

Comparison of Red Cell and Whole Blood Volume as Performed Using Both Chromium-51–Tagged Red Cells and Iodine-125–Tagged Albumin and Using I-131–Tagged Albumin and Extrapolated Red Cell Volume

HOWARD J. DWORKIN, MD; MARY PREMO, BA, CNMT, ARRT (N);
STUART DEES, CNMT, ARRT (N)

ABSTRACT:*Background:* Our facility's current blood volume measurement protocol has involved separate measurement of plasma and red cell volumes. The purpose of this study is to determine whether measurement with a recently FDA-approved, one-compartment semiautomated system provides similar accuracy. *Methods:* Blood volume measurement was performed on 27 volunteers using our current protocol followed immediately by semiautomated plasma volume measurement and red cell volume calculation with a recently available system (BVA-100). *Results:* Double labeling for red cell mass and plasma volume required approximately 5 hours of technologist and processing time; measurement with the BVA-100 required approximately 1.5 hours or less, a saving of 3.5 hours time

per test. Whole blood and red cell volume each exhibited a Pearson correlation of 0.96, and plasma volume exhibited a Spearman rank correlation of 0.90. Average percent difference between the measurement methods was 2.2% for whole blood volume, 0.9% for red cell volume, and 3.3% for plasma volume. The mean ratio between the mean body hematocrit and measured venous hematocrit (f ratio) was 0.91, with a standard deviation of 0.0405. *Conclusions:* The BVA-100 has significant advantages in terms of time and ease of use. The 2 tests can be considered equivalent methods for blood volume measurements. **KEY INDEXING TERMS:** Blood volume; Plasma volume; Red cell volume; I-125 RISA [Am J Med Sci 2007;334(1):37–40.]

Blood volume derangements occur in a variety of conditions^{1–7} and are most accurately assessed through radioisotopic blood volume measurement.⁸

Currently, measurement of whole blood volume at William Beaumont Hospital includes red cell mass (RCM) measurement with Cr-51 labeled autologous red cells and plasma volume (PV) measurement with I-125 labeled human serum albumin (RISA) (cite). There are periodic lapses in the availability of Cr-51 and I-125, of which we are notified by phone.

These lapses have on occasion interfered with performing studies in a timely manner.

A convenient I-131 blood volume measurement kit has recently become available (Volumex, Daxor Corporation). We compared results from our standard method and the I-131 kit to see if the tests are equivalent, and if the kit is appropriate to replace our current measurement system.

Materials and Methods

Subjects

Twenty-seven volunteers (19 male, 8 female, ages 42 to 79) were studied consecutively, first (method I) using the traditional ICSH protocol (with minor variation, as described in the methods section), followed immediately thereafter by the radioiodinated I-131 albumin kit (method II, according to manufacturer's directions). Twenty-five patients had been referred to the William Beaumont Nuclear Medicine Department for suspected polycythemia or for follow-up of polycythemia treatment. The remaining two patients were healthy volunteers.

The study was approved by the William Beaumont Hospital Human Investigation Committee. All patients signed an Informed Consent Form after reading the form and having an opportunity to ask questions.

From the Department of Nuclear Medicine, William Beaumont Hospital, Royal Oak, Michigan.

Correspondence: Dr. Howard J. Dworkin, Department of Nuclear Medicine, William Beaumont Hospital, 3601 W. Thirteen Mile Road, Royal Oak, MI 48073-6769 (E-mail: hdworkin@beaumont.edu).

This work was supported in part by a grant from Daxor Corporation. There is no corporate relationship; the authors were not paid by Daxor Corporation with money provided only for supplies, radiopharmaceuticals, and equipment. Daxor did not participate in the performance of the studies. The work itself was not part of a larger clinical trial.

Comparison of Red Cell and Whole Blood Volume Using Tagged Red Cells, Iodine-125, and Albumin

Double-Labeled Blood Volume Measurement (Method I)

A sample of patient blood was removed. A portion was set aside for a patient blank, and a portion was mixed with Cr-51 sodium chromate. Two identical doses of chromium labeled red blood cells were drawn. One dose was used to prepare the standard and the other was reinfused into the patient. Both doses equaled about 30 μ Ci of Cr-51 labeled RBCs. Similarly, two doses of I-125 labeled human serum albumin (RISA) were drawn. One was used to prepare a standard and one to be injected into the patient. The RISA doses equaled about 8 μ Ci.

The patient was injected with both the Cr-51-labeled red cells and the I-125 iodinated human serum albumin. The Cr-51 RBCs were injected first, followed by 5 mL of saline. Then the RISA (contained in a separate syringe) was injected, followed by 5 mL of saline. Both radiopharmaceuticals were injected through the same IV line.

A total of 3 samples were then withdrawn, at 10, 20, and 30 minutes.

The hematocrit of the samples was determined.

The radioactivity of duplicate 3 mL aliquots of whole blood, I-125 and Cr-51 standards, and a patient blank were measured in a dual channel scintillation well counter. One channel is set to count I-125 and another to count Cr-51.

Red cell volume was calculated as follows. (These counts were measured in the Cr-51 Channel.)

$$\text{Red Cell Volume} = (C_{\text{Cr51std}} - C_{\text{rbkg}}) * 1000 \text{ Ml} * \text{Hct} (C_{\text{Cr51pt}} - C_{\text{pbkg}})$$

where C_{Cr51std} was the number of counts from the Cr-51 standard, C_{rbkg} was the number of counts from the **room background**, 1000 ml represents the volume of the standard (equal doses of the radiopharmaceutical are injected into the patient and the standard), and C_{Cr51pt} was the average count of the 3 blood samples counted in the Cr-51 channel of the well counter, **C_{pbkg} was the number of counts from patient background.**

For plasma volume, the patient sample counts were plotted semilogarithmically against time to extrapolate the counts at time $t = 0$. This corrects for loss of albumin from the intravascular space through transudation.

The plasma volume was then calculated as follows. (These counts were measured in the I-125 channel.)

$$\text{Plasma Volume} = (C_{\text{I125std}} - C_{\text{rbkg}}) * 1000 \text{ Ml} * (1 - \text{Hct}) (C_{\text{I125pt}} - C_{\text{pbkg}})$$

where C_{I125std} was the number of counts from the I-125 albumin standard, C_{rbkg} was the number of counts from the **room background**, 1000 ml represent the volume of the standard (again, equal doses of the radiopharmaceutical are injected into the standard and into the patient), C_{I125pt} represents the counts from the patient sample extrapolated back to $t=0$ and corrected for Cr-51 spill down into the I-125 channel; **C_{pbkg} was counts from patient background.**

Blood Volume Measurement with the Albumin I-131 Kit (BVA-100, Method II)

The BVA-100 is an integrated system consisting of a scintillation detector specifically designed for measuring plasma I-131 aliquots, a computer program that controls the detector and calculates blood volume from measurements of plasma radioactivity, and single-use kits of albumin I-131 injectate (in a flow chamber designed to deliver 99.8%+ of the radiolabeled albumin) and a matching standard in a pre-measured aliquot. Based on

studies of individual blood volume measurements submitted for FDA approval, the accuracy of the BVA-100 is $\pm 2.5\%$.

The patient was injected with approximately 10 μ Ci of I-131 iodinated human serum albumin. Five samples were withdrawn at 12, 18, 24, 30, and 36 minutes after injection, and 1 ml plasma aliquots were prepared in duplicate for each sample. The use of 5 sample points in the BVA-100 system (rather than the 3 sample points commonly used in double labeling) is designed to enable easier identification and possible discarding of outliers without loss of accuracy. The radioactivity of the sample aliquots, the standard, and a patient blank were measured.

These measurements were entered into the BVA-100 computer program and used to calculate blood volume. The BVA-100 calculations extrapolate plasma counts to zero time, to correct for albumin transudation. The whole blood and red cell volumes are determined using multiple hematocrit measurements; the ratio between venous (measured) hematocrit and whole body hematocrit is assumed to be 0.91. The program also incorporates a correction for trapped plasma.

Statistical Analysis

Whole blood (TBV), plasma (PV), and red cell volume (RCM) were expressed in milliliters. The difference between the 2 methods for each compartment was calculated for each of the 27 subjects using the following equation:

$$\frac{(\text{Mean BVA-100 Volume} - \text{Mean double-labeling volume})}{\frac{1}{2}(\text{Mean BVA-100 Volume} + \text{Mean Double-Labeling Volume})}$$

Whole blood volume and red cell volume distributions could be treated as normal distributions and compared with the paired t test. The plasma volume distribution was not normal. Its skewness was within 2 standard deviations of skewness for a sample size of 27 and could thus be treated as a symmetrical distribution and compared using the Wilcoxon signed rank test.

To compare possible variations arising from using a one-compartment versus a 2-compartment method,⁹⁻¹³ the venous hematocrit as measured for the double labeling technique was compared with the mean body hematocrit for each patient. A mean body hematocrit for each patient was calculated by dividing the Cr-51 measured red cell volume by the I-125 measured plasma volume. The ratio of the mean body hematocrit to the measured venous hematocrit (f ratio) was calculated.

Results

The technologist time for method I, which includes Cr-51 red cell labeling, was approximately 3.5 hours, with a total processing time of 5 hours for available results. For method II, the technologist time, including processing time for available results, was 1.5 hours.

Table 1 shows the mean, standard deviation, and correlation coefficient for each blood compartment measurement. Graphical comparison of the 2 sets of values is presented in Figures 1 through 3. The correlation between the methods for each compartment meets (PV) or exceeds (TBV and RCM) 0.90.

Table 1. Mean, Standard Deviation, and Correlation Coefficient for Each Blood Compartment Measurement

	Whole Blood Method I	Whole Blood Method II	Red Cell Method I	Red Cell Method II	Plasma Method I	Plasma Method II
Mean	5439	5320	2285	2306	3034	3134
Standard deviation	1268	1175	701	656	640	610
Correlation		0.96		0.96		0.90

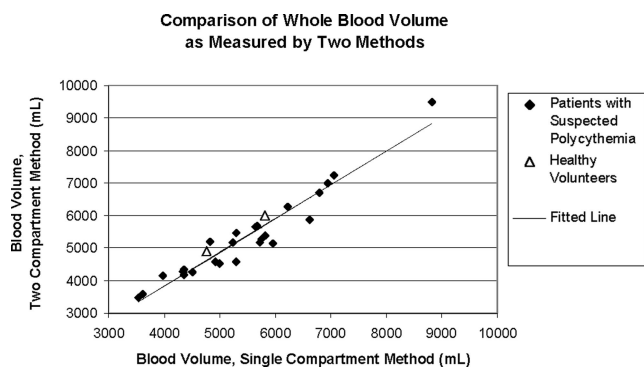


Figure 1. Whole blood, red cell, and plasma volume as measured by Method I compared with whole blood, red cell, and plasma volume as measured by method II. The fitted lines were calculated by the least-squares method.

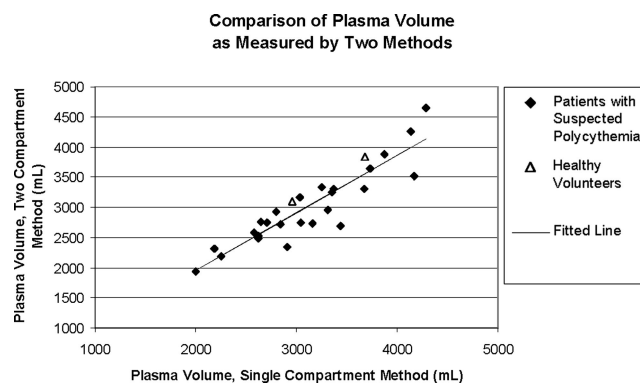


Figure 3. Whole blood, red cell, and plasma volume as measured by Method I compared with whole blood, red cell, and plasma volume as measured by method II. The fitted lines were calculated by the least-squares method.

The average percent differences were 2.2% for whole blood volume, 0.9% for red cell volume, and 3.3% for plasma volume.

The average f ratio of mean body hematocrit to measured hematocrit was found to be quite close to 0.91. Variations that occurred in f ratio did not correlate with differences between the 2 methods of measuring blood volume. Thus, individual differences in f ratio did not have a significant effect on the final accuracy of the results.

Discussion

The BVA-100 offers a considerable time saving for blood volume measurement. Additional time may be saved in emergency situations; preliminary results from the BVA-100 may be obtained from the first 2 to 3 samples, which can be withdrawn less than half an hour after injection. Results from the initial 2-3 samples are usually concordant within, respectively, 4% and 5% of the final results.

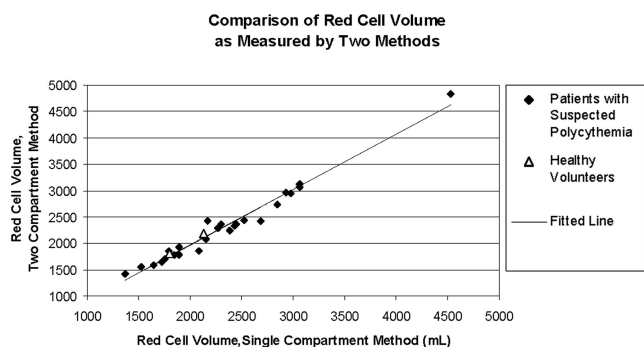


Figure 2. Whole blood, red cell, and plasma volume as measured by Method I compared with whole blood, red cell, and plasma volume as measured by method II. The fitted lines were calculated by the least-squares method.

Measurements for whole blood volume, red cell volume, and plasma volume exhibited a very high correlation between the 2 methods. Further, the percent differences between the 2 methods for whole blood volume and red cell volume were smaller than the reported accuracy of $\pm 2.5\%$ of the BVA-100 itself. The 2 tests can be considered equivalent.

It should be noted that this is a small study. Although generally the correlation of the data is tight, it would seem imperative that similar comparisons be done in other populations where “pathologic process may be a different etiology.”

The cost of re-agents were comparable.

Conclusion

From these tests, it can be seen that the BVA-100 correlated very strongly with double labeled radioisotopic blood volume measurement and can be treated as an equivalent test. The test is simpler and quicker to utilize and does not require the re-infusion of autologous cells, eliminating the concern of injectate mix-up. This test offers the opportunity for the incorporation of accurate radioisotopic blood volume measurement into clinical situations in which blood volume derangements are known to occur.

However, it should be noted that only a small number of normal patients were included in this study, and that a larger number of normal controls are necessary for complete validation. In addition, comparison of these 2 techniques needs to be extended into other disease states of altered blood volume.

Acknowledgments

The authors would like to acknowledge the assistance of the following people in the preparation of this manuscript: Conrad Nagle, MD, corporate chairman of Imaging Services at William Beaumont Hospital Daniel Soltis of Daxor Corporation.

References

1. **Androne AS, Hryniewicz K, Hudaihed A, et al.** Relation of unrecognized hypervolemia in chronic heart failure to clinical status, hemodynamics, and patient outcomes. *Am J Cardiol* 2004;93:1254–9.
2. **Androne AS, Katz SD, Lund L, et al.** Hemodilution is common in patients with advanced heart failure. *Circulation* 2003;107:226–9.
3. **Boussat S, Jacques T, Levy B, et al.** Intravascular volume monitoring and extravascular lung water in septic patients with pulmonary edema. *Intensive Care Med* 2002;28:712–8 Epub 2002 May 18.
4. **Gentilini P, Vizzutti F, Gentilini A, et al.** Update on ascites and hepatorenal syndrome. *Dig Liver Dis* 2002;34:592–605.
5. **Nair N, Padder FA, Kantharia BK.** Pathophysiology and management of neurocardiogenic syncope. *Am J Manag Care* 2003;9:327–34; quiz 335–6.
6. **Nakagawa A, Su CC, Sato K, et al.** Evaluation of changes in circulating blood volume during acute and very acute stages of subarachnoid hemorrhage: implications for the management of hypovolemia. *J Neurosurg* 2002;97:268–71, 512–7.
7. **Shevde K, Pagala M, Tyagaraj C, et al.** Preoperative blood volume deficit influences blood transfusion requirements in females and males undergoing coronary bypass graft surgery. *J Clin Anesth* 2002;14:512–517.
8. Recommended methods for measurement of red-cell and plasma volume: International Committee for Standardization in Haematology. *J Nucl Med* 1980;21:793–800.
9. **Chaplin H Jr, Mollison PL, Vetter H.** Body/venous hematocrit ratio: its constancy over wide hematocrit range. *J Clin Invest* 1953;32:1309.
10. **Nielsen S, Rodbro P.** Validity of rapid estimation of erythrocyte volume in the diagnosis of polycythemia vera. *Eur J Nucl Med* 1989;15:32–7.
11. **Najejan Y, Deschrywer F.** The body/venous haematocrit ratio and its use for calculating total blood volume from fractional volumes. *Eur J Nucl Med* 1984;9:558–60.
12. **Balga I, Solenthaler M, Furlan M.** Should whole-body red cell mass be measured or calculated? *Blood Cells Mol Dis* 2000;26:25–31; discussion 32–6.
13. **Fairbanks VF, Klee GG, Wiseman GA, et al.** Measurement of blood volume and red cell mass: re-examination of ⁵¹Cr and ¹²⁵I methods. *Blood Cells Mol Dis* 1996;22:169–86; discussion 186a–g.