ACCURATE METHOD FOR DETERMINATION OF RED CELL VOLUME IN POLYCYTHEMIA VERA

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ABSTRACT

The detailed procedure was performed to determination of blood volume in polycythemia vera. The usual protocol takes 3 hours to run with patients but the new procedure takes only 1.5 hours, 87% of patients who come to section of nuclear medicine, Siriraj Hospital, for blood volume measurement are polycythemia vera. This special development saves 45% of time without using any money because this research comes from routine work. It is an example for young staffs who always complain about lacking of money and time to do research.

INTRODUCTION

A simple hematocrit reading can mislead the doctors in estimation of red cell mass ; for example, if the patient has a low plasma volume the hematocrit will be falsely high. Keith N.M.et al.proposed dilution principle for clinical investigation.[1] Application of techniques in nuclear medicine has improved understanding and knowledge of accurate measurement of the circulation volume. The first Thai originators in using ^{51}Cr in estimation of blood volume and red cell volume are Suwanik Romsai[2] and Intrasupta Somlak.[3] Ruksawin Nisarut[4] used ^{99m}Tc instead of ^{51}Cr after Sukavatsesiri Valeerat[5] proved that no sinificant difference of blood volume value between the use of ^{51}Cr-RBC and ^{99m}Tc- RBC as tracers. The new process described here has developed to the most natural one so the clot or aggregation of RBC should not occured during the process.

MATERIALS

1. Heparin 5000 I U per ml.
2. 5 ml of sterile water for injection in sterile serum vial.
3. Terumo Surflo Int Typr Wing Infusion Set. (21G, 9 cm tubing with releasable injection site)
4. Normal saline
5. Stannous kit[6]
6. Falcon plastic centrifuge tube # 2098
7. ACD solution formula A 2.5 ml ; This amount can be prepared by aseptically transfer 5 ml serum vials and reautoclave. Store at room temperature.
8. ^{99m}Tc pertechnetate 200 μCi ; this amount can be prepared by using $Na^{99m}TcO_4$ 10 mCi/ml 2drops from tuberculin syringe with needle.
9. Analytical balance
10. 100 ml volumetric flask
11. Hematocrit determination set
12. 1 ml automatic pipette with replaceable plastic tip
13. Test tubes
14. Gamma well counter

METHODS

1. No preparation of the patient is required.

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2. Preparation of dilute heparin by adding 0.25 ml of heparin to 5 ml of sterile water for injection.

3. Insert infusion set in the patient's arm vein, 5 ml sample of blood is removed from the patient to establish patient background level.

4. Flush the infusion set with 0.5 ml of dilute heparin to keep it patent.

5. Add 1.5 ml of normal saline to stannous kit, mix the vial and withdraw the solution from the vial then inject to the releasable injection site, flush with normal saline to push all of the stannous solution in the circulation followed by 0.5 ml of dilute heparin.

6. Allow a circulation time of 10 minutes before withdrawing a 12 ml blood sample, put the blood sample in sterile Falcon plastic tube containing 2.5 ml ACD solution. Mix the sample and immediately put 200 mci of Na $^{99m}TcO_4$ and mix well.

7. Incubate(6) for 5 minutes for ^{99m}Tc to tag to red blood cells (^{99m}Tc-RBC). Mix and use syringe to draw 6 ml of ^{99m}Tc-RBC.

8. Weigh(7) by analytical balance Weight of syringe + needle + ^{99m}Tc-RBC = $\mathbf{W_1}$ g

9. Preparation of standard solution by filling 100 ml volumetric flask with 95 ml of tab water. Inject ^{99m}Tc RBC about 0.2 ml into the volumetric flask, make the total volume to 100 ml by adding tab water.

10. After discarding about 0.2 ml of ^{99m}Tc-RBC in (9), the rest of ^{99m}Tc-RBC is weighed again as $\mathbf{W_2}$ g $\mathbf{W_1}$-$\mathbf{W_2}$ is the weight of the amount of ^{99m}Tc-RBC in volumetric flask.

11. Inject normal saline 2 ml via the releasable injection site for checking the patency.

12. Inject ^{99m}Tc-RBC via the releasable injection site, flush with normal saline to push all of ^{99m}Tc-RBC in the 9 cm tube into blood circulation. Remove the infusion set. Weigh the syringe containing residual of ^{99m}Tc-RBC as $\mathbf{W_3}$ g $\mathbf{W_2}$-$\mathbf{W_3}$ will be the exact weight of ^{99m}Tc-RBC injected in the blood circulation of the patient.

13. Allow a circulation time of 10 minutes before withdrawing 5 ml blood sample with heparinized syringe from a vein on the opposite limb in order to avoid contamination.

14. Triplicately pipette (3), (9) and (13) 1 ml of each into 9 test tubes and mark each as to their content. Count each sample in ^{99m}Tc window by autogamma well counter.
Let the average activity from (3) be **Bg** cpm
Let the average activity from (9) be **Std** cpm
Let the average activity from (12) be **Sample** cpm

15. Calculate total activity injected to blood circulation of the patient

$$= (\text{Average } \mathbf{Std} - \text{Average } \mathbf{Bg}) \times 100 \left[\frac{\mathbf{W2 - W3}}{\mathbf{W1 - W2}} \right] = \ldots\ldots\ldots\ldots \text{ cpm}$$

16. Dilution Volume

$$= \frac{\text{Total activity injected to blood circulation of the patient}}{(\text{Average } \mathbf{Sample} - \text{Average } \mathbf{Bg})} = \ldots\ldots\ldots \text{ ml}$$

17. Red Cell Volume = $\frac{\text{Dilution Volume x Hematocrit}}{100}$ = ... ml

RESULTS

The new protocol for measurement of red cell mass in patients with hematocrit higher than 50% was prepared for teaching session on radiopharmaceuticals at division of nuclear medicine, department of radiology, Siriraj Hospital. Excellent result were obtained with a modification of this method for polycythemia vera. The labeling procedure described here is not identical to other procedures because no centrifuge is used. With 5 minute incubation time and no washing of red blood cells saves 45% of time.

DISCUSSION

There are three reasons to discuss about the advantages of the new protocol. First reason , the Surflo Int type Wing Infusion Set was used to reduce three venipunctures to one, it is not only to create the non-invasive investigation but also to establish the most accurate way to put all ^{99m}Tc-RBC in the blood circulation. Second reason, Sn-kit was used instead of MDP-kit, the Sn-kit is cheaper but more powerful in labeling that incubation time could be reduced to 5 minutes. Third reason, thismethod does not use centrifuge to wash the red cells, so it reduces time and mechanical steps.

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