COMMENTARY

This paper by Fairbanks et al. takes the position that measurement of red cell mass (RCM) by the ICSH procedure is unnecessarily duplicative in that the procedure requires the use of both ^{51}Cr labeled red cells and ^{123}I labeled albumin. The authors are of the opinion that measurement of the hematocrit, along with measurement of total blood volume using the dilution of a known amount of ^{123}I labeled albumin, provides an estimation of RCM that is adequate for clinical purposes. In this process the measurement of an accurate whole body hematocrit is obviously critical.

Whole body hematocrit cannot be measured directly. It is usually calculated from a measurement of the venous hematocrit which is known to be higher than whole body hematocrit by a factor which has been estimated by several investigators. The authors use:

0.864 x venous hematocrit = whole body hematocrit

The centrality of hematocrit measurement to a determination of RCM is underscored by the frequency with which the authors mention the process and its pitfalls:

1. page 170, left column, lines 5-11. "...hematocrit was measured by Coulter Counter, rather than by centrifugation...(for) elimination of plasma trapping..."
2. page 170, right column, lines 5, 6. "Centrifuged hematocrit values introduce a significant non-rectilinear error."
3. page 177, Fig. 5 legend, lines 15-18. "Normal erythrocytes are deformable discocytes that pack tightly on centrifugation, so that trapped fluid (“plasma trapping”) is about 4% of the normal hematocrit...much less if there is anemia...much greater if there is polycythemia...errors are inevitable if calculations are made using centrifuged hematocrit values".

and finally

4. page 178, left column, lines 23-26. "To whatever extent there may be an error in measurement of the venous hematocrit there will be a proportional error in the calculated RCM".

Clearly the authors are firm in their conclusion that the entire process of measuring RCM (whether by ^{51}CR and ^{123}I, or by ^{123}I alone) is crucially dependent upon an accurate hematocrit measure-ment. It is therefore puzzling that they insist upon use of the one approach to hematocrit measurement -- use of a multichannel hematology analyzer (Coulter Counter) -- that is known to be seriously flawed. They thus introduce a significant amount of avoidable random error in the process of measuring RCM.

The authors suggest that a multichannel hematology analyzer be used to measure hematocrit because they believe it to be immune to an error caused by variable plasma trapping in centrifuged microhematocrits. All hematology analyzers, including the Coulter, are calibrated against a centrifuged microhematocrit -- the same centrifuged microhematocrit that the authors denigrate. It is true that at one time the Coulter corporation did inflate the centrifuged microhematocrit values by 4% during the calibration process. They abandoned this correction more than a decade ago when it was realized that the 4% figure came from an archaic process of hematocrit measurement using Wintrobe sedimentation tubes spun for 20 minutes in a swinging bucket centrifuge. Like Coulter, other manufacturers of calibration materials, both in this country and abroad, now also use the microhematocrit as their calibration standard as specified by the National Committee for Clinical Laboratory Standards (NCCLS) (1).

Despite assertions of freedom from centrifuged hematocrits, the authors are likely to have used (apparently unknowingly) some derivative of a centrifuged hematocrit in all of their RCM measurements. This is because centrifuged microhematocrits will have been used to assign hematocrit values to the materials used to standardize Coulter Counters.

What is of considerably greater moment, however, is that the cause for discrepancies between the Coulter hematocrit and centrifuged micro-hematocrits is known -- and it is not, as the authors infer, a problem with the centrifuged microhematocrit measurement. It is, rather, due to the way in which most conductance-orifice analyzers register the volume of cells with an unusually high or unusually low mean corpuscular hemoglobin concentration (MCHC). High MCHC cells are internally more viscous than normal and do not deform rapidly into a maximally streamlined shape as they traverse the conductance orifice of the analyzer. Because of this high internal viscosity they appear larger and their cell volume is overestimated. The analyzers determine hematocrit by multiplying the average cell volume by the red cell count (or some mathematically equivalent procedure). Hence, the hematocrit of a high MCHC sample will be overestimated. Conversely, low MCHCs will result in an underestimation of the hematocrit.

This effect and its cause was first described in 1981 by Arnfred et al. in a paper entitled "Coulter...measurements of MCV are influenced by the mean erythrocyte haemoglobin concentration (MCHC)" (2). These observations were confirmed by Crawford et al. in 1987 in a paper that delineated the difficulties that varying MCHCs introduce into the calibration process for multichannel hematology analyzers (3). The minimal amount of trapped plasma that does remain in a properly centrifuged microhematocrit tube and the compensating errors that result from red cell dehydration resulting from contact with the tube wall were explored by Karlow and coworkers in the course of measuring the effect of the diameter of the microhematocrit tube on the registered hematocrit (4). For accurate measurement of the hematocrit, recent pronouncements by standards organizations such as NCCLS specify the use of the centrifuged microhematocrit (1).

The reason that these standards organizations eschew the use of secondary measurement devices such as the Coulter analyzer for measurement of hematocrit has been underscored by Bull et al. (5), who used reference methods to compare the performance of several multichannel analyzers with respect to their ability to measure an accurate MCHC and hematocrit. Several of the analyzers, including the Coulter, performed poorly. They performed so poorly, in fact, that they introduced an entirely new and significant source of error into the hematocrit measurement (and, by extension, into the calculation of the RCM). This new error -- the hematocrit registration error -- was occasioned by varying MCHC levels in the patient samples. The extent of this error can be estimated from the figures provided by Bull et al. (5) In their data set (using samples chosen specifically for high and low MCHC values) the hematocrit registration error would have contributed an additional 2.9% CV to the estimation of RCM. This is a source of error that can be completely eliminated by using centrifuged microhematocrits to measure venous hematocrit when RCM is measured.

REFERENCES

1. National Committee for Clinical Laboratory Standards. Procedure for determining packed cell volume by the microhematocrit method - second edition; approved standard. *NCCLS, 771 East Lancaster Avenue, Villanova, Pennsylvania 19085* 13 no. 9, 1-21, 1994.
2. Arnfred T, Christensen SD, Munck V. Coulter counter model S and model S-plus measurements of mean erythrocyte volume (MCV) are influenced by the mean erythrocyte haemoglobin concentration (MCHC). *Scand J Clin Lab Invest* 41, 717-721, 1981.
3. Crawford JM, Lau YR, Bull BS. Calibration of hematology analyzers -- role of the microhematocrit. *Arch Path Lab Med* 111, 324-327, 1987
4. Karlow MA, Westengard JC, Bull BS. Does tube diameter influence the packed cell volume? *Clin Lab Haemat* 11, 375-383, 1989
5. Bull BS, Aller R, Houwen B. MCHC - Red Cell Index or Quality Control Parameter? In: McArthur JR, Lee SH, Wong JEL, Ong YW, eds. *Haematology 1996, Education Programme of the 26th Congress of the International Society of Haematology,* pp. 43-43, 1996

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