
Evaluation of Technetium-99m Red Blood Cell Labeling Efficiency in Adults Receiving Chemotherapy and the Clinical Impact on Pediatric Oncology Patients

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Objective: Technetium-99m-labeled RBCs are used to evaluate ventricular function and are the preferred method for monitoring the cardiac function of patients receiving chemotherapy. Optimal imaging quality is critical for monitoring small but important changes in ventricular function. The labeling efficiency of three products from two manufacturers and images from 30 patients (21 men, 9 women; age 60.3 ± 11.9 yr) referred for clinical radionuclide ventriculograms before chemotherapy were evaluated to determine the best labeling technique.

Methods: Patients received RBCs labeled in one of three ways. Two pyrophosphate methods used a modified in vitro method and the manufacturer's instructions were used for the in vitro method. Imaging was performed and, upon completion (42.1 ± 9.6 min), blood samples were drawn, separated and counted to determine labeling efficiency.

Results: The labeling efficiencies were: (a) $88.1\% \pm 4.2\%$ and (b) $88.4\% \pm 4.8\%$ for the two modified in vitro methods; and (c) $95.3\% \pm 1.7\%$ for the in vitro method. The difference between the methods was statistically significant ($p = 0.019$). Twenty pediatric oncology patients (6.4 ± 5.2 yr) received in vitro labeled RBCs through their Hickman catheters. All 20 pediatric studies were of high quality.

Conclusion: In vitro labeling demonstrated a higher labeling efficiency than the modified in vitro methods. In vitro labeling also yielded high-quality images when the labeled RBCs were injected through existing chronic in-dwelling catheters.

Key Words: red blood cell labeling; radionuclide ventriculography

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The last 30 yr have seen major advances in the management of a variety of cancers using combination chemotherapy (1). These therapies have become more aggressive and new agents have been introduced continually. Unfortunately, along with

these advances has come an increase in cardiotoxicity (2). Noninvasive techniques for monitoring cardiac performance are essential for the functional assessment of patients undergoing chemotherapy. In particular, doxorubicin hydrochloride (adriamycin) is highly efficacious in treating a variety of cancers. Its effectiveness is dependent on high cumulative doses which may be limited by the development of often irreversible cardiomyopathies, congestive heart failure and death (3-7).

Several techniques have been used to assess left ventricular dysfunction during the course of chemotherapy. These have included systolic time intervals, echocardiography, biopsy and radionuclide ventriculography (RVG) (8-15). RVG with technetium-labeled RBCs is the most widely used technique and has proven to be an accurate and highly reproducible tool for evaluating ventricular function over time (16). Investigators have shown a good correlation between ejection fraction by this method and other modalities such as echocardiography and contrast ventriculography (17,18). When using RVG to assess the impact of chemotherapy, the detection of small changes in function is essential in preventing permanent cardiomyopathies while allowing treatment to continue if no changes are identified. High-quality imaging is important.

Labeling RBCs with ^{99m}Tc is a standard procedure performed daily in most nuclear medicine departments. The increasing number of pediatric oncology patients being given potentially cardiotoxic drugs which require evaluation with gated blood-pool imaging is a unique challenge for the technologist. Often such patients have chronic in-dwelling venous access devices, such as Hickman catheters. Previous attempts to label RBCs through these catheters (in vivo method) resulted in suboptimal or uninterpretable images due to the high concentration of ^{99m}Tc in the catheter. Reluctance on the part of parents and patients to have additional peripheral intravenous access established when such a catheter is in place is common.

Several methods are available for binding technetium to the RBCs, including in vivo and in vitro labeling and modified methods for each (19,20). To produce high-quality gated

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blood-pool studies, it is imperative that a high labeling efficiency between the ^{99m}Tc and the patient's RBCs be achieved. Several investigators have compared RBC labeling efficiencies using multiple methods (22–24). The purpose of this study was twofold: (a) compare the labeling efficiency of three RBC-labeling techniques supplied by two different manufacturers; and (b) evaluate the administration of in vitro-labeled RBCs through an in-dwelling catheter on pediatric oncology patients.

MATERIALS AND METHODS

Study Design

In Phase I, adult patients referred to the nuclear cardiology laboratory at Hartford Hospital for routine resting radionuclide ventriculography before chemotherapy were enrolled prospectively in one of three groups: (a) Group 1 patients underwent modified in vitro labeling with Technescan PYP (Mallinckrodt, Inc., St. Louis, MO); (b) Group 2 patients underwent modified in vitro RBC labeling with CIS-PYRO® (CIS-US, Inc., Bedford, MA); and (c) Group 3 patients underwent in vitro labeling with UltraTag® (Mallinckrodt, Inc., St. Louis, MO). A blood sample was collected from each patient and labeling efficiency was determined from this sample.

In Phase II, pediatric patients referred for routine prechemotherapy RVG who had an existing in-dwelling, long-line catheter underwent in vitro labeling with the UltraTag method. Labeling efficiency was determined from residual blood in the reaction vial for each pediatric patient.

Study Protocol

A total of 50 patients was enrolled in this study (30 adults, 20 children). Ten adult patients were placed randomly into each of the three groups for evaluation of RBC labeling efficiency. Blood from patients in Groups 1 and 2 was prepared using a modified in vitro technique (25,26). Group 3 labeling was performed according to the manufacturer's instructions (27). All pediatric patients underwent in vitro labeling with UltraTag. After administration of labeled RBCs (30.7 ± 0.8 mCi for adults and 8.1 ± 4.1 mCi for pediatric patients), standard equilibrium gated blood-pool imaging was performed in the left anterior oblique, anterior and left posterior oblique positions on an ADAC Cirrus (Milpitas, CA) camera system. Images were acquired for 4 million counts per view using general all-purpose collimation and a $64 \times 64 \times 16$ matrix.

Analysis of Labeling Efficiency

On completion of imaging (42.1 ± 9.6 min postinjection), 7 cc blood were drawn from each adult patient using a vein from the arm opposite the one used for the radionuclide injection. Determination of labeling efficiency for pediatric patients was performed using the blood remaining in the reaction vial after reinjection. The blood samples were centrifuged immediately and the plasma pipetted and placed into a clean test tube and capped. The plasma and red cells were placed separately into the well counter, counted twice, averaged and background subtracted. Values were recorded for net

TABLE 1
Phase 1 Patient Demographics

	Technescan n = 10	CIS PYRO® n = 10	UltraTag® n = 10
Male	8	6	9
Mean age (yr)	61 ± 4	63 ± 6	60 ± 9
Range	55–66	59–71	50–76
Injected dose (mCi)	30.3 ± 1.5	30.5 ± 0.8	30.1 ± 1.1
Time to blood draw (min)	43.3 ± 9.0	43.6 ± 6.2	45 ± 2.4
IV heparin therapy	2	3	3
Prior MI	6	7	7
Prior chemotherapy	4	3	3
Hx diabetic	8	5	3

RBC counts and net plasma counts. Labeling efficiency was calculated as:

$$\frac{\text{Net RBC counts}}{\text{Net RBC counts} + \text{Net plasma counts}} \times 100.$$

A paired Student's t-test was used to determine the statistical significance of the labeling differences observed among the three groups. A p-value of < 0.05 was predetermined for significance.

Each image set was interpreted by two experienced nuclear cardiologists without knowledge of the labeling method used. Image quality was classified as excellent, good or poor.

RESULTS

Patient Demographics

Demographics for the 30 adult patients in the Phase 1 study are summarized in Table 1. There were no statistical differences noted between the groups.

Quantitative Results of Labeling Efficiency

The mean labeling efficiency for each of the three groups is illustrated in Figure 1. Groups 1 and 2, the modified in vitro (pyrophosphate) methods, had identical labeling efficiencies at $88.1\% \pm 4.2\%$ and $88.4\% \pm 4.8\%$, respectively. Group 3, with UltraTag®, demonstrated a higher labeling efficiency with a

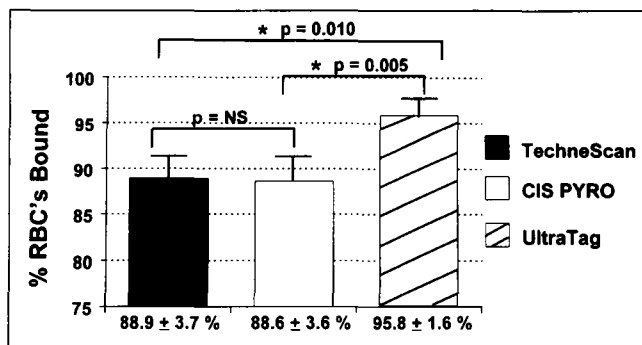


FIGURE 1. Labeling efficiency expressed as a percentage for each of the 30 adult patients. UltraTag® had a labeling efficiency consistently above 95%.

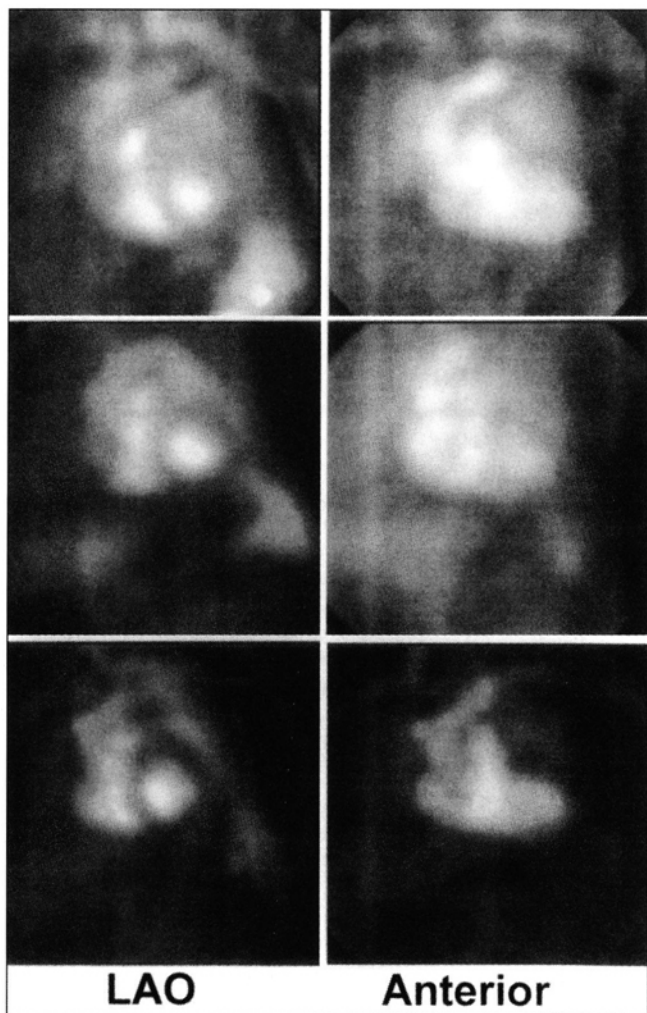


FIGURE 2. Equilibrium gated blood-pool images in left anterior oblique (LAO) and anterior projections from three patients. TechnoScan PYP (top), CIS-PYRO® (middle) and UltraTag® (bottom).

mean of $95.3\% \pm 1.7\%$. Labeling efficiency was significantly greater in Group 3 than in Group 1 ($p = 0.010$) and Group 2 ($p = 0.005$). The qualitative evaluation of the acquired images demonstrated less background activity when the UltraTag method was used (Fig. 2).

All 20 pediatric studies in Phase 2 were determined to be of excellent quality (Fig. 3). Residual activity in the in-dwelling catheters was not identified in any patient during image acquisition. The mean labeling efficiency was calculated for the 10 pediatric patients and was 96.2% (range 94.8–98.8). The results are consistent with those seen in the adult patients with the same labeling method.

DISCUSSION

Although excellent binding rates were observed with all three of the preparations we evaluated, Ultra-Tag® yielded consistently higher labeling efficiencies than the modified in vitro method. The differences were statistically significant.

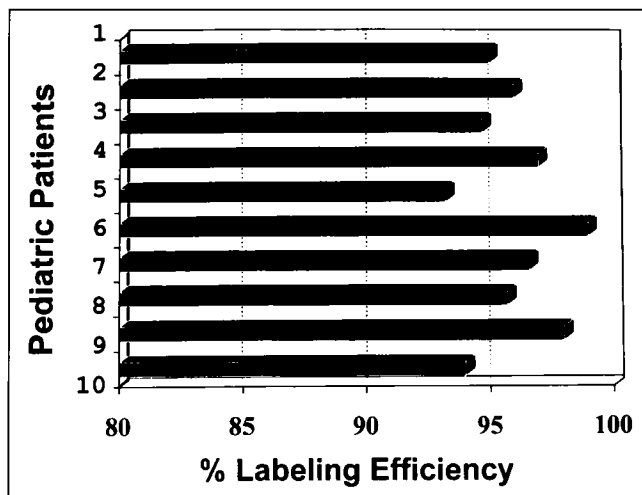


FIGURE 3. Labeling efficiency for 10 of the pediatric patients. All patients had labeling efficiencies above 94%.

Comparison with the Literature

The incidence of cardiomyopathy after chemotherapy with doxorubicin is estimated to be less than 2% in patients receiving a total dose of less than 400 mg/m^2 and as much as 30% in those whose dose exceeds 550 mg/m^2 (28). The occurrence of cardiomyopathy is serious, resulting in cardiac death in over half of identified cases (28). The importance of these drugs in cancer chemotherapy and the potential for serious cardiac toxicity has led to several approaches for early detection of this complication (29,30). Radionuclide methods have been shown to reliably detect doxorubicin cardiotoxicity before clinical signs of left ventricular dysfunction are manifest (2). Properly performed, this technique correlates well with other invasive and noninvasive modalities. Even when care is taken to properly label the RBCs, other factors can adversely affect labeling efficiency (31). Intravenous heparin therapy is perhaps the most documented source of reduced labeling efficiency. It is hypothesized that heparin competes with the RBCs for the Sn(II) ion and creates a situation where insufficient Sn(II) ion is available for tinning the RBCs (32–35). This did not appear to reduce the labeling efficiency in our study. Although 27% of the patients were receiving heparin therapy at the time of imaging, no impact on labeling efficiency was observed.

Clinical Relevance

Many laboratories opt to use either in vivo or modified in vitro methods for labeling RBCs because of convenience. We demonstrated that although each method provided the diagnostic information required, in vitro labeling with UltraTag provided consistently high labeling efficiencies above 95% while the other method ranged from 82%–94%.

Impact on Pediatric Oncology

The physical and psychological trauma associated with establishing peripheral intravenous access when a pediatric patient has an in-dwelling catheter is difficult to explain to an already frightened and concerned parent even though earlier

attempts to label RBCs through these catheters were unsuccessful. On two occasions attempts were made to use an existing Hickman catheter to administer the pyrophosphate. This resulted in both patients returning for a repeat study due to the high concentration of activity in the catheter, which lies curled in front of the ventricle making it impossible to visualize the heart. Our laboratory has evaluated patients as young as 3 mo. Efforts to eliminate the time and trauma associated with the need for additional peripheral intravenous access led us to an evaluation of in vitro RBC labeling for patients with in-dwelling catheters. The mean labeling efficiency of 96.2% (range 94.8%–98.8%) correlated well with our earlier findings.

CONCLUSION

In vitro labeling and reinjection through existing chronic in-dwelling long-line catheters demonstrated consistently high labeling efficiencies. This practice may be ideal for use in pediatric oncology patients needing accurate monitoring of ventricular function while preventing the necessity for additional intravenous access.

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