MCHC - Red Cell Index or Quality Control Parameter?

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Abstract

Clinicians frequently review red cell indices when they are trying to diagnose a patient's anemia. In so doing, most clinicians assume that all three indices - MCV, MCH and MCHC - are equally reliable and that each conveys as much useful information as does the hemoglobin or the hematocrit. If the indices have been measured on a multichannel hematology analyzer (as is almost always the case), this assumption on the clinician's part is in error.

Just how misleading the results may be depends largely on the sensitivity of the analyzer in question to the internal viscosity of red cells. Some analyzers are severely affected and significantly overestimate the MCV of highly viscous (high MCHC) cells. These same analyzers typically underestimate the MCV of low MCHC cells. Because of a compounding effect, the accuracy of MCHCs reported by such analyzers is very substantially impaired.

Of the red cell indices, the MCH is the only one that approaches the accuracy achieved by such routine hematology measurements as the hemoglobin and hematocrit. The MCV is poorer but still clinically useful as measured by virtually all multichannel analyzers. The MCHC is, by far, the least accurate of the three indices and, as reported by some analyzers, is virtually devoid of clinical usefulness. Surprisingly enough, on these analyzers the MCHC is typically highly useful as a quality control parameter. The analyzer errors that make the MCHC less useful clinically simultaneously enhance its stability and thus render it more useful as an analyzer calibration control.

Through the years, the red cell index mean corpuscular hemoglobin concentration (MCHC) has enjoyed considerable favor as a diagnostic tool. Along with the mean corpuscular volume (MCV), it has served to pigeon-hole the various anemias into smaller and more manageable groupings. The primary usefulness of the MCHC has been to distinguish between those anemias in which the red cells were normochromic versus those where the red cells were hypochromic.

More recently, the MCHC has found an additional use as a quality control parameter. This has come about because the three red cell indices - MCV, MCH and MCHC - are very tightly controlled physiologically with a coefficient of variation of less than 6%. It is thus the case that for a typical patient population an average value for the red cell indices will fall within very narrow limits if those indices are determined on a properly calibrated multichannel analyzer. Of the three red cell indices as measured on a multichannel analyzer, the MCHC is the most stable and thus the first to reflect any drift in analyzer calibration - hence its more recent use as a quality control parameter.[1,2]

Mathematically, the derivation of the MCHC is simple. It is derived by dividing the hemoglobin level of a blood sample (the amount of respiratory pigment) by the hematocrit (the space occupied by the cells that carry that pigment). The resulting ratio reflects the concentration of hemoglobin in the average red cell. Since hemoglobin is the

major intracellular protein, the MCHC reflects the protein content of the red cell - the rest of the intracellular contents being largely water. Thus, the MCHC reflects, very sensitively, the intracellular viscosity and for that reason, is not physiologically often found to exceed 40%. Above that level the intracellular protein content (and hence viscosity) is so high that red cell flexibility is too compromised to allow the cell to circulate. Thus, a theoretically possible MCHC category - hyperchromia - probably does not exist.

This tidy conceptual picture of the derivation and diagnostic usefulness of the MCHC was very significantly upset with the arrival of the multichannel hematology analyzers. Shortly after their introduction into the routine hematology laboratory, it became clear that the MCHC as registered by some multichannel analyzers differed significantly in certain blood samples from the MCHC that was calculated by dividing the hemoglobin of that sample by a spun hematocrit.

Nor was this the only problem that the new analyzers posed. Something very odd had also happened to the hematocrit. Unlike the MCHC measurements, where the machine had narrowed the range over which the MCHC could vary in a patient population, the hematocrit values generated by the conductance-orifice analyzers showed increased variability.

The increased variability of the hematocrit was initially explained by the notion that some plasma in spun hematocrits remained trapped in the red cell column and thus inflated the hematocrit as determined by centrifugation. Plasma trapping as the cause of at least the hematocrit part of this problem seemed intuitively correct. Unfortunately, like so many intuitively appealing solutions, the notion of plasma trapping as an explanation of the increased hematocrit variability turned out to be wrong.

Consider for a moment how the hematocrit and the MCHC are related. In the normal course of events the spun hematocrit is divided into the hemoglobin in order to determine the MCHC. An increased variability in the hematocrit would lead to an increased variability in the MCHC assuming the hemoglobin values were stable. That, however, is not the way in which the analyzers that utilize a conductance orifice determine the hematocrit. They do it by measuring the average volume of the red cells in a blood sample and multiply that volume by the red cell count (Coulter and similar machines) or, alternatively, by summing the volume of each red cell as it passes through the orifice during a counting cycle to arrive at the hematocrit directly (TOA-Sysmex analyzer).

If there were some way in which a high MCHC cell were to look larger than it deserved - if the fundamental error was one that affected the size determination - then both the range narrowing of the MCHC and the range widening of the hematocrit would be explained.

There is, indeed, just such an error, and its cause is well known. As a cell in suspension approaches the measuring orifice of the analyzer, the fluid in which it is suspended accelerates. This acceleration deforms the red cell from its normal morphology. It is stretched lengthwise and rounded in cross-section so that it assumes a shape something like an elongated cigar.

The rapidity with which a cell can undergo this transformation into and out of an elongated cigar-like shape is largely determined by its internal viscosity - in other words,

its MCHC. High MCHC cells don't slim down as rapidly as do low MCHC cells and thus look larger as they zip through the orifice. Why is looking larger a problem? It is a problem because the measurement of red cell volume seems to relate more nearly to the cross-sectional area of the cell than it does to true red cell volume. Thus, a cell that is irregularly shaped and nondeformable - say, a fixed reticulocyte - will appear to be considerably larger than a cell of equal volume that flexibly deforms into a streamlined, cigar-shape during its passage. This phenomenon has been well recognized for many years and it is the reason why it is so difficult to calibrate conductance-orifice analyzers with rigid latex spheres of known volume. It is why all analyzer calibrating solutions use the much less stable and much more troublesome partially embalmed red cell suspensions for this purpose. The end result is that cells in which the internal hemoglobin solution is more viscous than usual (high MCHC) will appear to be larger than they really are. Cells in which the internal hemoglobin solution is more dilute than usual (low MCHC) will appear to be smaller than their true volume.

When the individual cell volume is overestimated, the hematocrit of the blood sample from which the cells come is also overestimated. What is the effect on the MCHC? Well, since it is the high MCHC cells that fool the sensing orifice into seeing them as bigger than they really are, it is these same cells that have their hematocrit overestimated and their MCHC underestimated as a consequence. The opposite phenomenon occurs with low MCHC cells.

If the primary problem lies in the estimation of the cell volume, it seems logical that the range of the MCV would be affected along with the MCHC. Surprisingly, the range of the MCV is relatively unaffected. The reason is simply that errors in the MCV, while just as frequent as in the MCHC, are not as well organized and are therefore virtually undetectable. It is just as likely that a high MCHC cell will be big as it is that it will be small; thus, the overestimation errors are spread throughout the entire set of blood samples. The same is true of the underestimation errors. In the case of the MCHC determination, however, it is only the high MCHC cells that are erroneously oversized and only the low MCHC cells that are erroneously undersized. Thus, the clamping effect on the range of MCHC values in a patient population is very obvious indeed.

These errors in the measurement of the MCHC by many of the presently available conductance-orifice analyzers makes this red cell index much less useful as a diagnostic index for differentiating amongst the various types of anemia. This is unfortunate because the primary use of the MCHC is for this diagnostic purpose. The secondary use of the MCHC as a quality control parameter is, surprisingly, not degraded at all. In fact, because the errors clamp the MCHC and reduce its physiologic range, this error enhances the usefulness of the MCHC for quality control purposes. The reduced physiologic range makes the index much more sensitive to drift in analyzer calibration and reduces the number of samples that must be averaged in order to determine how well the analyzer is holding to its calibration set point.

We undertook to compare the performance of five different analyzers at this task of measuring MCHC. First, however, we needed to validate the accuracy of the MCHC we intended to use as a reference. Fortunately, a reference MCHC is possible because of the existence of reference procedures for both the hemoglobin and the hematocrit. The hemoglobin reference procedure used was that described by the National Committee for

Clinical Laboratory Standards (NCCLS).[3] The hematocrit reference was validated by means of hemoglobin ratios in the following manner. Two hemoglobin measurements were made on each blood sample for which a reference hematocrit was desired. The first measurement was made on the well-mixed whole blood. The second measurement was made on the packed red cell portion from a microhematocrit on that same sample. The reference hematocrit is the ratio of the first hemoglobin divided by the second. The hemoglobin value obtained on cells packed to 100% is the same as the MCHC. This approach provides, with no known biases, a direct measurement of both the MCHC and the hematocrit of a blood sample. Thus, it serves to validate spun hematocrits and MCHCs obtained in the more usual fashion.[4]

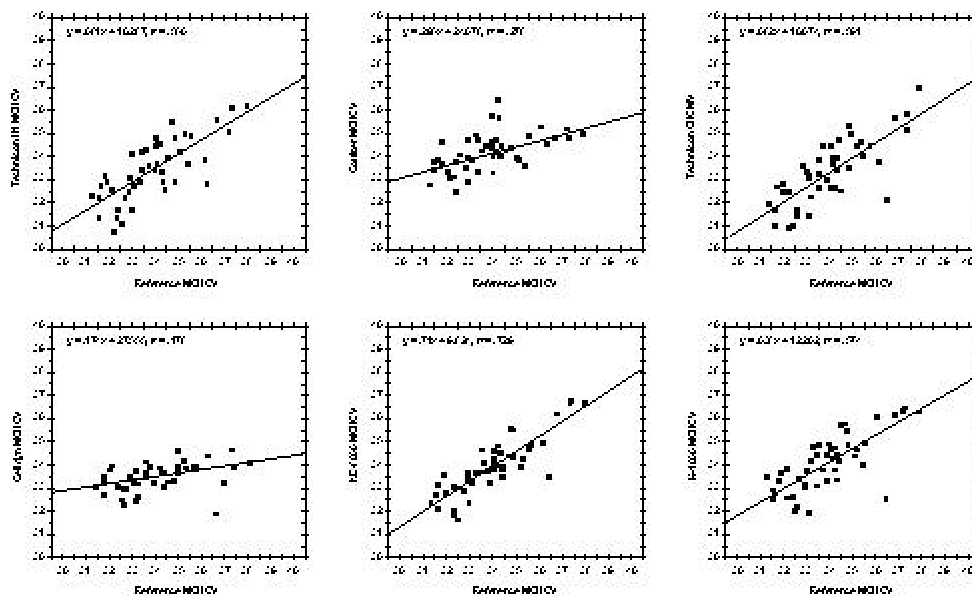

Figure 1. Six graphs of the MCHC as measured by various analyzers.

The behavior of the five different hematology analyzers on MCHC measurement of samples from 46 patients is shown in Figure 1. Six data sets are shown as the Technicon H1 produces two variants of the MCHC index (MCHC and CHCM). The r^2 values for MCHC measurements on each of the analyzers are shown in tabular form in Table 1. For comparative purposes, similar data sets for the MCV and the MCH are also shown in Table 1. An r^2 value approximately reflects the fractional information about true MCHC (MCV, hematocrit, etc.) available in the estimate of MCHC given by the particular analyzer.

This study did not compare all analyzers on a level playing field, nor did it include all available analyzers. The technology employed in several of the analyzers was 10-15 years old, whereas the technology employed in others was much more recent. We make no claims about a random selection process; we studied only those analyzers to which we had ready access.

The poor performance of several of the conductance-orifice machines is quite discouraging. An r^2 value of 0.2 (20%) means that only 20% of the information conveyed by an MCHC value is actually coming from the true MCHC - the remaining 80% is, in effect, random noise.

Table 1. Validity of MCHC as Reported by Various Analyzers.**Error! Reference source not**

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Analyzer	**r**	**2**
Coulter S Plus IV		.278
Sysmex NE-8000		.729
Technicon H1 MCHC		.556
Technicon H1 CHCM		.591
Sysmex K-1000	.574	
Celldyne 3000	.178	
Direct MCHC Method		.917

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Analyzer		**r2**
	.776	.602
	.927	.860
	.859	.738
	.902	.813
	.842	.709

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	r	**r**
Coulter S Plus IV	.901	
Sysmex NE-8000	.951	
Technicon H1	.931	
Sysmex K-1000	.928	
Celldyn 3000	.951	

Clearly an r2 of 0.2 or 20% makes an MCHC value little more than a guess. On the other hand, some conductance-orifice analyzers can, by orifice shaping, flow control,

the true MCHC information. It is thus clear that conductance-orifice machines are capable of producing better and more meaningful MCHC values than they are, at present,

marked clamping effects on the range of the MCHC are also the machines that deliver MCHCs most useful for quality control purposes.

While this may be good news for the laboratory hematologist, it is obviously not good news for the clinician. The clinician is, indeed, in trouble. Most clinicians are not aware that some of the red cell indices are much more diagnostically useful than are others. Most clinicians assume that all three indices are equally reliable and that all are at least 80% truthful (less than 20% consisting of random noise unrelated to the particular sample on which the index was generated). That clinicians should have this impression is not surprising. The hemoglobins and red cell counts produced by these same machines have r^2 values on the order of 95%.

This is clearly an undesirable state of affairs. Ideally the manufacturers will address this problem in the newer models of analyzers presently in use. Some machines are capable of measuring a reasonably accurate MCV that is relatively unaffected by the internal viscosity of the cells in a blood sample. Thus, it is clearly an achievable goal for all manufacturers. In the meantime, each manufacturer, upon the introduction of a new multichannel analyzer, should inform the hematology laboratory, and through the laboratory, the clinician end-user of the MCHC and MCV r^2 values to be expected from the new machine in a typical population of hospitalized patients.

References

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