Advantages of Multiple Sampling in Red Blood Cell Volume Determinations

Wayne R. Kasecamp and Rosemary A. Longo

The Johns Hopkins Hospital, Baltimore, Maryland

A patient with an elevated hematocrit was referred to the clinical nuclear medicine laboratory for evaluation of possible polycythemia. A ^{51}Cr red blood cell mass measurement was performed. Multiple postinjection samples proved to be necessary for precise determination of the red blood cell mass by the ^{51}Cr radioisotope dilution technique.

The total circulating red blood cell (RBC) volume can be precisely measured by use of the radioisotope dilution technique using ^{51}Cr-labeled red blood cells and the body hematocrit *(1, 2)*. In normal subjects, 10 min is a sufficient period to insure complete mixing of the labeled erythrocytes within the body. Since mixing can be delayed in some patients, we routinely obtain delayed postinjection samples. This report describes a patient with polycythemia in whom it was demonstrated that complete mixing of the ^{51}Cr-labeled cells had not occurred within the first 30 min after injection.

Case Report

An 82-year-old male with a four-year history of headaches was evaluated at another hospital in Jan. 1975 and was found to have polycythemia. The patient was treated with phelobotomy with removal of four units of blood over the next four months. The headaches persisted and in May 1975 the patient was referred to this hospital for further evaluation.

The admission physical examination revealed a robust patient with striking plethora. The pulse was 94 and regular with a blood pressure of 190/110. The conjunctivae were suffused and the tongue appeared deep red. The palms were red and the nail beds dusky. The spleen was palpable. The initial laboratory data revealed a hematocrit of 61, white cell count of 13,000, and a platelet count of 660,000. The RBC indices were normal and the blood smear showed thrombocytosis with numerous giant and abnormal platelets. The blood gases were normal. The serum iron was low with a high iron-binding capacity.

Methods

The RBC volume was determined by adding 10 ml of the patient's whole blood into a sterile tube containing 2 ml ACD solution *(3)* and 30 μCi of ^{51}Cr sodium chromate. After thorough mixing, the solution was incubated for 15 min. Further labeling of the erythrocytes was stopped by adding 50 mg of ascorbic acid. Exactly 5 ml of the sterile labeled RBC solution was injected intravenously. A standard was prepared by pipetting 2 ml of the labeled whole blood from a dose tube into a 100-ml volumetric flask already containing 50–60 ml of distilled water and a pinch of saponin. The volume in the flask was brought to the calibrated mark with distilled water. After it was well mixed, a 2-ml sample was pipetted from the flask into the counting tube. This was the whole blood standard. The remaining whole blood in the dose tube was centrifuged and 2 ml of plasma was pipetted. This was the plasma standard. Because the patient was known to have polycythemia, multiple postinjection blood samples were obtained at 30, 60, 90, and 270 min. The total blood volume for each sampling time was calculated according to the following formula:

$$\text{Total blood volume} = \frac{\text{volume injected} \left[\left(\text{standard whole blood counts} \times \text{dilution factor} \right) - \left(\text{standard plasma counts} \times \frac{\text{standard plasma}}{\text{crit}} \right) \right]}{\text{postinjection whole blood counts} - \left(\text{postinjection plasma counts} \times \text{patient plasma crit} \right)}$$

For reprints contact: Wayne R. Kasecamp, Div. of Nuclear Medicine, Johns Hopkins Hospital, Baltimore, MD 21205.

$$\text{RBC volume} = \text{blood volume} \times \text{patient decimal hematocrit},$$

$$\text{Plasma volume} = \text{blood volume} - \text{RBC volume}.$$

The patient's hematocrit on the day of the study was 63%.

Discussion

It has been stated that in normal subjects 10 min postinjection is a sufficient period of time to insure proper mixing. In certain disease states, such as hypersplenism or polycythemia, it may be necessary to wait 30–60 min postinjection.

In this case report, although the RBC volume is increased initially, there is incomplete mixing at 10, 30, and 60 min postinjection. Only after 90 min does complete mixing occur as proved by the sample obtained at 270 min (Table 1). Because of the markedly increased RBC volume, a longer period of time is needed to insure proper mixing.

This case illustrates the importance of collecting multiple delayed blood samples in patients suspected of having polycythemia. Patients who demonstrate an increased RBC volume at early sampling times should then have an additional delayed sample obtained. As our data demonstrates, this sample may be obtained several hours later.

TABLE 1. Calculated Volumes of Postinjection Samples

Time interval postinjection (min)	Total blood volume (ml/kg)	Total RBC volume (ml/kg)	Total plasma volume (ml/kg)
10*	69	44	26
30	72	46	27
60	76	48	28
90	81	51	30
270	81	51	30
Normal range for males	55–75	25–35	30–45

**Ten-minute sample in this table was calculated from data obtained with 30-, 60-, 90-, and 270-min samples.*

References

1. Berson JA: Blood volume determination. *Bull NY Acad Med* 30: 755–762, 1955

2. Korst DR, Knorpp CT, Bolt RJ: Circulating red cell mass determination using radiochromate. *Int J Appl Radiat Isot* 2: 156–164, 1957

3. Cunningham R, McGirr EM, Clement WE: The effect of prior contact between acid citrate dextrose and sodium radiochromate solutions on the efficiency with which Cr51 labels red cells. *J Lab Clin Med* 50: 778–787, 1957