European Review for Medical and Pharmacological Sciences 2020; 24: 12788-12793

Quantitative analysis of gastrointestinal bleeding with technetium-99m–labeled red blood cells in an experimental model

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Abstract. – OBJECTIVE: The aim of this study was to propose a quantitative analysis method using technetium (Tc)-99m-labeled red blood cell RBC imaging to allow calculation of the amount and the rate of gastrointestinal (GI) bleeding.

MATERIALS AND METHODS: Rabbit models of fixed-GI and continuous-GI bleeding were created using catheter infusion. The amount and rate of bleeding were calculated and compared with the infused amount and rate.

RESULTS: No significant differences in calculated or actual amounts or rates of GI bleeding were observed after 2, 4, and 6 hours of catheter infusion of Tc-99m-RBCs.

CONCLUSIONS: The proposed quantitative analysis method using Tc-99m-RBCs GI scintigraphy can accurately calculate the actual amount and rate of GI bleeding.

Key Words:
Gastrointestinal bleeding, Tc-99m–RBCs, Gastrointestinal scintigraphy, Calculated amount of bleeding.

Introduction

Gastrointestinal (GI) bleeding is a serious clinical problem with a mortality rate of 4% to 10%[1]. For the detection of GI bleeding, existing methods can be classified into one of three categories: (1) methods based on the observation of clinical manifestations of peripheral circulatory failure caused by a blood volume decrease, especially the dynamic observation of blood pressure and pulse[2]; (2) methods based on red blood cell (RBC) count, mean corpuscular hemoglobin, and hematocrit[3]; and (3) methods based on imaging modalities such as endoscopy, angiography, barium chloride examination, computed tomography (CT) small bowel reconstruction, CT vascular reconstruction, and GI scintigraphy[4-7].

Of the existing methods, the advantages of technetium (Tc)-99m-RBC GI scintigraphy methods include its sensitivity to bleeding rates as low as 0.05-0.1 mL/min[8], while CT angiography can only detect bleeding rates as low as 0.5 mL/min[9]. However, Tc-99m-RBC GI scintigraphy methods often fail to determine the amount and rate of bleeding. It is important to correctly estimate them to guide clinical treatment, because the amount and rate of bleeding are closely related to severity. Thus, effective methods for quantitatively estimating the amount and rate of GI bleeding are still needed.

In our study, we aimed to quantitatively analyze the amount and rate of GI bleeds using Tc-99m-RBC imaging in a rabbit GI bleeding model. The underlying basis is radionuclide imaging, which can be analyzed by calculating functional parameters, such as quantification of regional cerebral blood flow[10], Gates' method of measuring glomerular filtration rate (GFR)[11,12], and standardized uptake value of positron emission tomography imaging[13]. For this reason, we proposed a quantitative analysis method.

Materials and Methods

Materials

Materials included 12 rabbits, six male and six female, each weighing 2 or 3 kg; 10% chlo-

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ral hydrate, and sodium stannous pyrophosphate 99mTc pertechnetate. This study was approved by the Animal Ethics Committee of Dalian Medical University Animal Center.

Methods

The rabbits were anesthetized with 10% chloral hydrate, 3mL/kg. A catheter was inserted and fixed in the colonic cavity through a midline abdominal incision in five rabbits and the small intestine of seven rabbits. Autologous rabbit RBCs were labeled using the *in vivo* Tc-99m-RBC labeling method[14]. After 20 minutes, 10 to 15 mL of blood was withdrawn from the rabbits' hearts for infusion through an intraluminal bowel catheter.

Labeling efficiency of the Tc-99m–RBC was determined in vein samples before reinjection and 20 minutes after by placing two drops of blood in a test tube and adding 2 ml physiological saline[15]. Cells and supernatant were then separated by centrifugation and assayed using a scintillation probe counter. Binding percentage was determined using the following formula:

$$\%\ \text{labeling efficiency} = \frac{RBC}{RBC + Sup +} \times 100$$

where is RBC activity and is supernatant activity. Labeling efficiency was more than 80%.

The rabbits were imaged in the supine position, and the camera was placed anteriorly with the abdomen and pelvis in the field of view. To simulate GI bleeding, the Tc-99m-labeled blood was withdrawn from the rabbits and infused through the intraluminal bowel catheter with a variable rate setting. To prevent blood clotting, the extension tubing and syringe used to inject the labeled RBCs were filled with 4,000 USP units of heparin.

Five-minute frames for static images of rabbits and 1 ml Tc-99m-labeled blood were recorded 0.5 hour and 2, 4, 6, and 10 hours after injection. After static imaging, a positive marker was placed on the surface of the region above the bleeding point. CT was performed, and the distance between the bleeding point and the surface of the abdomen was measured to determine the bleeding depth using the following formula:

$$\text{Bleeding depth (cm)} = \frac{D1 + D2}{2}$$

where D1 is the distance from the skin to the area anterior to the bleeding point, and D2 is the distance from the skin to the area posterior to the bleeding point (Figure 1).

Based on the above experimental preparations, we proposed using the quantitative analysis method. Using this method, scintigraphic images were read by three experienced nuclear medicine physicians independently. Each physician manually outlined the region of interest (ROI) around the bleeding and the background region. The average value of the count ratios and pixels in the ROI and background area were recorded. Then, the amount of bleeding was calculated according to:

$$\text{Bleeding amount (ml)} = \frac{\left(\frac{CR\ of\ \text{ROI}}{P\ of\ ROI} - \frac{CR\ of\ BG}{P\ of\ BG}\right) \times P\ of\ \text{ROI}/e^{-ux}}{1\ \text{ml}\ Tc{-}blood\ CR}$$

where CR is count ratio, P is pixels, and BG is background, u = 0.153, × = Bleeding depth.

Rates of bleeding were calculated according to:

$$\text{Bleeding rates for 2 to 4 hours (ml/h)} = \frac{\text{CBA at 4 hour-CBA at 2 hour}}{\text{2 hour}}$$

$$\text{Bleeding rates for 4 to 6 hours (ml/h)} = \frac{\text{CBA at 6 hour-CBA at 4 hour}}{\text{2 hour}}$$

where CBA is the calculated bleeding amount.

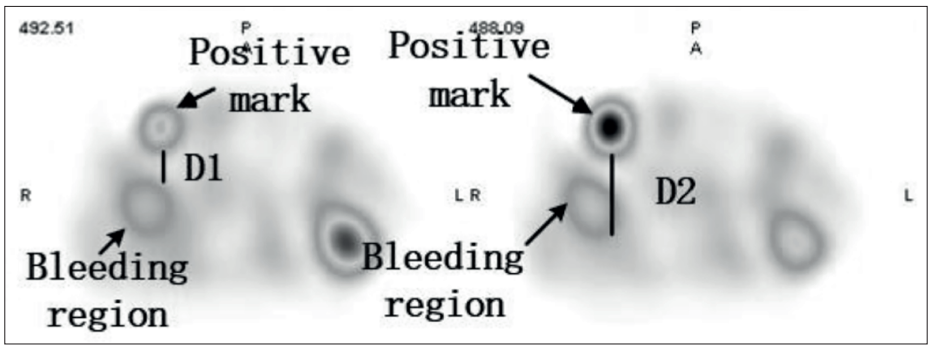

Figure 1. Distance from the skin of the abdomen to the bleeding region determined by placing a positive mark on the surface of abdomen above the bleeding region. The average of these two values (D1, D2) was calculated to obtain the bleeding depth.

Table I. Comparison between the calculated amount of bleeding and the actual blood amount of the fixed amount bleeding group.

	Calculated amount of bleeding (mL)	Actual blood amount (mL)	*p*
0.5 h	3.38 ± 1.46	5.2 ± 1.33	< 0.001
2 h	5.29 ± 1.39	5.2 ± 1.33	0.628*
4 h	5.36 ± 1.28	5.2 ± 1.33	0.358*
6 h	5.35± 1.30	5.2 ± 1.33	0.061*
10 h	2.92 ± 0.99	5.2 ± 1.33	< 0.001

*: No significant differences at $p > 0.05$.

Experimental Group

Six rabbits each were assigned to the continuous-bleeding or fixed-bleeding group. Rabbits in the continuous-bleeding group were studied at a variable rate using a Harvard pump.

Statistical Analysis

Data were analyzed using Statistical Product and Service Solutions (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA) statistical software. All data are expressed as *t*-test. A *p*-value greater than 0.05 indicated that the data in the two groups were not significantly different.

Results

The results of Tc-99m-RBC imaging were positive for all rabbits, and the shape of the bleeding sites was clearly shown in 0.5 to 10 hours.

In the fixed-bleeding group, 2, 4, and 6 hours post-injection, the calculated and actual bleeding amounts were not significantly different (Table I, Figures 2 and 3).

In the continuous-bleeding group, at 2, 4, and 6 hours post-injection, the calculated and actual bleeding amounts were not significantly different (Table II, Figures 4 and 5).

The calculated and actual bleeding results were not significantly different 2 to 4 hours or 4 to 6 hours after injection (Table III). At 0.5 to 10 hours post-injection, the calculated results in the fixed-bleeding and continuous-bleeding groups were statistically different from the actual amount of bleeding (Tables I and II, Figure 6).

Discussion

A variety of imaging modalities have been used to localize GI bleeding. The diagnosis of GI bleeding can usually be made on the basis of the course of the disease, physical examination, stool guaiac tests, and serial blood counts. Endoscopy and contrast angiography are commonly used methods to find the site of active GI bleeding, although false-negative results are common because the hemorrhage is intermittent. In animal models, Tc-99m sulfur colloid scintigraphy has been able to detect bleeding rates as slow as 0.1 mL/min. Tc-99m-RBC scintigraphy can localize the GI bleeding on repeat imaging for 24 hours because of the retention of circulating Tc-99m-RBC. However, these procedures can only yield a diagnosis at the time of tracer injection, and upper abdomen bleeding may be masked due to the high background in the liver and/or spleen[16-18]. Effective methods for quantitatively estimating the amount and rate of GI bleeding are still needed. This study proposed a method for accurate calculation of the amount and rate of GI bleeding.

To calculate the amount of bleeding, we used the quantitative analysis method formulated in

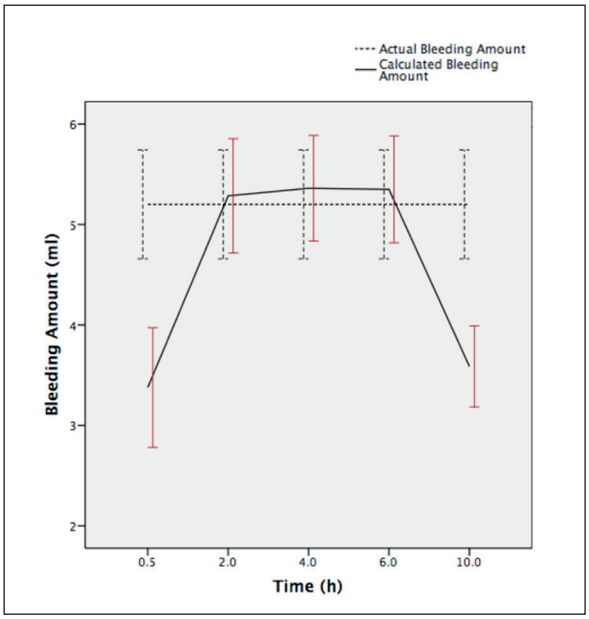

Figure 2. Comparison between the calculated and actual amount of bleeding in the fixed-bleeding group.

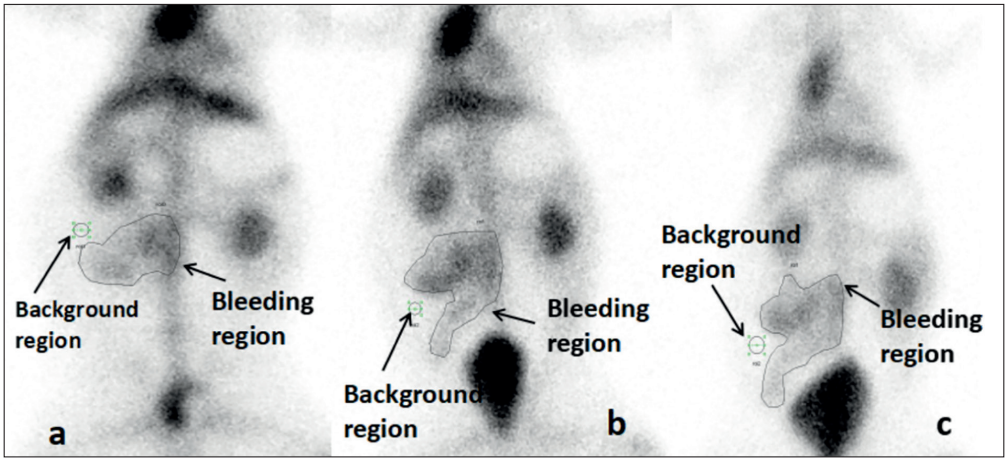

Figure 3. Rabbit 3 of the fixed-bleeding group was imaged after 2, 4, and 6 hours. **A,** Two-hour image shows flaky activity in left quadrant. **B,** and **C,** Images show increasing activity and movement to the distal bowel.

Table II. Comparison between the calculated amount of bleeding and the actual blood amount of the continuous bleeding group.

	Calculated amount of bleeding (mL)	Actual blood amount (mL)	*p*
0.5 h	2.24 ± 0.5	3.5 ± 0.44	< 0.001
2 h	3.6 ± 0.5	3.5 ± 0.44	0.115*
4 h	6.51 ± 0.98	6.42 ± 0.86	0.516*
6 h	8.98 ± 1.0	8.75 ± 0.88	0.181*
10 h	5.76 ± 1.54	8.75 ± 0.88	0.001

*: No significant differences at $p > 0.05$.

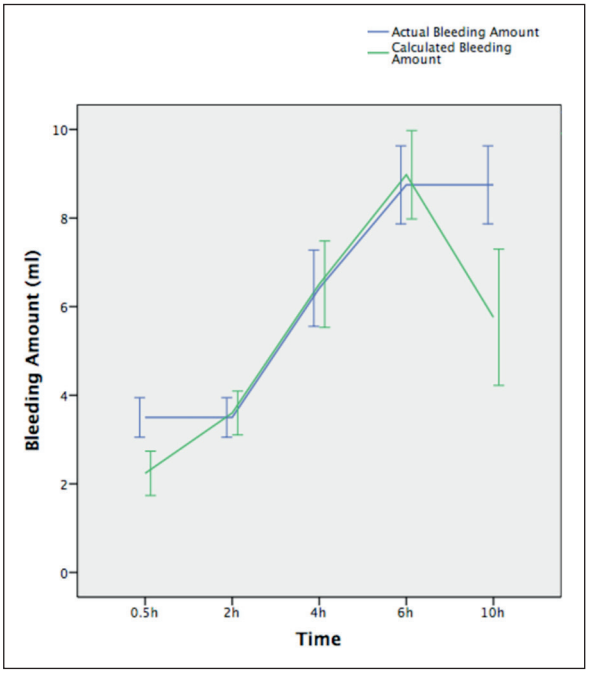

Figure 4. Comparison of the calculated and actual amount of bleeding in the continuous-bleeding group.

Eq. (3), which is analogous to Gates' method of measuring GFR. Based on the calculated bleeding results at each time point, we used Eqs. (4) (5) to calculate the bleeding rate of the continuous-bleeding group. The results show that the calculated results are consistent with the actual bleeding rate after injecting Tc-99m-labeled blood into intestine at 2 to 6 hours (Table III). The data from our study show that quantitative analysis of Tc-99m-RBC imaging is sensitive in calculating bleeding rates as low as 0.02 mL/min. Our findings show that the calculated results are consistent with the actual amount of bleeding 2 to 6 hours after injection (Tables I to III, Figures 1 and 2).

After 0.5 and 10 hours of injection, the calculated results were statistically different from the actual bleeding amounts. This is due to the free Tc-99m pertechnetate in blood taken up by the salivary glands and gastric mucosa and secreted into the GI tract in early imaging. As a result, the activity of background in the abdomen is high shortly after Tc-99m-RBC infusion. The

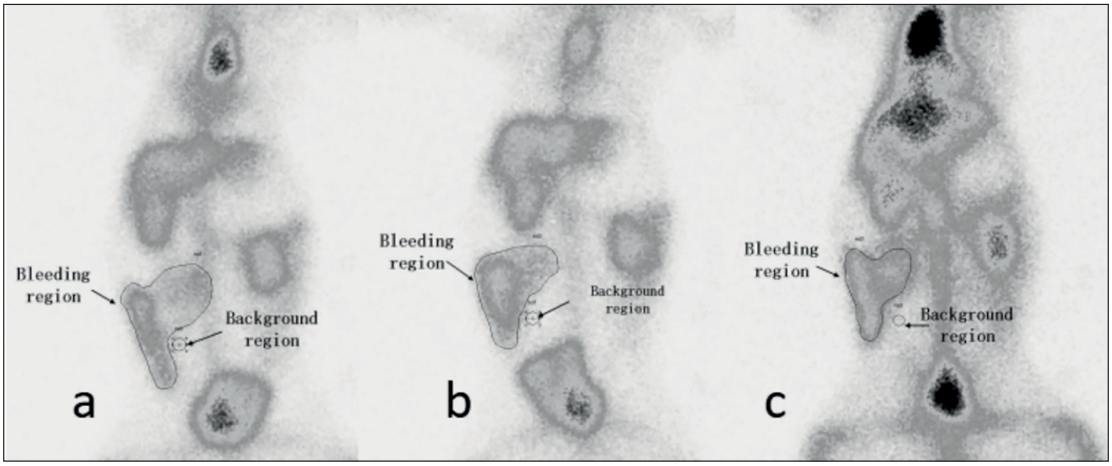

Figure 5. Rabbit 7 of the continuous-bleeding group was imaged after 2, 4, and 6 hours. **A**, Two-hour image shows flaky activity in left quadrant. **B,** and **C,** Images show increasing activity. Regions of interest are drawn for bleeding and background regions.

Table III. Comparison between calculated bleeding rate and actual bleeding rate.

	Calculated bleeding rate (mL/h)	Actual bleeding rate (mL/h)	*p*
2-4 h	1.454 ± 0.27	1.458 ± 0.246	0.952 *
4-6 h	1.235 ± 0.147	1.25 ± 0.316	0.903 *

*: No significant differences at $p > 0.05$.

radioactivity of the bleeding region is comparatively low, which leads to great errors in calculation. Over time, the more free Tc-99m pertechnetate is cleared, the lower the activity of the background. The activity in the bleeding region significantly increases, resulting in a significant reduction in calculation errors. Ten hours after Tc-99m-RBC injection, intestinal secretions are stimulated by the infused blood, resulting in dilution of infused blood and a significantly reduced count ratios of the bleeding region. Consequently, the calculated results are significantly less than the actual amounts 10 hours after blood injection.

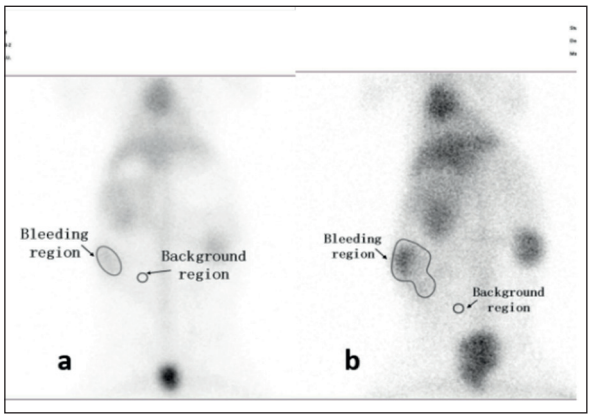

Figure 6. Rabbit 8 of the continuous-bleeding group is imaged after 0.5 and 10 hours. Images show blurry bleeding regions.

Conclusions

Treatment plans depend on the amount of GI bleeding. Our experimental results show that the calculated results are consistent with the actual amount and rate of bleeding 2 to 6 hours after Tc-99m-labeled blood injection. Thus, we observed that the formulas for quantitative analysis of Tc-99m-RBC imaging can accurately estimate the actual amount and rate of GI bleeding.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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