

Effects of In Vitro Versus In Vivo Red Cell Labeling on Image Quality in Gastrointestinal Bleeding Studies

A.H. Maurer, J.L. Urbain, B. Krevsky, L.C. Knight, George Revesz and K. Brown

Department of Diagnostic Imaging, Division of Nuclear Medicine and Internal Medicine, Section of Gastroenterology, Temple University School of Medicine, Philadelphia, Pennsylvania

Objective: Both in vivo and in vitro red cell labeling methods are available for performing gastrointestinal bleeding studies. While in vitro labeling has been shown to result in higher binding efficiency, no comparison of clinical image quality has been reported between those techniques. This study compares in vivo and in vitro methods using both subjective and objective measurements of image quality.

Methods: A consecutive series of gastrointestinal bleeding studies performed on 23 patients using in vivo labeling was compared to a series of 23 studies using in vitro labeling. Images at 30 min postinjection were randomized and analyzed by two observers. Subjective evaluation of image quality, as well as renal activity, was based on a comparison of femoral vein and inferior vena cava activity to adjacent background using numerical scores with a scale of 0–3. Image quality using the subjective scores was further classified as acceptable and poor. Target-to-background measurements of femoral vein to adjacent soft tissue in the thigh and inferior vena cava to adjacent abdominal background also were made.

Results: In vitro label subjective image quality was acceptable in 87% and 91% of cases for vascular and renal activity, respectively, but only 35% and 52% for in vivo labeling. In vitro label target-to-background ratios were significantly better than in vivo label for the femoral vein and inferior vena cava, as well as for the subjective assessment of vascular image quality, but not for renal activity.

Conclusion: In vitro red cell labeling improves clinical image quality as compared with in vivo labeling. Both subjective and objective measurements of image quality are useful for comparing the results of labeling methods.

Key Words: gastrointestinal bleeding; red blood cell labeling

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There have been more than two decades of research and development on the use of ^{99m}Tc labeling of red blood cells

For correspondence or reprints contact: Alan H. Maurer, MD, Director, Nuclear Medicine, Temple University Hospital, Broad and Ontario Streets, Philadelphia, PA 19140.

(RBCs). Radiolabeled red cells have been used for a wide range of clinical applications including cardiovascular disease, cerebrovascular disease, evaluation of liver masses (hemangioma), and the detection of gastrointestinal bleeding (1). The earliest methods for RBC labeling with technetium did not make use of reducing agents and resulted in suboptimal labeling (2,3). Pretreatment of the red cells with stannous ion for in vivo labeling results in clinically acceptable labeling efficiencies, however, yields are variable between 60% and 90% (1,4,5). Both in vivo and in vitro red cell labeling methods have been used to perform gastrointestinal bleeding studies. In vitro red cell labeling has been shown to result in higher binding efficiencies (>95%) (6,7), but no comparison of clinical image quality using these two methods has been reported.

Recently a commercial kit preparation for in vitro red cell labeling has become available (UltraTag® RBC, Mallinckrodt, Inc., St. Louis, MO). This study was designed to compare the image quality of clinical gastrointestinal bleeding studies using subjective and objective measurements after in vivo and in vitro labeling.

MATERIALS AND METHODS

Before 1992 all gastrointestinal bleeding studies at Temple University Hospital were performed using in vivo labeling of the patient's red blood cells. In vivo labeling was performed by first injecting stannous ion (20 $\mu\text{g}/\text{kg}$ patient weight) from a commercial pyrophosphate kit (TechneScan® PYP, Mallinckrodt, Inc., St. Louis, MO) followed 20–30 min later by 925 MBq (25 mCi) ^{99m}Tc -pertechnetate. The reaction vial of the pyrophosphate kit was reconstituted with 3 ml of sterile, non-pyrogenic normal saline which contained no preservatives.

Beginning in 1992 all studies were performed using in vitro labeling with the UltraTag® RBC kit (Mallinckrodt, Inc.). For in vitro labeling we followed the package insert instructions and likewise used 925 MBq ^{99m}Tc -pertechnetate.

To compare image quality with these two methods, we performed a retrospective analysis of the stored digital images from gastrointestinal bleeding studies that had been acquired as routine clinical studies. Our institutional review board

granted permission to perform this retrospective review of patient data. All bleeding studies were acquired and processed using NuLear MAC® software (Scientific Imaging, Denver, CO). The images were obtained in a 128 × 128-byte matrix with a large field-of-view camera (GE 535 or GE 500, General Electric Medical Systems, Milwaukee, WI). The images were acquired dynamically in sets of 15-sec images for 15 min (60 images per set) up to 1 hr or until a bleeding site was identified as previously described (8).

All bleeding studies archived from 1990 through 1991 were recalled from digital storage for analysis. Within this group, 23 gastrointestinal bleeding studies with *in vivo* labeling were found which contained images at least 30 min postinjection. These images were compared to a consecutive series of 23 *in vitro* labeled gastrointestinal bleeding studies performed in 1992 which also contained images to at least 30 min postinjection. All images were reformatted as 30-sec static images. A single, summed 30-sec image at 30 min postinjection was used for both the subjective and objective image analysis. Before analysis all 46 images were placed in randomized order. They then were blindly analyzed by two experienced observers.

Each observer performed independent subjective visual evaluations by grading vascular to adjacent soft tissue (target-to-background) image quality using a numerical scoring system. Normally the femoral veins are easily defined as symmetric linear bands of activity in the thigh that unite to form the inferior vena cava in the abdomen (9). Scoring was based on visual evaluation of the vena cava and femoral veins. The image was graded with a numerical value of 3 for high image contrast defined as vascular blood-pool activity much greater than the adjacent soft tissue background (Fig. 1D). The image was graded with a numerical value of 2 for moderate image contrast defined as vascular blood-pool activity significantly greater than soft tissue background but not as high as the highest image contrast (Fig. 1C). The image was graded with a numerical value of 1 for low image contrast defined as vascular blood-pool activity slightly greater than the soft tissue background (Fig. 1B). Finally, the image was graded with a numerical value of 0 for very poor vascular image contrast, that is, vascular blood-pool activity equal to or only minimally greater than the soft tissue background (Fig. 1A).

The renal uptake of free pertechnetate also was visually graded with a numerical score (Figs. 1A–1D). If no renal uptake was seen, the image was given a score of 3. For minimal renal activity the image was scored as 2. For renal activity equal to adjacent vascular blood-pool activity the score was 1 and for renal activity greater than the adjacent vascular blood-pool the score was 0.

To further classify the images' clinical value, the subjective scores were grouped into two groups, acceptable or poor image quality. This was done both for vascular to soft tissue contrast and renal activity. Images with mean scores from the two readers of greater than 2.0 were graded as acceptable and those less than 2.0 were graded as poor.

Each reader also performed quantitative target-to-background measurements. These were performed by drawing manual regions of interest (ROIs) to analyze vascular struc-

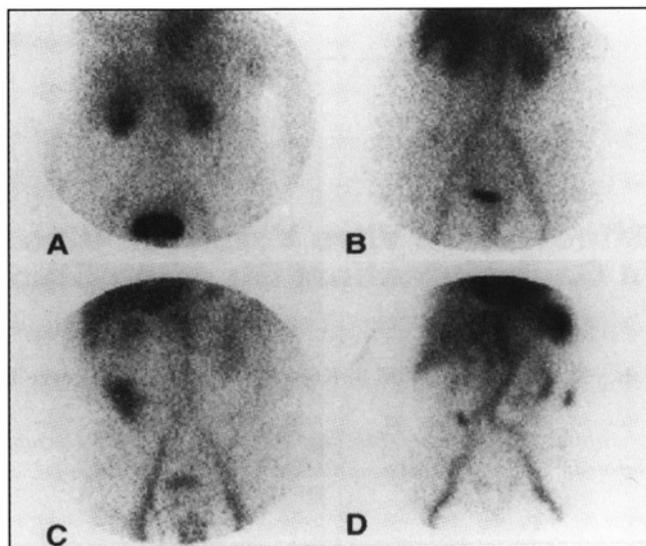


FIGURE 1. Case examples of scoring vascular and renal image quality. (A–C) *In vivo* labeling and (D) *in vitro* labeling. (A) Vascular activity is only minimally greater than the soft tissue background. Both readers recorded a score of 0 for vascular image quality. The renal activity was greater than the adjacent vascular blood pool and also received a score of 0 by both readers. (B) Vascular activity is slightly greater than background in the thigh and abdomen and was given a score of 1 by both observers. The renal activity is greater than the adjacent vascular blood pool and was scored as 0. (C) Moderate image contrast (score = 2) defined as vascular blood-pool activity significantly greater than the soft tissue background but not as high as the highest image contrast seen in D. The renal activity is equal to the blood-pool activity and was scored as 1. (D) Excellent image quality with high image contrast and the vascular blood-pool activity much greater than the adjacent soft tissue (score = 3). There is minimal renal activity (score = 2). The presence of an active bleed and blood in the gastrointestinal tract demonstrates the difficulty encountered when trying to choose a background ROI in the abdomen.

tures in the thigh and abdomen. In the thigh, the ROIs were drawn first to encompass a segment of the femoral vein and then a second region to include adjacent soft tissue. Ratios of vascular-to-soft tissue counts were obtained by dividing the average counts per pixel in the vessel ROI by the average counts per pixel in the adjacent soft tissue ROI. Similarly, counts from a segment of the inferior vena cava were compared to an adjacent nonvascular area in the abdomen. One observer also repeated each measurement at a different time to determine intraobserver variability.

Statistical Analysis

Interobserver and intraobserver agreement were compared using linear regression. The mean \pm s.d. was calculated for all subjective and quantitative measurements and the nonparametric Mann Whitney U test was used to determine if significant differences were present. Chi-square analysis was performed to determine significance when the results were grouped for the analysis of acceptable versus poor image quality. Results were considered significant at $p < 0.05$.

RESULTS

The readers were able to subjectively and visually assess vascular activity of the femoral vein and inferior vena cava in all cases. In three of the 46 cases the readers felt unable to make a visual, subjective score of renal activity due to difficulty in assessing renal uptake compared to adjacent soft tissue or vascular activity. They were able to quantitatively analyze the vascular-to-soft tissue background ratios for the femoral vein in all cases. In six of the 46 cases they were unable to obtain count ratios for the inferior vena cava either due to blood in the abdomen or tortuosity of the inferior vena cava and proximity to adjacent vascular structures (Fig. 1D).

The mean values obtained with the in vitro labeling method were significantly better than the values obtained with the in vivo method for the target-to-background ratios of the femoral vein (3.0 ± 0.72 vs. 2.2 ± 0.51 ; $p < 0.01$) and inferior vena cava (2.4 ± 0.55 vs. 2.0 ± 0.60 ; $p < 0.01$).

The mean values for the subjective assessment of in vitro vascular image quality were better for the femoral vein (2.4 ± 0.61 vs. 1.6 ± 0.57 ; $p = 0.02$) but not for renal activity (2.5 ± 0.62 vs. 2.0 ± 1.1 ; $p = 0.07$).

When using a mean score greater than or equal to 2.0 as acceptable and less than 2.0 as poor from the subjective scores, in vitro vascular image quality was acceptable in 87% (23 of 26 cases) but for in vivo labeling in only 35% (9 of 26 cases; $p = 0.0003$). Similarly, when using a mean score greater than or equal to 2.0 as acceptable and less than 2.0 as poor, in vitro renal image quality was acceptable in 91% (21 of 23 cases) but in only 52% (12 of 23 cases; $p = 0.003$) with in vivo labeling.

There was good interobserver agreement on the measurement of the femoral vein to background ratios in the thigh ($r = 0.75$; slope 0.82) but poor interobserver agreement in measuring the inferior vena cava to background ratios in the abdomen ($r = 0.54$; slope = 0.40). The intraobserver agreement was good for the femoral vein ratio measurements ($r = 0.82$; slope = 0.74) and the inferior vena cava abdominal ratios ($r = 0.77$; slope = 1.0).

For the subjective visual scoring there was only fair interobserver agreement on the vascular image quality ($r = 0.65$; slope = 0.84) but excellent agreement on the assessment of renal activity ($r = 0.86$; slope = 0.91). The intraobserver agreement was good for both the subjective visual scoring of vascular image quality ($r = 0.82$; slope = 0.73) and renal activity ($r = 0.86$; slope = 0.81).

DISCUSSION

Gastrointestinal bleeding studies require high red blood cell labeling efficiency to assure good image quality and permit imaging over long periods of time. There was early recognition that low binding efficiency and radiopharmaceutical breakdown of technetium-labeled red cells in gastrointestinal bleeding studies could lead to difficulties in image interpretation. Winzelberg described gastric mucosal uptake, renal activity, and the appearance of activity in the bowel in delayed images due to unbound technetium (10). Abello et al. also reported

that renal excretion could lead to incorrect localization of a bleeding site (11).

A commercial kit for the in vitro labeling of red blood cells with ^{99m}Tc has become available relatively recently. Before its availability most gastrointestinal bleeding studies were performed with an in vivo labeling method developed by Pavel which involves first preparing the patient's red blood cells with an injection of stannous ion followed 20–30 min later by an injection of ^{99m}Tc -pertechnetate (5). The stannous ion usually is obtained from a reconstituted commercial tin-pyrophosphate kit. It diffuses into the red cell and then remains intracellular. Technetium-99m-pertechnetate will diffuse readily in and out of the red cell membrane unless it is reduced. The intracellular stannous ion provides a reducing environment for the pertechnetate which, after diffusing into the red cell, becomes reduced and bound to the beta chain of the hemoglobin molecule (12). Although the in vivo method represents a quick and convenient method of labeling, labeling efficiencies have been reported as variable and unreliable, ranging from 60%–90% (13).

The first method developed to significantly improve labeling efficiency was the introduction of a modified in vivo/in vitro (in vitro) method (14). With this method the stannous ion was administered in vivo but the ^{99m}Tc -pertechnetate labeling took place in a sample of blood that had been withdrawn from the patient and mixed outside in a syringe that was then used for reinjection. Labeling efficiencies with this method approached 95%, with 5% of the activity injected as free pertechnetate.

The in vitro method is performed completely outside of the body in a sterile closed system using a sample of red blood cells taken from the patient. First, an anticoagulant and stannous solution are added to the blood sample and time is allowed for the stannous ion to diffuse into the red cells. Sodium hypochlorite and ACD solution then are added in sequence to rapidly oxidize the extracellular stannous ion. Technetium-99m-pertechnetate then is added to the blood sample and it diffuses inside the red blood cells where it is reduced and bound with very high labeling efficiency. Two in vitro preparations have been reported showing high labeling efficiencies in the 95%–98% range (6,7). The first study reported with the UltraTag® RBC kit showed a mean labeling efficiency of 97% (15).

While studies have confirmed high labeling efficiency with in vitro labeling kits, their routine use raises additional considerations in regard not only to the cost of the kits but also to the need for added handling of patient blood for withdrawal, kit preparation and reinjection. In addition, most previous studies comparing red cell labeling have focused only on measuring binding efficiency. There are few studies that have attempted to measure image quality and these were performed to evaluate cardiac gated blood-pool imaging for regional wall motion and ejection fraction calculation (16–19). No standardized method for measuring labeled red blood cell image quality has been used.

For these reasons we attempted to determine if the added cost of in vitro red cell labeling could be justified by showing improved image quality in gastrointestinal bleeding studies.

We evaluated both subjective and objective measurements of image quality. By setting a threshold level for acceptable versus poor image quality, we found that in a significantly greater percentage of cases acceptable image quality was attained with the in vitro labeling method. The quantitative measurements of vascular target-to-background activity also were better for the in vitro label in both the thigh and abdomen. As it was not a goal of this study, no attempt was made to determine whether this improvement in image quality led to higher diagnostic accuracy in localizing a bleeding site.

There was better inter- and intraobserver reproducibility for the ratios measuring vascular-to-soft tissue activity in the thigh than in the abdomen. This is easily understood as placement of the thigh ROIs were not complicated by variable renal activity and the presence of blood in the gastrointestinal tract. The readers in this study made a judgment that they were unable to perform the count ratio measurements in six of the 46 cases in the abdomen due to tortuosity of the inferior vena cava and its proximity to adjacent structures. The overall subjective scoring of vascular activity showed poorer interobserver agreement while the visual assessment of renal activity showed better agreement.

CONCLUSION

These findings suggest that quantitative measures of vascular image quality are best made in an area such as the thigh where the femoral vein can be compared more easily to soft tissue background. The subjective assessment of renal activity showed both good inter- and intraobserver reproducibility.

These results confirm that the higher labeling efficiency achieved with an in vitro label results in improved image quality for gastrointestinal bleeding studies. The subjective and objective measurements used in this study may be useful for comparing image quality as other alternative blood-pool agents are developed for detecting gastrointestinal bleeding (20).

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