

## Red Cell Mass: The Sleeping Beauty

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**Objective:** One of the more neglected procedures in nuclear medicine is the radioisotopic determination of red cell mass (RCM) which is a valuable, cost-effective and easily performed procedure that helps establish the diagnosis of true polycythemia and distinguish it from relative erythrocytosis. Early diagnosis of polycythemia is important since a delay in treatment may be associated with serious complications.

**Methods:** Whole blood (15–25 ml) was withdrawn and added to a small volume of ACD solution. Chromate-51 (1.48–1.85 MBq) was added to the blood with continuous mixing at 37°–39°C. After incubation, ascorbic acid (50–100 mg) was added to reduce the  $^{51}\text{Cr}$  to the trivalent state and stop it from binding to the red cells. The blood was reinjected into the patient and a whole-blood sample was drawn from a different vein at 45 min postinjection. The aliquots from the sample were washed three times in saline and then counted.

**Results:** If the RCM exceeds the predicted mean normal value by more than 12.5%, the patient is thought to have a high-normal RCM, which may be a physiologic variable, transient anomaly or an early stage of an absolute polycythemia.

**Conclusions:** Although many laboratories express the results of RCM in terms of ml/kg body weight, this approach may be inaccurate for the individual patient. Based on the literature, a more accurate and practical method of predicting and interpreting RCM is presented.

**Key Words:** red cell mass; polycythemia; chromate-51

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Polycythemia vera is a chronic myeloproliferative disorder characterized by an increase in all cellular bone marrow elements, specifically in the red cell mass (RCM), caused by a clonal stem cell disorder. There is a true increase in the RCM and plasma volume (PV). A mild granulocytic leukocytosis and marked thrombocytosis occur frequently. Relative polycythemia is associated with an elevated hematocrit secondary to decreased PV rather than an increase in RCM.

Secondary polycythemia is an increase in RCM with some known cause. One of the most common causes for secondary polycythemia is smoking (1,2).

The usual laboratory parameters used for evaluation of blood volume like hemoglobin, red cell count and packed cell volume (PCV = hematocrit) do not always reflect the total RCM (3) due to PV fluctuations. It has been shown that absolute polycythemias were increasingly frequent at higher PCV levels but a definite diagnosis of polycythemia could only be made at a PCV of 0.60 (4). Patients with hematocrits in the range of 0.500–0.599 may have an increased, normal or low red cell volume.

### CLINICAL USEFULNESS

The most common use of RCM measurements is for the diagnosis and management of polycythemia vera. However, RCM measurements are also useful in surgical patients, where the evaluation of oligemia may be inaccurate by means of clinical signs and symptoms or central venous pressure (5). Post-trauma patients and bleeding patients may also benefit from RCM measurements.

### RADIOPHARMACEUTICALS

The ideal radionuclide for RCM determination must be stable, nonantigenic, sterile, nonpyrogenic and have a high specific activity. The amount of free radionuclide should be known and specific activity measurable. Equilibrium in the blood compartment must be attained in a short time (30–45 min) before samples are withdrawn.

Several different radiopharmaceuticals have been used in the past to measure red cell volume including radiolabeled carbon-monoxide, radiophosphorus,  $^{99m}\text{Tc}$ -pertechnetate and indium (6–9). Technetium-99m-pertechnetate has a short half-life and can be reinjected for repeated measurements, although 4%–10% of the activity elutes from the cells in the first hour after injection. Indium-113m is more suitable for short-term measurements since elution is less than that with technetium.

Currently, the most commonly used tracer is sodium-chromate-51 ( $^{51}\text{Cr}$ ). Its elution in vivo is negligible during the test period (60–90 min) and the gamma photons (320 keV) can

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easily be distinguished from those of  $^{125}\text{I}$  (35 keV) which is frequently used for simultaneous plasma volume determinations. However, the long half-life of  $^{51}\text{Cr}$  precludes frequent repeat measurements due to residual activity in the blood.

The estimated mean radiation dose from  $^{51}\text{Cr}$  to the spleen (critical organ) is 1.05 mGy per MBq administered, with 0.2 of the administered activity reaching the spleen (10).

## RCM MEASUREMENTS WITH CHROMATE-51

### Principles

The volume of a closed system can be determined by using a tracer and the dilution principle (10). Although the vascular system is an open system, its volume can be determined if the tracer leaves the system at a slow rate and the samples are taken only after complete mixing. This principle was adapted by the International Committee for Standardization in Hematology (ICSH), and is being used for RCM measurements.

### Procedure

Different laboratories use somewhat different activities and techniques. In general, 15–25 ml of whole blood is withdrawn from the patient and added to a small volume of ACD solution. A dose of 1.48–1.85 MBq of  $^{51}\text{Cr}$  (at least 0.2 ml) is added to the blood with continuous mixing at 37°–39°C. Following the incubation period, ascorbic acid (50–100 mg) is added to reduce the hexavalent chromium to the trivalent state and stop the binding of  $^{51}\text{Cr}$  to red cells. Aliquots are taken from the blood for background activity measurements and preparation of a standard. The pre-calibrated, well-mixed tagged blood is reinjected into the patient and a whole-blood sample is drawn from a different vein at 45 min post-injection. The aliquots from the sample, standard and background are washed three times in saline and then counted. It should be noted that labeling red cells by  $^{51}\text{Cr}$  is more rapid during incubation at 39° than at room temperature.

### Precautions

A careful technique is very important for accurate measurements as an excessive amount of anticoagulant may affect the calculated RCM. Red cell damage is minimized by using a high specific activity of  $^{51}\text{Cr}$  (1.85–3.70 MBq/ $\mu\text{g}$ ) and the patient should not receive more than 7.4 kBq/kg of body weight.

Since blood volume is affected by body position, all measurements should be taken after the patient has been supine for 20–30 min.

## CALCULATIONS

The measured RCM is the total amount of injected radioactivity (counts/minute) divided by the concentration of radioactivity in red cells of a blood sample drawn after mixing is complete (counts/minute per ml red cells) (11). Since we measure the radioactivity in a volume of a whole-blood sample, we have to multiply the result by the hematocrit of the

blood sample. Two important factors should be considered when measuring the hematocrit:

1. Trapped plasma between the red cells may overestimate the venous hematocrit and result in overestimation of RCM. It was estimated that in normal blood using the microhematocrit method, there is approximately 1% trapping (12). However, in polycythemia the trapping may rise to 3.5% and therefore correction for trapping may be appropriate. The extent of trapping also depends on the centrifugal force and time of centrifugation. Trapping also increases in spherocytosis, thalassemia and sickle cell anemia.
2. In normal subjects, the total blood volume (body) hematocrit is consistently lower than the peripheral venous hematocrit and the ratio (also known as the f-cell ratio) is approximately 0.9 (0.89–0.92) (6). This ratio, however, was found to rise in direct proportion to the size of splenic enlargement (13) and varies considerably between individuals. Due to the inherited variability of this ratio, some have proposed to abandon the use of the f-cell ratio for blood volume calculations.

It should be noted, however, that by measuring RCM and PV independently using two different tracers, the total body hematocrit can be estimated directly by the ratio  $\text{RCM}/(\text{RCM} + \text{PV})$ .

### Methods of Predicting RCM

Since body fat is relatively avascular, the more accurate predictions of expected normal values of RCM are obtained by derivation from the lean body mass of the individual (14–16). Since the measurements of lean body mass are cumbersome and time consuming, estimates were derived which have close similarity to the predictions based on lean body mass. Two commonly used predictions are those based on height and weight proposed by Nadler (17) and those based on body surface area proposed by Hurley (18). It has been demonstrated that predicting individual normal RCM by derivation from the Nadler tables gives almost identical results to the lean body mass method (4).

RCM reports commonly express the results in terms of ml/kg body weight. Many clinicians and laboratories prefer to interpret the results in this manner. This approach was also adapted by the polycythemia study group for the diagnosis of polycythemia vera (3) and in the report made by the ICSH. The mean normal value for males is commonly considered as  $30 \pm 5$  ml/kg and the mean normal value for females is  $25 \pm 5$  ml/kg (ICSH 1980).

Although this may be acceptable in screening large populations, expression of RCM in terms of ml/kg may have limitations in *individual patients*. Obese patients will tend to have low blood volume in relation to body weight.

It has been observed in our laboratory and by others (19) that in certain circumstances a considerable discrepancy may exist between the mean Nadler RCM predictions and

ml/kg predictions for individual subjects. This may be illustrated by using a fixed reference height and increasing the body weight. As body weight increases, it becomes more difficult to recognize polycythemia in the individual by using ml/kg predictions.

A method of predicting and interpreting RCM based on the blood volume prediction (PBV) of Nadler et al. was presented and expanded by Pearson et al. (19) who proposed that a more accurate determination of mean normal predicted red cell mass (MNRCM) is obtained by using:

$$0.47 * 0.91 * PBV = \text{MNRCM for males,}$$

$$0.43 * 0.91 * PBV = \text{MNRCM for females,}$$

where 0.47 and 0.43 are the mean hematocrits for males and females, respectively, and 0.91 is the mean predicted body-to-venous hematocrit ratio. The advantage of the prediction formulas is that they allow an individual subject's observed value to be interpreted by comparison with the appropriate reference value.

Examination of the 95% confidence limits of the Nadler predictions has shown that to be consistent with polycythemia, the measured RCM for males must exceed the predicted mean normal value by 25%, and for females the measured RCM must exceed the predicted mean normal value by 30%. This recommended approach is a satisfactory and convenient way to interpret RCM measurements.

When the measured RCM exceeds the predicted mean normal value by more than 12.5% but less than 25%, the patient may be regarded as having a high-normal RCM (4). The entity of high-normal RCM may be a physiologic variant, transient anomaly or an early stage of an absolute polycythemia.

## CONCLUSIONS

The radioisotopic determination of RCM is a useful and easy procedure. It is more accurate than the usual laboratory parameters used for evaluation of blood volume. With the appropriate corrections and acceptable normal prediction methods (e.g., Nadler et al.), it can substantially aid in the differential diagnosis and follow-up of polycythemia. It is recommended that in routine clinical practice, the upper limit of RCM should be defined as a percentage of the mean value, rather than expressing the results in terms of ml/kg body weight.

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