Techniques Used for the Determination of Blood Volume

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The assessment of blood volume is an important tool in clinical medicine for the evaluation of disorders ranging from polycythemia, anemia, blood loss, and endocrine disorders to congestive heart failure and syncope. Several articles in this issue of the *American Journal of the Medical Sciences* outline the value of blood volume assessment in different disorders and disease states. This article reviews various methods of blood volume assessment, including those methods used for both relative and absolute blood volume.

'Relative' Blood Volume Assessment

Whole blood comprises a liquid phase (plasma) and a solid (or cellular) phase. The latter includes red blood cells, white blood cells, and platelets. In terms of cellular mass, the red cells comprise almost the entire cellular mass of whole blood, with negligible contributions from the other components. Plasma volume is regulated by various hormones, which include the renin-angiotensin-aldosterone system, antidiuretic hormone, and atrial natriuretic factor, whereas red cell volume is regulated by different hormones (erythropoietin) and growth factors.

The hematocrit or packed cell volume (PCV) is a routine blood test that is available as a part of a complete blood count (CBC). The hematocrit is the ratio of red cell volume to whole blood volume and is expressed as a percentage. The white blood cell and platelet fractions are usually ignored because they comprise a relatively small volume compared with the red cells and plasma.

The hematocrit is often used as a "poor man's" assessment of red blood cell volume, with a low hematocrit used as a sign of anemia. These data are incomplete, and in some cases they can be very misleading. Since hematocrit is a proportion of red cell volume to total blood volume, it does not reveal any information about the actual red cell volume. Figure 1A shows 2 examples of samples with identical hematocrits. Compared with the sample on the left, the sample on the right shows a balanced reduction in both the red cell volume and the plasma volume with a preserved ratio between these 2 variables. Despite the similar hematocrit values between the two samples, it would be inaccurate to state that the red cell volumes were equal. The sample on the right may actually reflect a low red cell volume, or anemia, despite the normal hematocrit.

Figure 1B shows 3 examples in which the red cell volume is identical. Each sample, however, has a varying degree of plasma volume. The resultant differences in hematocrit give a superficial appearance of polycythemia (middle, M) and anemia (right, R) if the perturbations are isolated to plasma volume and not red cell volume.

Another consideration when using hematocrit is that it is sensitive to body position. We and others have shown that the hematocrit is significantly higher when measured with the patient in an upright position compared with a supine position. This phenomenon, which has been termed "postural pseudoanemia," is thought to be due to the gravity-dependent transudation of plasma from the intravascular space into the extravascular space. As an example, a change in body posture can result in an average absolute change in the hematocrit of over 4% (from 41.8% to 37.7%), which is a change that is potentially large enough to raise concerns about acute blood loss. Other technical considerations include the assumption that the ratio of central: peripheral hematocrit is 0.96 (F-cell ratio) when sampling in the peripheral vasculature such as the arm. Further, an excess of EJTA in the blood collection tube can cause a falsely low hematocrit and, conversely, the amount of "trapped plasma" within
the mass of red cells (by inadequate centrifugation) can lead to a slight overestimate of hematocrit.⁶

'Absolute' Blood Volume Assessment

Absolute assessments of blood volume use methods that follow the indicator and dye-dilution method. The basic principle is that if a known amount of indicator (A) is injected into a system, and the concentration (C) of the indicator can be measured, then one can solve for the unknown volume (V), such as plasma volume, using the formula $V = CA$ (a rearrangement of $A = V/C$). As seen in Figure 2, if a known volume of red dye is injected into a small volume of fluid, such as a glass of water, then the water will be crimson with a high concentration of red dye. If the same known volume of red dye is injected into a large volume of fluid, such as a lake, then the water probably would show only the lightest pink tinge, as the water would have a very low concentration of dye.

The use of a radiolabeled tracer as the indicator poses some unique challenges as compared with a dye. Due to radioactive decay, the measured activity will decrease with time, and thus the measured "concentration" of tracer will also vary. To circumvent this difficulty, one can simultaneously inject the same amount of tracer both into the patient (Ap - patient) as well as a "standard" solution (As - standard) of known volume (Vs - standard volume). The concentration of tracer, or activity, can be measured in both the patient (Cp) and standard (Cs). Since $\Delta A = Vs \times Cs$, $Ap = Vp \times Cp$, and $As$ and $Ap$ are equal, then $Vp \times Cp = Vs \times Cs$, which can be rearranged to solve for the patient volume, $Vp = (Vs \times Cs)/Cs$.

An underlying assumption of this method is that the red blood cell compartments and plasma compartments are "closed systems," or at least in a steady state. For short durations of time required to complete sampling in these studies, this assumption is reasonable in the absence of any changes in body position or significant hemorrhages. There is a minimal transfer of albumin out of the intravascular space to the extravascular space (albumin is used to tag the plasma volume) in this time, and one would not expect a sudden release of red blood cells into the circulating blood volume.

Specific Measurement Techniques

The commonly used techniques for the measurement of blood volume can be grouped into those methods that use a radioisotope tagged to a blood product (either red blood cells or albumin) and those that use a dye (Evans blue or indocyanine green). These techniques are discussed below. There are also other techniques that can be used to measure blood volume such as CO rebreathing⁷⁸ or ⁵¹Cr mass spectrometry,⁹ which are discussed elsewhere.

⁵¹Cr Red Blood Cell Volume

The gold standard technique of red blood cell volume measurement is the chromium isotope labeled red blood cell (⁵¹Cr-RBC) method.¹⁰ This technique involves collecting 30 to 50 mL of whole blood from the patient and labeling the red cells with ⁵¹Cr in
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vitro. The $^{51}$Cr penetrates the red cell membrane and binds to the hemoglobin $\beta$-chain. Once bound to the red cells, there is minimal (and very slow) elution of $^{51}$Cr from the red blood cells. This labeling takes about 30 to 60 minutes, after which the labeling is terminated, the cells are “washed” to remove unbound $^{51}$Cr, and the cells are then reinjected into the patient. The labeled red cells usually distribute uniformly in the vascular space within 10 to 20 minutes, and blood sampling should be performed after 20 minutes. The red cells are lysed before counting the radioactivity. Due to radioactive decay, a “standard dilution” is also required. The “standard dilution” consists of a 1 mL-aliquot of $^{51}$Cr-labeled autologous red cells diluted into 100 mL of water. Since the sampled blood is whole blood, the calculated patient volume (Vp) is the total blood volume. The hematocrit can then be used to estimate the red blood cell volume and the plasma volume (with the assumption that all of the non-red cell volume is plasma).

Radiolabeled Iodinated Human Serum Albumin

Plasma volume is usually assumed to be equivalent to the albumin circulating space. Commonly available techniques involve tagging iodine isotopes ($^{125}$I or $^{131}$I) to human serum albumin (HSA). The HSA is typically heat-treated at 60°C for 10 hours, a method thought to kill encapsulated viruses such as Hepatitis B, Hepatitis C, or the human immunodeficiency virus. A known small amount of the radiolabeled albumin is injected into the patient, and samples collected after the radiolabeled albumin has fully mixed throughout the plasma (which usually occurs within 10 to 30 minutes). Whole blood is then sampled, a capillary hematocrit measured, and then the sample is centrifuged to extract the plasma. The radioactivity of the plasma sample can be measured and compared with the activity of a “standard solution” (in which a known amount of radiolabeled albumin is injected into a known volume of water), and the plasma volume is calculated.

Some nuclear medicine laboratories will simultaneously measure blood volume with both the $^{51}$Cr-RBC and the $^{125}$I-HSA methods to provide optimal accuracy for measurements of both red cell and plasma volume.

DAXOR BVA-100 $^{131}$I-HSA Method

The BVA-100 $^{131}$I-HSA technique (DAXOR Corporation, New York) follows the principles outlined above for the measurement of plasma volume. The key difference is that the Food and Drug Administration-approved BVA-100 system provides the needed elements in an easy-to-use prepackaged kit. The $^{131}$I-HSA comes in a predosed syringe (Volume) with between 10 and 25 $\mu$Ci of activity and a “standard solution” created from the same batch of $^{131}$I-HSA. Blood is sampled from the patient before injection (blank) and then sampled 4 or 5 times after the injection of BVA-100 $^{131}$I-HSA. The samples are centrifuged and the plasma placed in a gamma counter that is connected to a computer for automated data acquisition. The use of multiple sampling allows for the individual blood volume values to be digitally plotted on a semi-log scale and then “back-extrapolation” from the time of sampling to the time of injection to correct for the errors introduced by radioactive decay and by a small time-dependent loss of albumin from the vascular space. Thus, multiple sampling improves the accuracy of the plasma volume measurement. Each sample is counted twice in reverse order (the first sample counted is again counted last) and the 2 values are averaged to minimize the effects of radioactive decay. As with other techniques, a microcapillary hematocrit measurement is used to calculate the total blood volume and red cell volume. Elsewhere in this issue of The American Journal of the Medical Sciences, Dworkin et al compared the BVA-100 based method with the more traditional $^{51}$Cr-RBC and $^{125}$I-HSA methods and found that the BVA-100 produced similar results in less time. One disadvantage of the BVA-100 system is that the $^{131}$I-HSA is more expensive than the other radioisotopes used for blood volume determination, although this may be offset by a savings in labor costs and time.

Evans Blue Dye Dilution Plasma Volume Measurement

Evans blue (EB) dye is a diazo chemical compound that strongly binds to plasma proteins. The volume of distribution of EB is the plasma volume; thus, it can be used to measure this space. After the injection of a known amount of EB into a vein, several minutes must be given to allow for adequate equilibration of the EB in the plasma. Blood is then sampled, using either a single timed blood draw (with a correction for the timing of the draw) or multiple blood draws with a back-extrapolation to the time-zero blood volume. Plasma volume can be calculated through spectrophotometry either directly from the plasma or from the EB eluted from the plasma. Spectrophotometric absorption of eluted EB dye is highest at 620 nm, but multiple wavelengths between 600 and 780 nm are used when using raw plasma samples (to avoid washing and eluting EB from plasma) due to potentially interfering substances in plasma. A preinjection blood sample (plasma blank) can be used to account for interfering substances and any residual EB from a prior blood volume assessment. Assuming that the EB binds tightly to plasma proteins that distribute evenly throughout the circulating blood volume, plasma volume can be calcu-
lates by 1) knowing the exact amount of dye that has been injected, 2) use of a correction factor to account for albumin leakage out of the blood vessels,15 and 3) spectrophotometric determination of the dye density in a postinjection sample. Hematocrit (Hct) must be obtained simultaneously to calculate total blood volume.

There are several caveats important to the measurement of plasma volume with EB. A large-gauge intravenous catheter is placed to ensure that the dye is injected into a patent vessel. Care must be taken to clear the injection site by saline flush so it will enter the circulation and is cleared so a postinjection sample will not be contaminated by the preinjection. The loaded syringes should be transported with the bevel of the injection needle maintained in upright position so dye is not lost. The same cap used on the preinjection weighed syringe should be used to minimize weight variations, although safety concerns may preclude recapping.

When the procedure is performed by experienced operators and care is taken to ensure the accuracy of the procedures, day-to-day variation in a volunteer can be measured with an accuracy of ±10 mL,17 although this variation will be much higher for most users. A primary advantage to the use of EB is that it does not involve any radioactive substances. A potential risk is that EB is a suspected carcinogen based on some animal studies.

Indocyanine Green Plasma Volume Measurement

Long used in determinations of cardiac output, the compound indocyanine green (ICG) also utilizes the indicator dilution method and the fact that it is tightly bound to plasma proteins. A key difference between ICG and other techniques mentioned above is that the halflife of ICG is very short due to rapid elimination of ICG by the liver. The rate of elimination decreases in liver failure.18 Thus, multiple postinjection samples are needed to enable back-extrapolation to calculate the plasma volume at the time of injection.19 Unfortunately, the use of back-extrapolation with a rapidly disappearing substance can decrease the accuracy of the measurement. One advantage of the rapid elimination of ICG is that this enables sequential determinations of plasma volume, since the dye does not accumulate in the blood. The peak spectrophotometric absorption of ICG in plasma is at 805 nm. Whereas the traditional method involves repeated arterial blood sampling, more recently, noninvasive ear oximetry and finger photosensor detection of ICG have been used11,20,21 to enable calculation of the protein bound dye decay curves in the circulation after injection. These noninvasive techniques are available in Europe and Japan but are not yet Food and Drug Administration-approved for use in the United States. ICG is again nonradioactive and appears to be non-toxic.

Ideal (or Expected) Blood Volumes

Blood volume measurements can be difficult to interpret in isolation (Table 1). This is because, as a patient or physician, we are not usually interested in knowing just the blood volume but whether the blood volume is abnormally high or low. Unlike some parameters with a static normal range (eg, serum sodium), expected blood volume changes with body size, lean versus fat body mass, and gender. One approach is to correct blood volume for body mass (mL/kg) or body mass index (mL/kg/m²). Unfortunately, the relation between height or mass and blood volume is not linear but rather it follows a curvilinear relation. The use of a linear correlation will tend to overestimate blood volume in an obese individual because fat does not increase blood volume as much as lean muscle. The BVA-100 blood volume analyzer calculates and reports expected blood volumes automatically. Feldschuh et al expand on this concept in an article in this issue of the American Journal of the Medical Sciences.

Conclusions

There are currently many techniques available to measure blood volume. Not every technique will be suitable for every purpose. Whereas a radiolotope-based assessment may give optimal results for a "routine" clinical blood volume assessment, a nonradioactive dye approach might be more suitable for some research applications. Similarly, ICG may be the agent of choice if multiple assessments of blood
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volume are needed over a short time span (eg, over a few hours).

Acknowledgments

We thank our research subjects, without whom this work would not be possible. We would also like to thank the dedicated staff of the Vanderbilt University Clinical Research Center. We would also like to thank Dr. Jeff Clinton for his thoughtful review of this manuscript.

References