ventriculography (*1*), two-dimensional echocardiography (*2*), or radionuclide ventriculography (*3*). These methods make geometric assumptions in invoking a standard configuration for every ventricle, and thus do not allow for complex variations of ventricular shape that occur in health and disease.

Radionuclide blood-pool images provide an alternative method of ventricular volume determination wherein counts arising from the ventricle divided by counts arising from a standard volume of the patient's blood (corrected for tissue attenuation) yield ventricular volume. This calculation is free of the above geometric limitations and the principle is now being widely used (*4–8*).

In order to invoke a protocol for serial determinations of relative ventricular volume by a count method, two practical problems must be addressed.

1. Left-ventricular (LV) end-diastolic count determination is dependent on accurate assignment of ventricular cavity edge and background. The various methods for edge assignment now used (manual method and semiautomated second-derivative algorithm) require objective comparison of their accuracy and reproducibility.

2. Serial ventricular volume measurements by count methods in a single patient (for example, during an intervention or during exercise) should allow for in vivo decay of blood-pool activity.

The problem of tissue attenuation is avoided in serial determinations of relative ventricular volume because this factor remains constant.

In this study we compared the reproducibility of LV end-diastolic count determination by a single observer using five standard methods of ventricular edge detection and background subtraction.

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TABLE 1. PATIENT DATA

Patient	Sex	Age	Dx.	Medication	Creatinine clearance (ml/min)
1	M	51	CAD	digoxin	58
2	M	61	CAD	propranolol isosorbide dinitrate perhexilene	87
3	M	58	CAD	metoprolol isosorbide dinitrate trimethropin and sulfamethoxazole heparin	73
4	M	40	CAD	digoxin furosemide hydralazine sodium warfarin amiodarone	69
5	F	68	presyncope	propranalol aspirin quinidine	101
6	M	72	CAD	propranalol digoxin quinidine levodopa and benserozide	38
7	M	66	CAD	propranolol isosorbide dinitrate	80
8	M	58	pericarditis	alphamethyldopa salbutamol ibuprofen	133
9	M	58	CAD	metoprolol isosorbide dinitrate digoxin furosemide	83

STUDY POPULATION

This comprised nine adults, two females, and seven males, aged 40 to 72. Seven patients had clinical and/or angiographic evidence of coronary artery disease, one had presyncope of unknown origin, and one had pericarditis. All were able to lie supine comfortably, were in sinus rhythm, were free of congestive heart failure, and had no significant "third space" fluid retention. Serum electrolytes, hemoglobin, and red blood cell (RBC) indices were normal. Creatinine clearance was measured by 24-hr urine collection. Medications were as listed in Table 1. Each patient provided informed consent to the following protocol.

PROTOCOL

The procedure was carried out on two occasions in each subject, with at least 24 hr between studies. Eighteen patient studies were thus obtained in nine patients.

Patients fasted for at least 4 hr before each study, then were positioned supine on an imaging table. A catheter was inserted in an antecubital vein and stannous pyrophosphate (2.5 mg $SnCl_2 \cdot 2H_2O$, 40 mg $P_2O_7^{4-}$, 100 mg $Na_4P_2O_7 \cdot 10H_2O$) was administered. After 15 min 25 mCi of Tc-99m as approximately 1 ml of $NaTcO_4$-saline solution was injected to effect blood-pool labeling. The catheter was flushed with 10 ml of heparinized saline. Blood aliquots (3 ml) were withdrawn at 5, 15, 30, 60, 90, and 120 min after labeling. Resting gated radionuclide ventriculograms were acquired at the same six times that the blood samples were obtained, and heart rate and blood pressure were recorded.

In three patient studies blood samples were taken before and 1 hr after labeling for measurement of RBC osmotic fragility (*9*).

Venous blood counting. The hematocrit of each sample of labeled venous blood was measured in triplicate by centrifugation in a capillary tube. One-ml blood aliquots were pipetted from each sample into a preweighed, heparinized counting tube and reweighed with an analytical balance. These samples were then lysed with saponin. The remaining portion of each blood sample was

separated by centrifugation and 1-ml samples of plasma were drawn off and weighed in the same manner.

Counts per second per gram of each weighed sample of blood and plasma were measured in a single-channel scintillation counter and corrected for in vitro decay from the time of withdrawal by the formula:

$$a_0 = ae^{\lambda t}$$

where a_0 = corrected activity (cps/g)
a = measured activity (cps/g)
λ = >0.1155/hr
t = time from specimen withdrawal to well counting (hr).

Imaging technique. Resting ECG-gated radionuclide ventriculograms were obtained using a mobile small-field-of-view camera with a general-purpose parallel-hole collimator. A 45° left anterior oblique projection with 5° to 15° of caudal angulation was used, with adjustment to optimize ventricular separation and minimize left-atrial overlap. Counts were collected in histogram mode in a 64 × 64 matrix, with 28 frames per cardiac cycle and a total of 500 cardiac cycles. Patients were able to rest comfortably on large cushions without moving during and between acquisition images.

Left ventricular end-diastolic count determination. Five methods of determining LV end-diastolic count (LVEDC) were applied to each radionuclide ventriculogram.

Method 1. The end-diastolic image was identified from a time-activity curve generated from a single, manually designated LV region of interest (ROI). A nine-point spatial smoothing filter was applied and the count thresholds were adjusted to facilitate manual identification of the LV border. A background ROI was then automatically assigned outside the LV free wall at end-diastole, using user-modified commercial software. Background was assigned as a two-pixel-wide region removed from the assigned LV border by two pixels between 3 and 5 o'clock. Background-corrected LVEDC was computed and LVEDC per second was then calculated by the formula:

$$\text{LVEDC/sec} = \frac{\text{LVEDC} \times 1000}{\text{end-diastolic image time (msec)} \times 500 \text{ cardiac cycles}}$$

Method 2. This differed from Method 1 only in its strategy for background ROI. This was assigned by the same computer algorithm but it was applied to the manually identified end-systolic LV ROI. The background activity was used to correct the unprocessed LVEDC as in Method 1.

Method 3. This method also differed only in its strategy for background correction. The mean of the average background counts per pixel of Methods 1 and 2 was used to correct LVEDC as in Methods 1 and 2.

Method 4. The LV end-diastolic and end-systolic images were identified from an automatically generated time-activity curve using multiple regions of interest. The LV edge was assigned by a standard commercial computer program that calculated the second derivative of count profiles through the LV and designated points where the second derivative equaled zero above a minimum-count threshold that was independently designated for each quadrant. A background ROI was assigned outside the end-systolic ROI in a manner similar to that in Method 2, and LVEDC/sec (corrected for background) was calculated as before.

Method 5. This method was identical to Method 4 except that a 5 by 5 point spatial smoothing filter and a 5-point temporal image smoothing filter were used to facilitate computer-automated edge identification.

DATA ANALYSIS

Venous blood counts. For each patient study, venous blood cps/g, back-corrected to time of sampling, was plotted against time. Venous blood counts varied unpredictably between samples at 5 and 15 min and at 15 and 30 min, with dramatic increases and rapid decreases seen in a number of patient studies (Fig. 1). Between 30 and 120 min, semilogarithmic plots of these counts against time satisfied Fisher's analysis-of-variance test for linearity, showing that the in vivo decay of Tc-99m activity bound to erythrocytes by our method is monoexponential. In vivo half-time of this activity was calculated from the slope of this line in each patient study.

In every patient study, RBC labeling efficiency was calculated at each sampling time using the formula:

$$\text{Labeling efficiency (\%)} = \frac{\text{BC} - [\text{PC}(1\text{-hematocrit})] \times 100\%}{\text{BC}}$$

Where:

BC = blood cps/g
PC = plasma cps/g

Left-ventricular end-diastolic counts. In a manner analogous to that used for venous blood counts, semilogarithmic regression lines of LVEDC/sec against time were created for the LVEDC determinations at 30, 60, 90, and 120 min of ten patient studies by each of the five methods. LVEDC "half-times" were then calculated. The other patient studies were excluded from this analysis as explained below.

Comparison of methods of LVEDC determination. If a method of LV edge detection and background subtraction is reproducible, and if LV end-diastolic volume does not change, then the half-time of decay of LVEDC should equal the half-time of decay of venous blood counts. We assumed that LV end-diastolic volume did

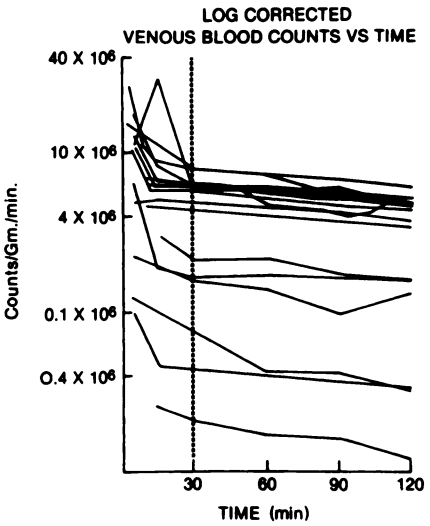

FIG. 1. Semilog plot of venous blood cpm/g plotted against time in 18 patient studies. Decay is monoexponential from 30 to 120 min after labeling (time 0).

not change significantly between 30 and 120 min after RBC labeling if heart rate and blood pressure remained stable over this period—that is, within 5% of the mean value during the study. These exclusion criteria removed seven patient studies from further analysis. One further patient study was excluded (see Results—Venous blood counts).

To test the decay relationships, we plotted venous blood count half-times against LVEDC "half-times" for the ten patient studies in which LV volume may be assumed to have been constant. A plot was constructed for each of the five methods of LV end-diastolic count determination (Fig. 2 shows the plot for Method 5). Each point in this plot represents a patient study in which venous blood count half-time is derived from four serial venous blood counts between 30 and 120 min, and

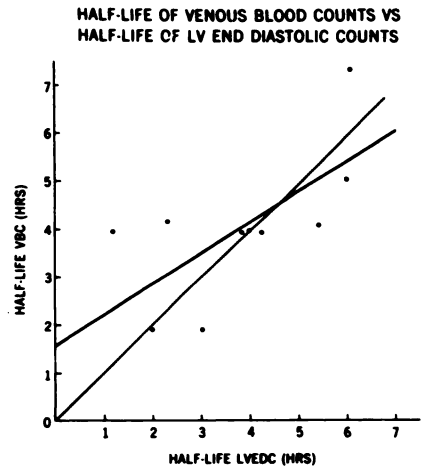

FIG. 2. Example of plot of venous blood count half-times plotted against half-times of left-ventricular end-diastolic count (this being for Method 5) in ten patient studies where left-ventricular end-diastolic volume is assumed to have been constant.

TABLE 2. HALF-TIMES FOR VENOUS BLOOD ACTIVITY

Patient	Half-time (min) Study No. 1	Study No. 2
1	118	84
2	239	302
3	253	251
4	239	116
5	207	237
6	239	226
7	202	248
8	146	1319*
9	441	287

* Excluded from further analysis.

LVEDC half-time is derived from four corresponding serial radionuclide ventriculograms. A line of identity (shown as a broken line) would result in the ideal case of a method that reproducibly identified the LV blood pool, whose volume did not change over time.

RESULTS

Venous blood counts. The half-times of decay of venous blood counts for each patient study are tabulated in Table 2. One result has been excluded as it was not within 4 s.d. of the mean. The mean in vivo half-time of Tc-99m bound to red blood cells by our technique is 4.1 ± 1.4 hr, compared with the Tc-99m physical half-life of 6 hr. The interpatient variability, calculated as the mean difference in each patient's mean half-time (two patient studies per patient) from the overall mean half-time, was 0.9 ± 0.8 hr. The intrapatient variability calculated as the mean difference between each patient's two studies was 1.0 ± 0.9.

Means and standard deviations of RBC labeling efficiency at the time of each sample withdrawal are

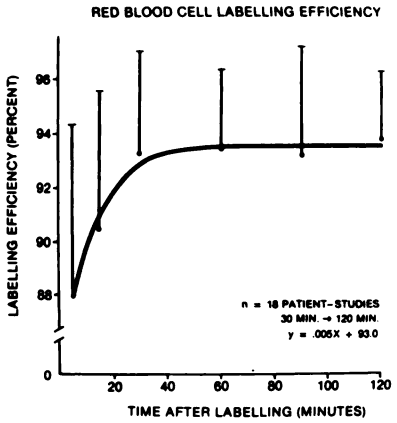

FIG. 3. RBC labeling efficiency (mean and s.d.) plotted against time after labeling. Equation represents least-squares regression line between 30 and 120 min (Upper half of s.d. shown).

TABLE 3. HALF-TIMES (min) DETERMINED FROM VENOUS BLOOD COUNTS AND LEFT-VENTRICULAR END-DIASTOLIC COUNTS

Patient study	Venous blood cps/g	Method 1	Method 2	Method 3	Method 4	Method 5
1	118	144	102	120	583	181
1	239	103	151	125	94	239
2	302	338	69	316	507	359
2	251	219	182	199	185	138
1	239	247	224	235	229	255
1	116	235	373	289	202	120
2	237	223	281	249	424	230
1	239	74	70	73	86	101
2	248	723	344	456	359	325
1	441	145	83	102	497	364
Mean	243	245	188	216	416	231
Standard deviation	91	185	114	118	418	97

plotted in Fig. 3. Labeling efficiency increased until 30 min after injection of Tc-99m. The higher proportion of unbound Tc-99m up to this time corresponds to the duration of nonlinear exponential decay of blood-pool activity. From 30 to 120 min after labeling, mean labeling efficiency is constant at 93% (Fig. 3). Over the same period, decay of blood activity is monoexponential (Fig. 1).

Left-ventricular counts. LVEDC half-times were determined from the semilogarithmic least-squares regression lines of LVEDC against time. The half-time for the ten patient studies using the five methods of LVEDC determination are shown in Table 3, with the corresponding venous cps/g half-times, and the mean values.

Comparison of methods of LVEDC determination. The data from plots of LVEDC half-time against venous cps/g half-time for each method of LVEDC determination are shown in Table 4. The only statistically significant correlation was that provided by Method 5, that is, when LVEDC determination used a semiautomated second-derivative edge-detection algorithm on temporally and spatially filtered images.

DISCUSSION

This study demonstrates significant interpatient and intrapatient variability of the half-time in venous blood for Tc-99m labeled to red blood cells in vivo. Substantial variation in the stability of RBC labeling with minor procedural variations has been observed previously (*10,11*). In spite of adherence to uniform labeling technique there can still be a wide variation in decay of activity (*12*).

It has been speculated that this variation reflects individual differences in fragility of labeled RBCs (*11*). This is supported by the fact that a fall in blood-pool counts is accompanied by increase in splenic activity, with possible sequestration of "altered" cells (*12,13*). We measured RBC fragility before and 1 hr after labeling by an autohemolysis technique in three patient studies, and found no abnormalities. This method, however, might not be sensitive enough to detect ab-

TABLE 4. CORRELATION DATA: LEFT-VENTRICULAR END-DIASTOLIC HALF-TIME COMPARED WITH VENOUS BLOOD COUNT HALF-TIME

	Half-time (min) Mean ± s.d.	Correlation coefficient Method vs. VBC	P
VBC	243 ± 91	N.A.	—
Method 1	245 ± 185	0.02	NS
Method 2	188 ± 114	−0.43	NS
Method 3	216 ± 118	−0.12	NS
Method 4	416 ± 418	0.26	NS
Method 5	231 ± 97	0.69	<0.03

normalities in the relatively small proportion of labeled red blood cells.

Another mechanism causing variability of in vivo decay is distribution into extravascular spaces. In comparison with cells labeled with chromium-51, it is known that Tc-99m-labeled RBCs distribute progressively into a "larger space" (*14*). This unknown "space" is likely to consist of several compartments. Studies of Tc-99m binding have shown variable preferential binding to blood elements, liver, spleen, kidneys, stomach, and gut after pretreatment with various stannous complexes (*15*). Billinghurst and Somers (*16*) have shown that critical concentrations of stannous pyrophosphate are required in pretreatment of RBCs to prevent preferential labeling of elements in the plasma. They observed that circulating drugs might become labeled as well. In our study however, we are unable to correlate interpatient variability of the in vivo half-time with our patients' medications, which are listed in Table 1.

The complex kinetics of the concentration of Tc-99m bound to RBCs is still without a model. Possibly a major "compartment" of this model is that related to renal excretion. In patients whose cells label poorly, high bladder counts are seen after 30 min (*17*). Eckelman et al. feel that the "expanded blood pool obtained by Tc-99m-labeled red blood cells after 30 min in vivo is the result of urinary excretion and not of uptake by any organ" (*10*). Labeled molecules of a size falling between technetium pyrophosphate and pertechnetate, which would be filtered by the glomerulus, are found in labeled blood specimens at this time (*15*). We examined the relationship between the half-times of activity in our patients and their creatinine clearances (Table 1), and found no correlation.

We are thus unable to explain the variability of the half-time of Tc-99m bound to RBCs on the basis of altered RBC membrane integrity, circulating drugs, or variability of glomerular filtration rate. Ideally, serial measurements of LV counts should be accompanied by counting of blood samples, each obtained at the time of LV count determination, because there is at present no method to predict the rate of in vivo decay. This recommendation has recently been made for serial ventricular volume estimates in response to exercise stress (*18*); we extend it to incorporate serial measurements even in the absence of such an intervention. Also, because rapid fluctuations of blood-pool activity occur during the first 15 to 30 min after labeling, we suggest that time-averaged volume measurements from gated studies be made only after half an hour. Investigators using other labeling techniques should determine this "unstable" period independently.

Our data demonstrate that LV end-diastolic counts are most reproducibly determined using automated edge detection with a second-derivative algorithm on spatially and temporally filtered images. While the correlation of half-times of LVED counts by this technique is statistically significant, we concede that the LVED volume is unlikely to have been absolutely constant during these studies. However, this comparison provides a sensitive assessment of consistency of LVED counts.

The alternative of comparing count-derived volumes measured at one time against contrast-angiographic volume estimates measured at a different time is likely to incorporate different hemodynamic conditions as well as the errors implicit in geometric assumptions in contrast volume estimates. Furthermore, rapid infusion of angiographic contrast material is an intervention that rapidly increases resting end-diastolic pressure (*19*) and volumes above the resting values.

Links et al. (*8*) have suggested that the second-derivative technique for ventricular edge detection consistently underestimates absolute LVED volume. Their study, however, assumes that tissue attenuation of LV blood-pool counts is the same as attenuation by water, which is conceivably another source of systematic error. Our study shows that this method is preferred for serial, relative volume determinations. Manual edge detection is based on subjective image interpretation, which in this study often led to decreasing estimation of ventricular size as blood-pool activity fell. This is manifest as frequent low values for LVEDC half-time by Methods 1, 2, and 3 (Table 3). Whether or not the second-derivative algorithm accurately identifies the anatomic ventricular border is not known, but our results indicate that the border identified is the most reproducible.

In summary, the half-time Tc-99m bound to RBCs in vivo differs from the physical half-life of the label. Thus the 6-hr half-life ideally should not be used to correct for count decay in serial determination of LV volume by emission methods. There is, moreover, significant interpatient and intrapatient variability of the in vivo half-time.

This study also demonstrates that a more reproducible LVED count determination can result from use of a semiautomated second-derivative algorithm applied to temporally and spatially smoothed images, rather than by application of this algorithm to unfiltered images or by manual edge detection.

For measurement of serial, relative, count-based ventricular volumes using radionuclide ventriculography, we make the following recommendations. Samples of venous blood should be obtained over the period of investigation to establish the patient's particular half-time for blood-pool activity. This half-time is constant between 30 and 120 min after labeling by our technique and may then be used to calculate in vivo decay of ventricular end-diastolic counts that occur independently of ventricular volume changes. Left ventricular end-diastolic counts are most reproducibly determined by the semiautomated second-derivative method after spatial and temporal image smoothing.

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