RAPID BOLUS SINGLE-VENEPUNCTURE VERSUS TRADITIONAL TWO-VENEPUNCTURE TECHNIQUE IN MODIFIED IN-VIVO RED BLOOD CELL LABELING FOR RADIONUCLIDE VENTRICULOGRAPHY: IS THE IMAGE QUALITY ADEQUATE?

Charoonsak SOMBOONPORN; MD[1], James WESTCOTT; BAppSci[2], Nouria SALEHI; PhD[2], Nathan BETTER; MBBS, FRACP[2], Dan Bing ZHOU; MD[2], Dr. Krisana ROYSRI; MD[1], Meir LICHTENSTEIN; MBBS, FRACP[2]

ABSTRACT

Objective: In modified in-vivo red blood cell labeling, stannous pyrophosphate is traditionally injected through a metal needle, and a plastic catheter is used to take and re-inject the blood. This is problematic in patients with needle phobia or poor veins. To eliminate one venepuncture, the percentage labeling efficiency (% LE) of a new technique was ascertained and image quality of resting radionuclide ventriculography was compared with that of the traditional technique.

Methods: Technique A (n=20) included rapid injection of 20-ml diluted stannous pyrophosphate through an Optiva catheter and this retained catheter was later used for taking and reinjecting the labeled blood. Technique B used a traditional small volume of the stannous agent injected by metal needle and use of an Optiva catheter to manipulate pretinned blood and subsequently reinject it.

Results: The % LE of technique A at the time before reinjection and at the end of image acquisition was 75.0% ± 17.9% and 86.0% ± 11.4%, respectively. Mean left ventricle (LV) count, background (BG) count and LV to BG count was not significantly different between the two techniques (p = 0.414, 0.944, 0.338, respectively). Eighty and 20 percent of images were graded as good and fair quality in both groups, respectively.

Conclusions: This new technique provides a high and acceptable % LE for radionuclide ventriculography but needs only single venepuncture.

Key Words: red blood cell labeling; radionuclide ventriculography; plastic catheter; image quality

%LE = percentage labeling efficiency
LV = left ventricle
BG = back ground

[1] Department of Radiology, Faculty of Medicine, Khon Kaen University, Thailand
[2] Department of Nuclear Medicine, Royal Melbourne Hospital, Victoria, Australia
Author for correspondence: Charoonsak Somboonporn, MD
Address: Department of Radiology, Faculty of Medicine, Khon Kaen University, 40002 Thailand
Phone: 043 363 895 **Facsimile:** 043 202 472 **E-mail address:** charoonsak_s@yahoo.com

INTRODUCTION

Left ventricular ejection fraction (LVEF) is an important and universally accepted physiologic index of cardiac function. Radionuclide ventriculography (RNVG) is a highly reliable, widely accepted and noninvasive method for determining LVEF.[1] Image quality and the accuracy in calculating LVEF depend on the red blood cell (RBC) labeling yields.

Modified in-vivo labeling has been used worldwide with a high labeling yield and is less technical demanding than the classical in vitro method.[2-3] However, to achieve a good labeling, venepuncture is usually has to be done twice. Stannous agent as a reducing agent is intravenously injected by a metal needle to pretin the RBC. Then a plastic catheter is inserted to remove approximately 3 ml of blood to be labeled with ^{99m}Tc pertechnetate and the catheter is retained for injecting the radiolabeled RBC back into the patients. Separate stannous agent injection using a metal needle is mandatory; if injected through a plastic catheter made of teflon, it is postulated to form a complex with a compound leached from teflon of the catheter, resulting in a diminished and potentially insufficient amount of stannous agent to reach RBC in vivo and function as a reducing agent.[4] Although there is no published evidence that this negative effect can be found when a polyurethane catheter is used, this two-venepuncture technique has been adopted to guarantee a good labeling yield for modified in-vivo procedure in many nuclear medicine laboratories worldwide, regardless of the type of plastic catheter used.

A major group of patients referred for RNVG are cancer patients. Baseline study before and interval study during administration of certain chemotherapies to assess cardiac function by RNVG are crucial to early diagnose possible cardiotoxicity.[5-8] However, in cancer patients with poor veins and sometimes needle phobia due to multiple previous venepunctures, it may be difficult to perform a venepuncture procedure twice.

RNVG = Radionuclide ventriculography
LVEF = Left ventricular ejection fraction
% LE = % labeling efficiency

In our Nuclear Medicine Department, we had been using a polyurethane catheter in two-venepucture technique for RBC labeling. In order to eliminate one venepuncture, the performance of a new single -venepuncture technique for modified in-vivo RBC labeling was evaluated. The image quality of RNVG was compared with that of the traditional two -venepuncture technique.

PATIENTS AND METHOD

Patients

A prospective study of the performance of this new technique (technique A) was carried out in 20 consecutive patients referred for resting RNVG at the Department of Nuclear Medicine, Royal Melbourne Hospital during August 2003. The percentage labeling efficiency (%LE) of this technique was determined. The image quality of RNVG was also compared with that performed by the conventional two-venepuncture technique (technique B) in prior 20 consecutive patients. Related demographic data such as age, sex and clinical diagnosis were recorded. Previous chemotherapy administered within three months and concurrent medications taken that have been known to affect RBC labeling were also recorded.[9-10]

Autologous red blood cell labeling method

Technique A: A 22-gauge polyurethane catheter, (Optiva; Johnson & Johnson Medical), attached with a 3-way tap was firstly inserted into an available vein of the patient. Then 1 ml of stannous agent (1.1 mg stannous ion) (PYP; Radpharm Scientific, Australia), diluted with isotonic saline to 20 ml, was rapidly injected through a port of the tap, followed by a thorough flush with 5 ml of isotonic saline via another port of the tap. After 10-15 minutes to allow stannous ion to reduce the RBC, 5 ml of blood was drawn via this retained catheter into a syringe containing freshly

eluted ^{99m}Tc pertechnetate (11.4 MBq per kilogram of body weight) and 10 units of heparin. This syringe was incubated at room temperature for 15-20 minutes.

Before injecting the radiolabeled blood back to the patients through this catheter, 0.5 ml of the labeled blood was separated into a heparinized tube (sample 1) to measure %LE. Cardiac images were then acquired according to our usual RNVG protocol. At the end of image acquisition, 2 ml of blood was drawn via the catheter into another heparinized tube (sample 2) before removing the catheter.

Technique B: The same dose of stannous agent, derived from the same company as in technique A, in a traditional small volume (1 ml) without dilution was injected intravenously in a usual speed through a metal needle. After 10-15 minutes, a 22-gauge Optiva catheter was inserted into another vein for collecting 5 ml of the pretinned blood into a syringe containing the same dose of Tc-99m pertechnetate and heparin as in technique A. After incubation at room temperature for 15-20 minutes, the labeled blood was injected into the patient through the catheter.

RNVG image acquisition and processing

Acquisition was performed 10 minutes later using a Siemens Ecam gamma camera, equipped with a low-energy, all-purpose collimator (with ECG triggering and monitoring), using a 64x64 matrix and 8 frames/cardiac cycle. Three projections, anterior, left anterior oblique (LAO) and left lateral, were imaged for 7,500 Kcount per projection. For each study, the optimal LAO angle with the best visual demarcation of the left ventricle was chosen for positioning the head of the camera, with a caudal tilt of 0 to 10 degrees. Total acquisition time was about 10-15 minutes.

Processing was then performed to calculate LVEF by using a standard area-counts technique, in which background-subtract stroke counts (end -diastolic-end-systolic counts) were divided by end-diastolic counts. An experienced nuclear technologist manually drew separate end-systolic, end-diastolic, and pericardiac background (PCBG) regions of interest from the LAO projection. The region of interest for PCBG was drawn in a curvilinear fashion inferolaterally to the left ventricle. The interpreting physician reviewed region of interest placements and visually confirmed the quantified LVEF value.

Labeling efficiency measurement

Immediately after obtaining sample 2, both sample 1 and 2 were measured for the labeling efficiency. Both samples were diluted to 5 ml with saline solution and then were centrifuged at 3,000 g/ minute for 10 minutes and the plasma was then separated from the cells. In order to exclude technical error, the blood cell sample was washed again by dilution with 5 ml isotonic saline followed by repeated centrifugation. Radioactivity of plasma and cell fractions was counted for 60 seconds for two times in a dose calibrator (Capintec, CRC-120, USA) and the average values were used to calculate % LE according to the following formula:

$$\%\,LE = \frac{\text{radioactivity in RBC fraction}}{\text{radioactivity in RBC fraction+plasma fraction}} \times 100$$

Image quality assessment

The image quality was assessed both semi -quantitatively and qualitatively. For semi-quantitative method, mean left ventricular count, mean PCBG count, and mean left ventricular count to mean PCBG count ratio were used to indicate the image quality. Qualitative assessment was evaluated visually according to the previously published criteria11-12 as "good", "fair" or "poor" quality by two interpreters (CS and DZ) independently, blinded to the labeling technique and relevant clinical data. Any discordant grading was later reviewed and discussed to reach the consensus. Good quality was defined if the boundary of the left ventricle was clearly defined to draw the region of interest. Fair quality was defined if the image quality was reduced but left ventricular edge detection was still possible. Poor quality was defined

PCBG = pericardiac background

if left ventricular edge detection could not be separated completely from surrounding radioactivity.

Statistical analysis

Results were expressed as mean ± SD and percent for continuous and categorical data, respectively. Continuous data including age, LVEF and mean counts were compared using Student's *t*-test. Difference between sex, clinical diagnosis and medications used were evaluated using chi-square analysis or the Fisher exact test when appropriate. A probability value less than 0.05 was considered to be statistically significant.

RESULTS

Patient's characteristics were shown in Table 1. There was no significant difference between age, gender, primary clinical diagnosis and derived LVEF between the groups. Most of the patients in both groups were cancer patients. There was also no significant difference of the prevalence of medications used between the two groups.

Mean % LE (± SD) before re-injecting 99mTc labeled RBC into the patient (sample 1) and immediately after completion of the image acquisition (sample 2) of the technique A was 75.0% ± 17.9% and 86.0% ± 11.4%, respectively. Nineteen out of 20 cases showed a progressively increasing %LE from sample 1 to sample 2. (Figure1).

Table 2 compares the image quality semi -quantitatively between the two techniques. There was no significant difference of the mean left ventricular count, mean PCBG count and mean left ventricular count to PCBG count ratio between the two groups (P = 0.414, 0.944 and 0.338, respectively). In addition, by visual grading the results were equal in both groups. Sixteen of 20 images (80%) were graded as good quality and the other four images (20%) were graded as fair quality. No poor image quality was found in either group. A concordant rate between the two interpreters was found in 18 of 20 images (90%) and 19 of 20 (95%) images from technique A and B respectively. Figure 2 shows an example of a good and fair image quality derived from technique A.

Table 1 Characteristics of patients.

Characteristic	Single-venepuncture (n = 20)	Two-venepuncture (n = 20)
Age (y, mean + SD)	48.9 ± 12.3	47.9 ± 16.0
Gender (M/F)	8/12	12/8
Clinical diagnosis (n, %)		
Leukemia	9 (45)	6 (30)
Lymphoma	4 (20)	6 (30)
Breast cancer	5 (25)	4 (20)
Cardiac diseases	2 (10)	4 (20)
Medications (n)		
Beta-adrenergic blocker	3	1
Calcium-channel blocker	2	1
Furosemide	0	1
Aspirin	1	2
Benzodiazepine	2	2
Lipid lowering drug	1	3
Antibiotic	2	1
Anti-arrhythmic drug	0	2
ACE inhibitor	3	2
Chemotherapeutic agent	4	6
Coumadin	3	2
Iodinated contrast media	3	1
% LVEF (mean ± SD)	59.3 ± 11.7	59.8 ± 19.6

ACE, angiotensin-converting enzyme; LVEF, left ventricular ejection fraction
Data are not statistically significant.

Table 2 Semi-quantitative comparison of image quality between the two techniques.

	Single-venepuncture	Two-venepuncture	p-value
LV count	895.8 ± 132.6	918.9 ± 169.3	ns
PCBG count	439.4 ± 47.2	450.9 ± 68.7	ns
LV/PCBG count	2.0 ± 0.3	2.1 ± 0.3	ns

LV, left ventricle; PCBG, pericardiac background
Values are shown as mean ± SD.
ns, not significant

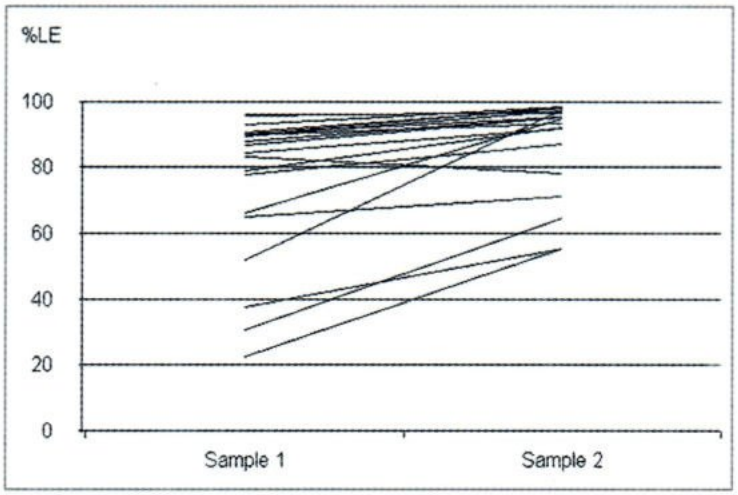

Fig.1 Labeling efficiency of technique A before re-injection of the labeled blood to the patient (sample 1) and after completion of image acquisition (sample 2). The mean %LE increased from 75.0 % to 86.0 % in concordance with similar increase using the two-injections technique in other trials.

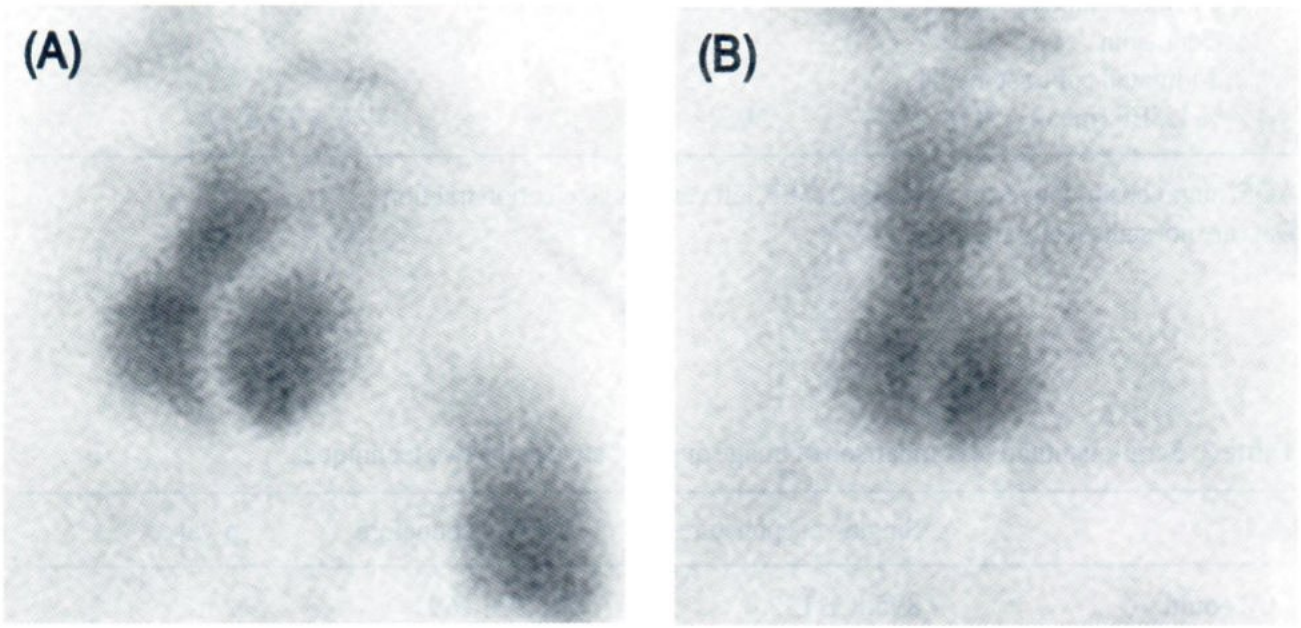

Fig.2 Example of RNVG images of the patients using single-venepuncture technique. (A) Good image quality: clear separation of radioactivity in the left ventricle from surrounding activity. (B) Fair image quality: minimal deterioration of image quality, but left ventricular edge detection was still possible without difficulty.

DISCUSSION

A large number of factors can affect the red cell labeling yield including concurrent medications and iodinated contrast media administered within 24 hours.[9,10,13] For the modified in vivo technique introduced by Callahan et al. in 1982,[2] an intravenous catheter is inserted to withdraw the blood and retained for later reinjecting the labeled blood. This catheter is therefore an inviting route for injection of the stannous agent. However, poor image quality and diminished RBC labeling efficiency was found when injecting the stannous agent through this catheter. Millar et al. reported a poor labeling and rapid clearance of ^{99m}Tc from the bloodstream when stannous agent was injected through a teflon cannula, which was significantly different from those obtained from stannous injection via a metal needle.[4] In addition, the experience of poor labeling and image quality from using the modified in vivo technique with injection of stannous agent through the Hickman catheter was also reported.[14] This had led to the adoption of the two-venepuncture technique to separate the administered route for stannous agent from the route for manipulating the blood.

Our study showed a new technique for such RBC labeling that needed only one venepuncture by inserting a conventional plastic catheter, which was retained for the entire processes of labeling. We prepared a large volume of stannous agent, with rapid injection through a plastic cannula, followed immediately by a thorough flushing the catheter with isotonic saline. We believe that this could reduce the chance of stannous agent to form a complex with the compound that may be leached from the plastic catheter and may also diminish the chance of stannous agent being adsorbed onto the catheter surface.

Since we retrospectively compared this new technique with the data of the prior patients undergoing the traditional two-venepuncture technique in our department, we therefore do not have the data regarding % LE of these prior patients to compare with those of the new technique. However, % LE obtained from this technique was comparable to those performed by traditional two-venepuncture technique reported in the literature.[15]

Regarding the image quality for RNVG, our study showed a satisfactory image quality in most of the patients performed by this new technique. Eighty percent of patients had a good image quality on RNVG and therefore allowed clear and easy delineation of the left ventricular activity for calculating the LVEF. Only a minority of patients was graded as fair image quality, but the left ventricular definition was still sufficient to calculate LVEF in all patients. By comparing this visual grading system between the two techniques, the same results were obtained.

It should be noted that the image quality is not only dependent on %LE but also the stability of the labeling. If significant amount of ^{99m}Tc is discharged from RBC, it can extravasate into the organs surrounding the heart and cause lower left ventricular count to PCBG count ratio and eventually deteriorating image quality. From our single-venepuncture technique, LE after completion of image acquisition was still high suggesting adequate retention time of the labeling for calculation of LVEF in the RNVG procedure.

CONCLUSION

We have shown in a limited number of patients that, a modified in vivo RBC labeling for RNVG can be performed by introducing a retaining polyurethane catheter into the patient, with a rapid bolus of the stannous pyrophosphate. It provides a high and acceptable %LE, without compromising the image quality. It would be very useful in patients with needle phobia or poor venous access. Further study should be carried out to validate this single -venepuncture, high-volume and rapid-injection technique in other kinds of catheter, in particular those made of teflon.

REFERENCES

1. Shaw LJ, Heinle SK, Borges-Neto S, Kesler K, Coleman RE, Jones RH. Prognosis by measurements of left ventricular function during exercise. Duke Noninvasive Research Working Group. J Nucl Med 1998; 39: 140-6.
2. Callahan RJ, Froelich JW, McKusick KA, Leppo J, Strauss HW. A modified method for the in vivo labeling of red blood cells with Tc-99m: concise communication. J Nucl Med 1982; 23: 315-8.
3. Bauer R, Haluszczynski I, Langhammer H, Bachmann W. In vivo/in vitro labeling of red blood cells with ^{99m}Tc. Eur J Nucl Med 1983; 8: 218-22.
4. Millar AM, Wathen CG, Muir AL. Failure in labelling of red blood cells with 99mTc: interaction between intravenous cannulae an stannous pyrophosphate. Eur J Nucl Med 1983; 8: 502-4.
5. Cottin Y, Touzery C, Coudert B, Gilles A, Walker P, Massing JL, et al. Impairment of diastolic function during short-term anthracycline chemotherapy. Br Heart J 1995; 73: 61-4.
6. Ganz WI, Sridhar KS, Ganz SS, Gonzalez R, Chakko S, Serafini A. Review of tests for monitoring doxorubicin-induced cardiomyopathy. Oncology 1996; 53: 461-70.
7. Stuckey SL, Pitman AG, Hicks RJ. Cardiac blood-pool imaging in oncology more than an ejection fraction? Clin Nucl Med 2001; 26: 18-26.
8. Mitani I, Jain D, Joska TM, Burtness B, Zaret BL. Doxorubicin cardiotoxicity: prevention of congestive heart failure with serial cardiac function monitoring with equilibrium radionuclide angiocardiography in the current era. J Nucl Cardiol 2003; 10: 132-9.
9. Tatum JL, Burke TS, Hirsch JI, Millar WW, Fratkin MJ. Pitfall to modified in vivo method of technetium-99m red blood cell labeling. Iodinated contrast media. Clin Nucl Med 1983; 8: 585-7.
10. Sampson CB. Complications and difficulties in radiolabelling blood cells: a review. Nucl Med Commun 1996; 17: 648-58.
11. Neumann P, Schicha H, Schumbrand P, Bahre M, Emrich D. Visualizing cardiac blood pool: comparison of three labeling methods. Eur J Nucl Med 1983; 8: 463-6.
12. Hambye AS, Vandermeiren R, Vervaet A, Vandevivere J. Failure to label red blood cells adequately in daily practice using an in vivo method: methodological and clinical considerations. Eur J Nucl Med 1995; 22: 61-7.
13. Kelly MJ, Cowie AR, Antonino A, Barton H, Kalff V. An assessment of factors which influence the effectiveness of the modified in vivo technetium-99m-erythrocyte labeling technique in clinical use. J Nucl Med 1992; 33: 2222-5.
14. White MP, Mann A, Cross DM, Heller GV. Evaluation of technetium-99m red blood cell labeling efficiency in adults receiving chemotherapy and the clinical impact on pediatric oncology patients. J Nucl Med Technol 1998; 26: 265-8.
15. Kuehne R, Reuter E. High RBC labeling efficiency by controlling pretinning with the modified in vivo/in vitro labeling method. J Nucl Med Technol 1999; 27: 222-6.