Variation in Gated Blood-pool Image Quality Using In Vivo, Modified In Vivo, and In Vitro Red Blood Cell Labeling Techniques

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The scintigraphic results of three methods of labeling red blood cells (RBCs) were compared. Image quality was evaluated in 200 patient studies by measuring the left ventricle to background ratio. These studies were performed using either the in vivo, modified in vivo or in vitro method for labeling RBCs. The average ventricle to background ratio for the in vitro method was 2.85 compared to 2.42 and 2.20 for the modified in vivo and in vivo methods, respectively. The in vitro method gave a statistically higher ($p < 0.01$) ratio than did either the in vivo or the modified in vivo methods.

Cardiac gated blood-pool imaging has become a useful diagnostic tool in quantitating ventricular ejection fraction (EF) and assessing cardiac function (*1-4*). It has not only been used for the calculation of EF (*5*), but is also used to calculate left ventricular volume (*6*). As this valuable noninvasive procedure becomes more widely used, there is a need to maximize the quality of the images.

Gated blood-pool imaging can be accomplished using several red blood cell (RBC) labeling techniques. The most popular of these is the in vivo technique (*7*). Imaging has also been done using ^{99m}Tc labeled human serum albumin, but this technique is not optimal (*8*). Another method of labeling RBCs is the modified in vivo technique (*9*). This technique is different from the in vivo technique in that it includes a single in vitro step in the labeling process.

A new method for labeling RBCs was developed at the Brookhaven National Laboratories (*10*). This technique is an in vitro method that is now available as a commercially supplied kit.*

The success of cardiac EF measurement is related to the clear visualization of the ventricle edge, which is provided by images with low background activity. Our study was undertaken to compare three labeling techniques, evaluating which of these techniques gave the best image quality based on left ventricle (LV) to background ratios.

MATERIALS AND METHODS

Three RBC labeling techqniues were compared: in vivo, modified in vivo, and in vitro.

In Vivo Labeling Technique

Patients were injected intravenously with 15 mg of stannous pyrophosphate† dissolved in 1.0 cc of nonbacteriostatic saline. Twenty minutes following the stannous pyrophosphate injection, 20–25mCi [^{99m}Tc]sodium pertechnetate were injected intravenously. Care was taken to avoid the site where the pyrophosphate had been injected. The pyrophosphate was injected directly into a vein and was never administered through an i.v. line.

Modified In Vivo Labeling Technique

A modification of the in vivo technique, as described by Callahan et al. (*9*), also began with the direct i.v. injection of 15 mg pyrophosphate. After a 20-min delay for equilibration, an i.v. line was established. A three-way stopcock was connected to this line and 5-10 ml of blood were withdrawn into a 10-cc syringe containing 20–25 mCi of [^{99m}Tc]pertechnetate and 1.0 ml of acid citrate dextrose. The mixture was gently rotated at room temperature for 10 min before reinjection through the i.v. line.

In Vitro Labeling Technique

The commercial kit tube contains 2.0 μg tin, 3.67 mg sodium citrate, 5.5 mg dextrose, and 0.11 mg sodium chloride. An i.v. line was established in the patient's arm and 4–6 ml of blood were drawn into a heparinized syringe. The contents of the syringe were then added to the kit tube and mixed gently on a rotator for 5 min at room temperature. While this tube was rotating, 1–3 ml of [^{99m}Tc]pertechnetate (20–25 mCi) were added to a sterile and pyrogen-free vial. When the tube had rotated for 5 min, 1.0 ml of a 4.4% EDTA solution was added to the tube. (Addition of EDTA to the blood reduces the SN^{+2} trapped within the packed RBCs by chelating the tin into a Sn-EDTA complex.) The tube was then inverted five times and centrifuged at 1300 G, stopper-end down, for 5 min. After centrifugation, the tube was removed from the centrifuge. Care was taken not to disturb the red cell pack in the tube. Using a 3-cc syringe, 1.5–2.0 ml of packed red cells were removed from the tube, injected into the vial containing the pertechnetate, and allowed to incubate at room temperature for 10 min with gentle inversion. The entire volume of the vial was then removed into a syringe, assayed in the dose calibrator, and injected into the patient through the i.v. line (*10,11*).

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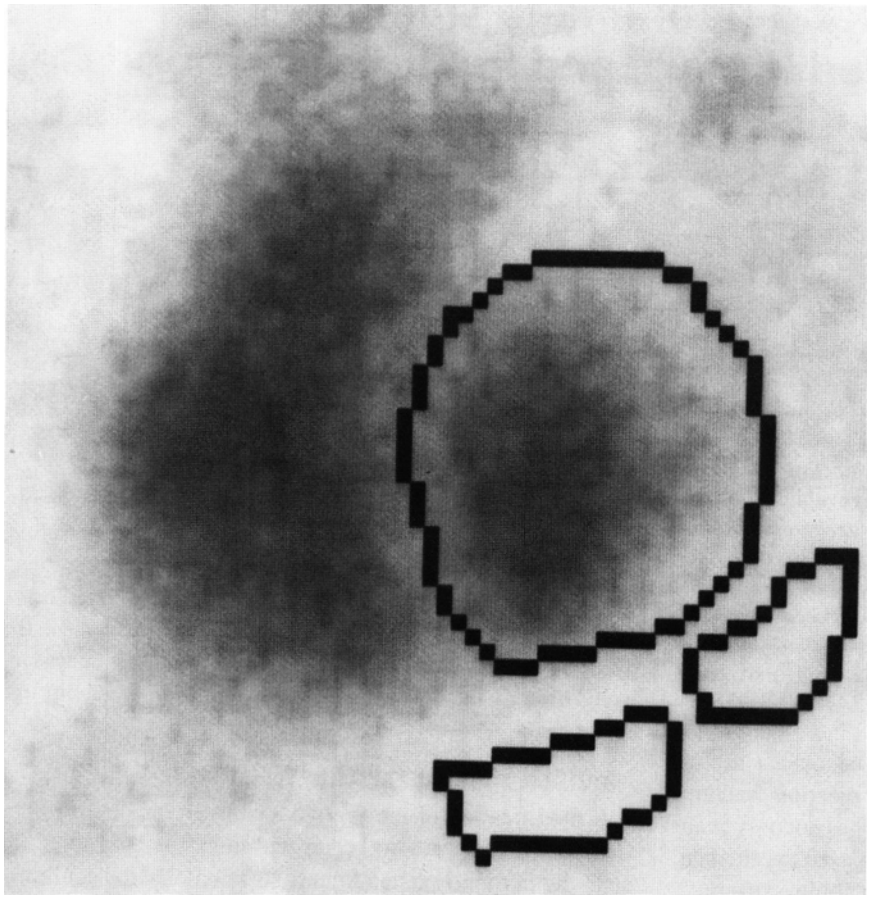

FIG. 1. Manually defined ROIs using an end-diastolic frame in the 45° LAO projection.

Patient Studies

A total of 200 studies were performed. Of these, 39 were done using the in vivo technique, 84 were done using the modified in vivo technique, and 77 were done using the in vitro technique. Fifteen of the patients evaluated using the modified in vivo technique were in a drug research protocol testing a cardiac drug, diltiazem hydrochloride. This protocol required imaging the patient, administering either diltiazem, propanolol, or a placebo, and imaging the patient again 3 hr later. This delayed imaging allowed comparison of the ventricle to background ratios to see if they deteriorated with time.

Gated radionuclide ventriculography was performed on each patient. A mobile scintillation camera fitted with a general all-purpose collimator was used. Multigated computer data were acquired for 16 frames per R-R interval using a 2:1 zoom. Images were acquired in the 45° LAO, anterior, and 70° LAO projections. In each view, data were acquired until a count density of 200 counts per pixel was achieved in the LV.

Three regions-of-interest (ROIs) were manually defined using the end-diastolic frame in the 45° LAO projection (Fig. 1). The first ROI included the entire LV. The second was a background region in the apical area adjacent to the LV (care was taken to exclude any portion of the spleen). The third ROI was a background region drawn inferior to the LV. The counts in the background regions were normalized to the number of pixels in the LV region and a LV-to-background ratio was calculated for each background ROI. These ratios were then statistically compared using the z score. The z score, also known as the standard score, describes the location of a value in terms of the number of standard deviations the value is located form the mean (*12*).

RESULTS

The results from each of the techniques are shown in Table 1. The mean LV-to-background ratios for the two background ROIs and their average were significantly higher ($p < 0.01$) using the in vitro technique than either of the two other techniques. The apical ROI gave a higher ventricle to background ratio than the inferior ROI for all labeling techniques.

For the 15 patients with immediate and time-delayed studies, the mean ventricle-to-background ratio was 2.42 ± 0.47 for the immediate study and 2.35 ± 0.37 for the 3-hr delayed study. There was a slight deterioration of the modified in vivo label in 3 hr, but this decrease was not statistically significant.

DISCUSSION

In order to use many of the automated computer generated LV edge-detection techniques, the quality of the image must be very high. Images with high radionuclide concentration in the blood pool and low background are dependent on the efficiency of the RBC label.

Several studies have previously demonstrated the improvement of the use of the in vivo and modified in vivo labeling techniques over the labeling of human serum albumin with

TABLE 1. Ventricle-to-Background Ratios

Labeling Technique	Apical	Inferior	Average
In vivo	2.50 ± 0.73	2.05 ± 0.46	2.20 ± 0.53
Modified in vivo	2.74 ± 0.67	2.16 ± 0.50	2.42 ± 0.62
In vitro	3.08 ± 0.81	2.70 ± 0.70	2.85 ± 0.68

^{99m}Tc (*8*). In addition, reports have described the use of a recently developed in vitro kit (*10,11,13,14*).

A recent study (*13*) showed that higher blood concentrations of radioactivity were obtained using an in vitro labeling technique than those obtained using an in vivo technique. These higher blood concentrations may facilitate the use of slightly smaller amounts of administered radioactivity or shorter acquisition times. In addition, there was reduced retention of the in vitro labeled RBCs, resulting in a lower radiation dose to the patient.

A study, similar to ours, was performed by Hegge et al. (*14*). In this study, they used an inferior apical ROI and calculated the LV-to-background ratio comparing the in vivo and in vitro labeling methods. Their results showed that the ratios increased from 2.3 for the in vivo technique to 2.5 for the in vitro technique.

Of the three techniques, the in vivo is by far the easiest and least time consuming method. This method, however, also produces lower quality images since the background is more apparent. The modified in vivo and the in vitro techniques take an equal amount of time, but the in vitro method produces the best quality images with a reduction in imaging time.

FOOTNOTES

*Cadema Medical Products, Inc., Middletown, NY.

†Squibb Diagnostics, New Brunswick, NJ.

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