Quantitative Radionuclide Angiocardiography in Animals with Experimental Atrial Septal Defects

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Atrial septal defects were created surgically in eleven healthy dogs. After a 4-wk period of postoperative convalescence, each dog had repeated determinations ($n = 10$) of left-to-right (L-R) shunt size using the gamma-function method of quantitative radionuclide angiocardiography (QRAC). Seven animals then underwent median sternotomy, and shunt size was determined simultaneously by QRAC and electromagnetic flow probes (EFP). During this portion of the study, the hemodynamic significance of the L-R shunt was altered by surgically created right- or left-ventricular outflow obstructions. The coefficient of variation for 110 L-R shunt measurements by QRAC was 11.2%. QRAC determinations of shunt size correlated closely ($r = 0.97$, $p < 0.001$) with those of EFP, and accurately reflected the hemodynamic significance of L-R shunts even in the presence of ventricular outflow abnormalities. QRAC is an accurate and reproducible method for determining the hemodynamic significance of L-R shunts.

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Radionuclide measurement of left-to-right (L-R) shunt size is a rapid, easily tolerated, low-radiation-dose clinical procedure that has been used in children for several years (*1–6*). Area-ratio techniques like the gamma-function (*4*) and exponential (*5*) methods have resulted in increased accuracy and fewer false-positive studies than were previously obtained using count-ratio (C_2/C_1 ratio) approaches (*6*). Although quantitative radioangiocardiography (QRAC) is now used extensively in some hospitals (*7*), several basic parameters of importance have not been investigated. The current study was undertaken to determine the reproducibility of the technique, to compare its reproducibility with that of standard oximetry, to determine its accuracy by comparing its results with shunt-size determinations made by electromagnetic flow probes, and to determine whether the accuracy of the technique is adversely affected by the presence of right- or left-ventricular outflow obstruction.

METHODS

In order to perform this study, an animal model of a L-R shunt was needed. The model chosen was atrial septal defect (ASD) in dogs. ASD was chosen because it can be surgically created in dogs without requiring cardiopulmonary bypass, and because ASD is a well-tolerated L-R shunt compatible with prolonged survival of the animal after surgery. Atrial septal defects were surgically created in 11 healthy beagle dogs (10–15 kg) using an inflow-occlusion technique. Each animal was anesthetized with sodium thiamylal (20 mg/kg) and ventilated through a cuffed endotracheal tube. Anesthesia was maintained with Halothane and oxygen. A right thoracotomy

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was carried out through the fifth interspace, and the pericardium was opened, exposing the right atrium. The venae cavae were isolated and a snare was placed around each. A right atriotomy was performed using a large vascular clamp for control. The cavae were then occluded, the heart was fibrillated, and the atriotomy was opened. An area, 1 cm in diameter, in the midportion of the atrial septum was excised to simulate an ostium secundum ASD, and the atriotomy was then approximated with a vascular clamp. The heart was restarted with a single DC countershock, after circulation had been interrupted for less than 1 min. The operation was then completed, and each animal was allowed to recover for 2 wk before studies were performed to determine whether a L-R shunt had been created.

Before surgery, each animal had a normal baseline radioangiocardiogram. After the initial postsurgical radionuclide studies had revealed evidence of a L-R shunt in the 11 dogs, they were allowed 2 more weeks of convalescence before radionuclide reproducibility studies were begun. All subsequent radionuclide angiocardiograms were performed with the dog lying supine beneath a gamma camera, which was fitted with a high-sensitivity parallel-hole collimator and interfaced to a small dedicated laboratory computer. The dogs were anesthetized for these studies using sodium thiamylal (20 mg/kg i.v.). Use of this preparation resulted in lightly sedated, spontaneously breathing animals that were fully awake within 20 min after the onset of sedation. To perform the study, a 3- to 4-mCi bolus of [^{99m}Tc] pertechnetate was rapidly injected into the external jugular vein of each animal using a scalp-vein needle set. A saline-flush technique was used to produce a compact bolus injection. Digitized data frames were collected at 0.4-sec intervals during the pulmonary transit of the bolus and stored on magnetic tape after being corrected for deadtime count losses. A summed image of the venous and arterial phases of the study was presented on the computer output oscilloscope, and an area of interest was outlined over the peripheral lung fields. The cardiac blood pool and other large vessels were excluded from the area of interest. Since the cardiac blood pool often obscured a portion of the left lung, the area of interest usually contained more of the right lung than the left. A pulmonary time-activity curve was generated from the outlined region. A smaller area of interest was placed over the superior vena cava to evaluate the bolus injection. Studies showing a prolonged (>2.0 sec) or double-peak bolus were not considered in the subsequent data analysis.

All radionuclide angiocardiograms were analyzed using a previously described algorithm that is a minor modification of the gamma-function method of Maltz and Treves (*4*). This method was chosen because an earlier comparative study of count-ratio and area-ratio techniques for shunt measurement (*6*) found it to be the most accurate. The Q_p/Q_s ratio calculated by the gamma-function method is limited to the range from 1:1 to 3:1. When a shunt is very large and the pulmonary time-activity curve falls very slowly from the peak, the program automatically designates the shunt as greater than 3:1. Similarly, time-activity curves showing virtually no abnormal early recirculation are designated as showing a Q_p/Q_s ratio of 1:1. Because of the 3:1 upper limit of L-R shunt size reported by the gamma-function method, shunts that measured larger than 3:1 by electromagnetic flow probes were designated as 3:1 during regression analysis. This convention has been employed in the previous reports (*4,6,7*) of the gamma-function method.

In order to test the day-to-day reproducibility of shunt-size determinations made by QRAC, each dog had ten radionuclide studies done on separate days over a 3-wk observation period. During this period all animals were clinically stable. Six of the dogs also underwent serial cardiac catheterization ($n = 3$). These were done in standard fashion using a femoral-vein approach. During the procedure the dogs were under general Halothane anesthesia and ventilated (on room air) by a Harvard respirator. At least two samples each from the superior and inferior venae cavae were obtained to calculate the venous oxygen saturation during each study. The equality of variability between QRAC and oximetry was assessed using the F-statistic.

To determine the reproducibility of radionuclide studies done on the same day, five dogs had multiple radionuclide injections ($n = 4$) and shunt determinations done within a 90-min period. Before each radionuclide injection a 60-sec pulmonary background count was made using the standard area of interest employed for each shunt determination. This background was subtracted from each data curve before analysis. The animal remained supine beneath the camera during this reproducibility study and did not move. Two to three additional i.v. doses of sodium thiamylal were usually required to maintain satisfactory anesthesia.

To determine the effect of coexistent cardiac disease (e.g., valvular disease) on the accuracy of radionuclide shunt-size determinations, and to measure the accuracy of radionuclide techniques against a more direct standard than oximetry, radionuclide shunt-size measurements were compared with results obtained by electromagnetic flow-probe (EFP) measurements of shunt size in seven of the dogs.

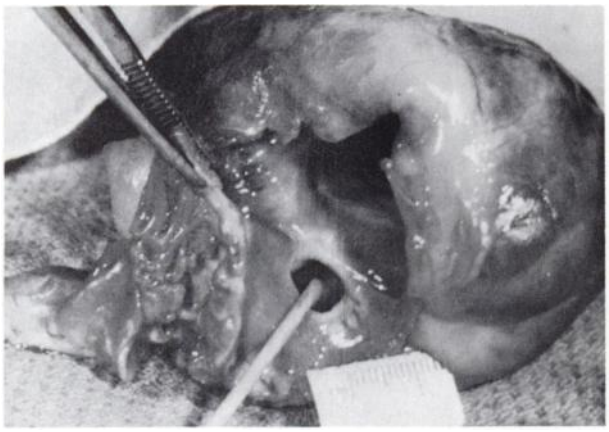

FIG. 1. Right atrium has been opened to expose atrial septal defect. A probe has been placed in defect.

Each animal underwent complete median sternotomy under general anesthesia. The pericardium was opened, and meticulous dissection was carried out around the aorta and pulmonary arteries to prepare them for appropriately sized flow probes. Five to seven flow measurements were made sequentially on each vessel. The mean of the readings indicated the flow (cc/min) through each vessel, and was used to calculate a Q_p/Q_s ratio ($\overline{X}$ PA flow/$\overline{X}$ Ao flow).

After the initial (baseline) EFP reading, four animals underwent successful banding of the pulmonary outflow tract. Pledgetted mattress sutures were placed along the short axis of the pulmonary outflow tract to decrease pulmonary blood flow. The shunt was then measured by both techniques. After removal of the band, one animal underwent supravalvular aortic obstruction using a snare placed on the ascending aorta. Again, shunt measurements were made by both techniques. Since the surgeon was working in close physical contact with animals containing several millicuries of radioactivity during these manipulations, an attempt was made to reduce his radiation exposure. Accordingly, each animal had a QRAC study no longer than 48 hr before median sternotomy to confirm that shunt size had not changed. Then, if the baseline EFP reading revealed a shunt of the same size (within ± 1 s.d. of the mean shunt determined by reproducibility studies), the initial (baseline) radionuclide injection was omitted. This occurred in four of the seven animals studied in this phase of the experiment.

Immediately following the completion of the surgical maneuvers and multiple Q_p/Q_s determinations, each of these seven animals was killed by injecting barbiturate intravenously. The heart was removed and opened to verify the presence and appearance of the atrial septal defect. Four additional animals had died during the 6–9 mo period after their initial surgery: two during the initial attempts at EFP placement and two from treatment-resistant pneumonias. The hearts of these four animals were also examined.

RESULTS

Each of the eleven dogs had an ASD. The defects had regular borders covered with smooth, glistening endothelium (Fig. 1).

The day-to-day reproducibility of the radionuclide shunt determinations was good, ranging from 9.0% to 14.2% of the shunt size measured (Table 1). The coefficient of variation for the 110 measurements in the 11 dogs was 11.2%. The reproducibility of shunt determinations made with repeated radionuclide injections on the same day was better than the day-to-day reproducibility. The s.d. ranged between 5.6 and 7.4% of the shunt size measured, and the coefficient of variation for the total of 20 measurements was 7.0%. This result not only reaffirmed the reproducibility of radionuclide shunt determinations, but also demonstrated that any changes in QRAC shunt size seen during the surgical intervention portion of the study would represent true changes in shunt size, not technical artifacts produced by the close proximity of the serial radionuclide injections.

TABLE 1. REPRODUCIBILITY OF RADIONUCLIDE ANGIOCARDIOGRAPHIC SHUNT DETERMINATIONS IN EXPERIMENTAL ASD

Dog no.	Q_p/Q_s ratio (± s.d.)	(% s.d.)
1	1.56 ± 0.14	9.0
2	1.70 ± 0.18	10.6
3	1.69 ± 0.24	14.2
4	1.86 ± 0.18	9.7
5	1.72 ± 0.17	9.9
6	1.56 ± 0.19	12.2
7	1.76 ± 0.22	12.5
8	1.68 ± 0.23	13.7
9	1.45 ± 0.13	9.0
10	1.71 ± 0.23	13.4
11	1.69 ± 0.20	11.8

TABLE 2. REPRODUCIBILITY OF SHUNT DETERMINATIONS BY OXIMETRY AND RADIONUCLIDE ANGIOCARDIOGRAPHY

Dog no.	Oximetry Q_p/Q_s (± s.d.)	Radionuclide Q_p/Q_s (± s.d.)
1	1.27 ± 0.12	1.56 ± 0.14
2	1.40 ± 0.12	1.70 ± 0.18
5	1.33 ± 0.15	1.72 ± 0.17
6	1.93 ± 0.38	1.56 ± 0.19
9	1.30 ± 0.36	1.45 ± 0.13
11	1.27 ± 0.31	1.69 ± 0.20

The day-to-day variability of oximetry determinations was larger than that of the radionuclide studies (Table 2): the coefficient of variation for QRAC in these six dogs was 10.2% against 18.5% for oximetry. This difference in variability, however, is not statistically significant. The correlation between the mean shunt sizes determined by oximetry and QRAC in these dogs as poor ($r = 0.16$, $p > 0.10$).

There was close agreement between the results of shunt determinations performed by EFP and radionuclides (Table 3). Three of the seven animals had significantly larger baseline L-R shunts during the EFP study than those measured during the earlier reproducibility study. This was presumably due to hemodynamic changes caused by anesthesia and/or the open chest. New baseline radionuclide studies were therefore performed in these dogs. The EFP-radionuclide regression analysis showed that the radionuclide method accurately reflected the hemodynamic significance of the L-R shunt under baseline conditions ($n = 7$) and in the presence of partial obstructions to right- ($n = 4$) or left- ($n = 1$) ventricular outflow (Fig. 2). This is clearly demonstrated by the sequence of pulmonary time-activity curves obtained in the one dog that underwent both pulmonary banding and left ventricular outflow obstruction (Fig. 3). The baseline shunt in this dog (Table 3, #3) was determined to be 1.7:1 by radionuclides and 2.0:1 by EFP. After banding, neither procedure detected a L-R shunt. After left ventricular outflow obstruction, however, the L-R shunt was greater than 3:1. The radionuclide study accurately reflected this change.

TABLE 3. SHUNT DETERMINATIONS BY RADIONUCLIDE ANGIOCARDIOGRAPHY AND FLOW PROBES

Dog no.	Procedure	Shunt by QRAC	Shunt by probes* (PA/Ao ± s.d.)
3	Baseline	1.69†	2.05 ± 0.24
	Band	1.00	0.86 ± 0.10
	LV obstruction	2.62	5.27 ± 0.57
4	Baseline	1.86†	1.69 ± 0.33
5	Baseline	1.72†	2.04 ± 0.17
	Band	1.00	0.90 ± 0.20
6	Baseline	2.97	3.56 ± 1.19
	Band	1.53	1.89 ± 0.59
9	Baseline	2.21	2.33 ± 0.30
	Band	1.00	0.84 ± 0.13
10	Baseline	>3	4.02 ± 1.95
11	Baseline	1.69†	1.74 ± 0.40

* Mean value of 5–7 measurements on each animal.
† Mean value from long-term reproducibility studies.

phasic variations by determining shunt size from the pulmonary time-activity curve.

Weldon (*10*) has demonstrated that the effect of pulmonary-artery constriction is to increase right ventricular (RV) pressure load, thus reducing the L-R component of an ASD shunt. The results of the current experiments were similar, and QRAC accurately reflected this change in shunt size. Similarly, aortic constriction raises LV pressure and augments L-R shunting (*10*). QRAC also reflected this hemodynamic change accurately.

The results demonstrate the close correlation be-

DISCUSSION

The results of the current study demonstrate that quantitative radionuclide angiocardiography is a reproducible and accurate technique for determining L-R shunt size through an ASD. The results also document the accuracy of QRAC in relation to direct flow measurements, and demonstrate that the technique accurately reflects the hemodynamic significance of a L-R shunt, even when other cardiac abnormalities (i.e., ventricular outflow obstruction) are present. Several authors (*8–10*) have demonstrated the dynamic nature of shunt flow across an ASD. Alexander et al. (*8*) and Levin et al. (*9*) demonstrated that both L-R and right-to-left (R-L) shunting occur during different phases of the cardiac cycle. When left-heart pressures are maximum during late ventricular systole and atrial contraction, L-R shunting occurs. However, small amounts of R-L shunting occur during the onset of ventricular systole. QRAC is monitoring the net effect of these

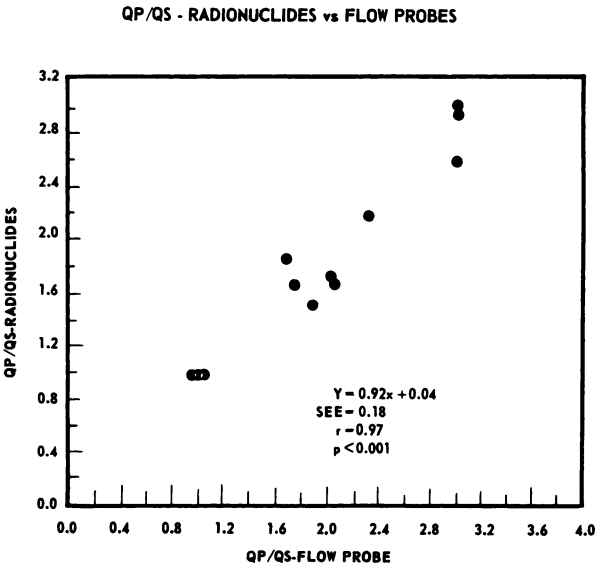

FIG. 2. Relationship of Q_p/Q_s determined by gamma-function radionuclide method and by electromagnetic flow probes.

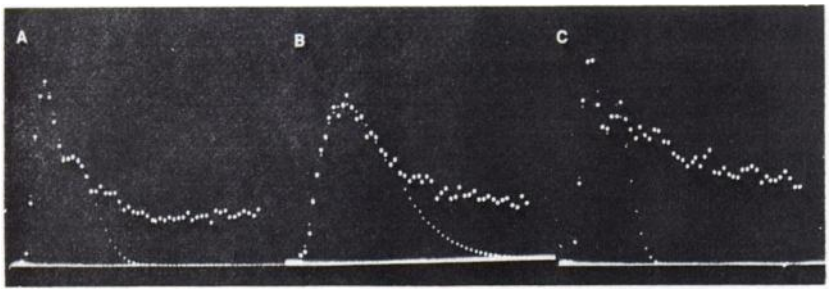

FIG. 3. Three time-activity curves obtained in same dog are shown. Curve (A) was "baseline." Curve (B) was obtained after pulmonary-artery banding, and (C) after left ventricular outflow obstruction. Large dots represent data curve, the smaller dots the fitted gamma-function curve. Gamma-function curves shown represent a summation of gamma function for normal first pulmonary transit and abnormal second transit due to L-R shunting.

tween QRAC determinations and hemodynamic shunt size, and confirm earlier clinical impressions (*6*) that QRAC can accurately measure L-R shunting in the presence of valvular heart disease. However, the results also clearly emphasize the functional nature of the QRAC determination. In the presence of severe ventricular outflow abnormalities (e.g., severe pulmonic stenosis, pulmonary banding) a Q_p/Q_s of 1.0 by QRAC in no way excludes the presence of an anatomic ASD with roughly equal L-R and R-L components, or an ASD with a net R-L shunt. This will not be a common cause for failing to detect an ASD in clinical studies. Nearly 70% of ASDs of the ostium-secundum type occur as solitary abnormalities, and only 10% are associated with pulmonic stenosis (*11*). Left-to-right shunt flow will cease, or a R-L shunt will result, only when the pulmonic stenosis is severe. The majority of patients with an ASD and pulmonic stenosis have persistent L-R shunting (*11,12*).

The correlation between oximetry and QRAC results ($r = 0.16$) was much lower than that between QRAC and EFP ($r = 0.97$). This finding seems to support the contention that oximetry is not a reliable means for measuring atrial-level shunts of less than 2:1 magnitude (*13*), and supports the hypothesis of Maltz and Treves (*4,7*) that QRAC is more reliable than oximetry in measuring shunt size in ASDs. However, the absolute size range of the shunts measured by oximetry was small (Table 2) and the experimental settings for QRAC and oximetry were somewhat different (i.e., barbiturate compared to Halothane anesthesia). Thus, the poor correlation coefficient does not prove that oximetry is inaccurate. Further studies will be needed to assess the relative efficiency of QRAC and oximetry in measuring ASD shunts.

The results of this study support clinical studies (*4,6,7*) that have recommended the use of QRAC. The procedure is noninvasive and rapid, and exposes the patient to only small amounts of radiation. It can therefore be repeated and used for long-term followup studies. QRAC procedures using ultrashort-lived radionuclides like iridium-191m (4.9-sec half-life) (*14*) or oxygen-15-labeled CO_2 (2 min half-life) (*15*) promise to reduce radiation doses even further. QRAC is an accurate and reproducible method for determining the hemodynamic significance of a L-R shunt, and is an excellent method for measuring L-R shunting through an ASD.

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