A Methodical Approach to Interpreting the Red Blood Cell Parameters of the Complete Blood Count

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LEARNING OBJECTIVES

1. List the red blood cell parameters of the complete blood count (CBC).
2. Describe the principle of analysis for each of the red blood parameters of the CBC.
3. Explain the disadvantages of relative reticulocyte counts.
4. Given relative reticulocyte counts and red blood cell counts, calculate absolute reticulocyte counts.
5. Given a relative reticulocyte count and patient hematocrit and morphology, calculate the corrected reticulocyte count and reticulocyte production index, when needed.
6. Given the red blood cell parameters of a CBC, compare each to the reference interval and apply proper terminology to the interpretation of results outside the reference interval.
7. Given red blood cell parameters of a CBC, apply a methodical approach to assess the validity and reportability of results.
8. Apply a methodical approach to red blood cell parameters of the CBC to assess diagnostic and clinical significance.

ABSTRACT

A methodical approach to interpreting the panel of complete blood count (CBC) results helps to ensure that spurious results are detected and corrected before results are reported and helps to ensure that no results are overlooked in a diagnostic analysis of the results. The steps to interpreting the red blood cell (rbc) parameters are:

1. Interpret the hemoglobin value relative to the appropriate reference interval.
2. Interpret the mean cell volume relative to the reference interval.
3. Interpret the mean cell hemoglobin concentration relative to the reference interval.
4. Interpret the red blood cell distribution width relative to the reference interval.
5. Examine the red blood cell morphology, if available, and correlate morphology with instrument parameters for consistency and quality purposes. Also, review for additional diagnostic findings.
6. As a final check on the numerical parameters, examine the rbc count, hematocrit, mean cell hemoglobin, and calculate the Rule of Three to ensure that the above interpretations are correct.
7. Use related test results when available, particularly reticulocyte parameters, to verify CBC findings and add diagnostic information.
8. Interpret the rbc parameters for diagnostic significance and correlate with results of white blood cell and platelet parameters.

Explanations for conducting the evaluations are provided and the above steps are applied to examples to demonstrate this approach to interpreting the rbc parameters of the CBC.

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INTRODUCTION

The complete blood count (CBC) or hemogram is a panel of test results on the cellular components of blood: the red blood cells (rbcs), white blood cells (wbcs), and platelets (plts). Each of the portions can be interpreted separately in a methodical fashion and the interpretation has two aims for laboratorians. The first is quality assessment. Laboratorians must review results before they are reported (or devise the autoverification rules) that will ensure accuracy. Once the results have been verified for accuracy, the second aim, diagnostic interpretation, can be conducted to assess whether the results require additional review, critical value actions, or notifications to other departments that rely on hematologic values, like the transfusion service.

Although diagnostic algorithms are common in the medical literature[1], the steps presented here are unique in focusing on the assessments that need to be made by laboratorians to ensure quality test result reporting. Modern computerized instruments provide flags that draw operators' attention to particular parameters that may have diagnostic significance (High and Low results flags) while other flags alert operators to possible spurious results (e.g. lipemia, rbc fragments). Many of these flags can be customized by the key operator, which eases the interpretive challenge for regular staff. Still, understanding of the inter-relationships among the test parameters is necessary in establishing those customized flags.

This article describes a methodical, step-wise approach to interpreting the rbc parameters of the CBC. The sequencing of the steps is not necessarily fixed. Completing them all is important but the purpose of the step may influence the sequence. For example, a

parameter that is quite sensitive to testing errors and interferences (often flagged) is the mean cell hemoglobin concentration (MCHC). For a laboratorian who is checking results for accuracy before reporting, starting with the MCHC could be quite sensible. But once results are verified for reporting, then beginning with the hemoglobin for clinical interpretation makes more sense and that is the order presented here. It is important that nothing is overlooked since the reported parameters provide related, but different information for quality control assessment and patient diagnosis.

The rbc components of the CBC are:
- Red blood cell count (RBC)
- Hemoglobin (HB)
- Hematocrit (HCT)
- Classic red blood cell indices: Mean cell volume (MCV), Mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC)
- Red blood cell distribution width (RDW)
- Red blood cell morphology

Interpretation of reticulocyte parameters will also be discussed.

The steps to interpreting the red blood cell components of the CBC are presented in Table 1 for easy reference and described, in detail, below.

Table 1. Steps in the methodical interpretation of the red blood cell parameters of the CBC.

1. Interpret the HB value relative to the appropriate reference interval.
2. Interpret the MCV relative to the reference interval.
3. Interpret the MCHC relative to the reference interval.
4. Interpret the RDW relative to the reference interval.
5. Examine the rbc morphology, if available, and correlate morphology with instrument parameters for consistency and quality purposes. Also review for additional diagnostic findings.
6. As a final check on the numerical parameters, examine the RBC, HCT, MCH, and calculate the Rule of Three to ensure that the above interpretations are correct.
7. Use related test results when available, particularly reticulocyte parameters, to verify CBC findings and add diagnostic information.
8. Interpret the rbc parameters for diagnostic significance and correlate with results of wbc and plt parameters.

Using the Steps for Interpretation of the Red Blood Cell Parameters of the CBC and Reticulocyte Counts

Step 1. Interpret the HB value relative to the

appropriate reference interval.

When the hemoglobin is below the reference interval it is described as anemia. The physiological definition of anemia is a decrease in the oxygen carrying capacity of the blood. Since the oxygen-carrying capacity depends on hemoglobin, it is the most reliable indicator of anemia and most instances of anemia will have an associated decrease in hemoglobin. The exception is with non-functional hemoglobin, such as carbon monoxide poisoning, in which the hemoglobin is adequate in amount but cannot carry oxygen. The patient is anemic physiologically, but the hemoglobin value will not reflect it. Fortunately, these instances are rare.

When the hemoglobin is above the reference interval it is described as erythrocytosis. While the strict definition of erythrocytosis is an increase in the number of erythrocytes and therefore, cannot be truly assessed by the hemoglobin value, in most instances when there is an elevated hemoglobin value, there is also erythrocytosis. Additionally, the term polycythemia is sometimes used when the hemoglobin is elevated. This risks confusion with polycythemia vera, a malignant condition in which the rbcs, wbcs, and plts are all elevated.

Step 2. Interpret the MCV relative to the reference interval.

When the MCV is below the reference interval it is called microcytosis (microcytic rbcs); within the reference interval, normocytosis (normocytic rbcs); and above the reference interval, macrocytosis (macrocytic rbcs). Red blood cell volume is an important parameter in distinguishing the cause of anemia since some anemias cause red blood cell volume to drop while others cause it to rise. For example, microcytic anemias are usually due to defective HB synthesis while macrocytic anemias are often the consequences of problematic cell development.

Step 3. Interpret the MCHC relative to the reference interval.

When the MCHC is below the reference interval it is called hypochromia (hypochromic rbcs); within the reference interval, it normochromia (normochromic rbcs); and above the reference interval, follow institutional protocol. It may seem natural to refer to cells with an MCHC above the reference interval as "hyperchromic" and indeed, some instrument manufacturers have adopted this term for new indices. Likewise, some authors and some institutional protocols do authorize the term. Others avoid it for the following reasons. Under healthy conditions, rbcs cannot accumulate more than a normal concentration of hemoglobin in the cytoplasm. Hemoglobin production is regulated by the accumulation of hemoglobin to a concentration of approximately 33%, at which point it remains soluble and function is optimized. In this view, red blood cells cannot have increased hemoglobin concentration and so, cannot be hyperchromic, even though they can stain more darkly on a blood film. The dark staining typically results from a change in the shape, and thus the depth of the cell, creating the impression that the hemoglobin is more concentrated.

A physiological rise of the MCHC does occur when RBCs are spherocytic, however. Two factors contribute to the MCHC rise in spherocytes: 1) the geometric efficiency of a sphere to enclose the largest possible volume with the smallest possible surface area and 2) some dehydration that causes true hemoglobin concentration within the cells to rise. While modest elevations of the MCHC, no higher than about 42%, can point to spherocytes, there is a physiological limit on the degree to which the hemoglobin can be concentrated. An elevated MCHC warrants a visual review of the blood film. If examined microscopically, spherocytes appear darker than other cells and thus are truly "hyperchromic." Therefore, an institutional protocol may authorize the use of the term hyperchromic if spherocytes are observed to be present.

Another instance in which the MCHC may rise above the reference interval is when one of the parameters used to calculate it has been falsely affected (e.g. a false elevation of hemoglobin due to lipemia). In the equation of MCHC = HB/HCT X 100% (HCT=hematocrit), a disproportionate elevation of the HB will cause a false rise of the MCHC. Examination of the red blood cell morphology will show that there are no spherocytes, hence, no hyperchromia. These instances often lead to a significant elevation of the MCHC above 42%, which can be expected to generate an instrument flag. Nevertheless, laboratorians should suspect a spurious instrument result with so high an elevation of the MCHC, even without an instrument flag.

A disproportionate drop of the HCT can also lead to a falsely elevated MCHC. This may occur with a hemolyzed sample in which the hemoglobin from the red cells is present in the plasma and still contributes to the HB value. The rbcs contributing to the hematocrit will be reduced, however. A similar situation occurs on some instruments with cold agglutinins that cause the RBC and calculated HCT to be falsely low while the HB is accurate for the patient.

Very often, instruments are able to flag the results when the MCHC is elevated, even suggesting a cause (e.g. Suspect lipemia). Following the protocol for that instrument in that institution, the corrective action should be taken. Once reliable results are attained, then the results should be reinterpreted using the Steps 1-3.

Step 4. Interpret the RDW relative to the reference interval.

An elevated RDW correlates to anisocytosis. An RDW within the reference interval indicates no anisocytosis. The RDW is an unusual parameter because it is a statistical calculation of the variability of the size of the red blood cells and as such, cannot drop below normal. It is expressed as either a standard deviation (RDW-SD) or coefficient of variation (RDW-CV), the latter being the SD divided by the MCV. In either case, as the size range between cells increases, the RDW rises. For the RDW to fall below the reference interval, the size differences within a patient's cell population would have to be reduced; the cells would need to be more uniform in size than is normal. Since cell production is a biological process, making cells more uniform than normal is not really possible, and so the RDW is a parameter that can only rise. Having said that, recall that the typical manner of determining reference intervals leaves about 2.5% of healthy, normal patients' results outside the reference interval on the lower end. Thus, an occasional patient will have an RDW value that is slightly below the reference interval, but will not indicate either an instrument error or a pathological condition.

Step 5. Examine the RBC morphology report, if available, and correlate morphology with instrument parameters for consistency and quality purposes. Also review the morphology for additional diagnostic findings that cannot be discerned by instrument parameters.

If the MCV indicates microcytosis or macrocytosis, then the morphology report may be expected to reflect that, unless the changes are too subtle for morphologic appreciation. If both are reported in the morphology, the MCV may still be within the reference interval since it is an average value. An elevated RDW with a normal MCV would be a clue to expect this situation.

An elevated MCHC can be evaluated for the presence of spherocytes and if not found, then a cause of spurious elevation should be investigated. Hypochromia on the blood film should be correlated to a reduced MCHC.

An elevated RDW should be expected to correlate with a visual assessment of anisocytosis.

With the exception of the relationship between spherocytes and MCHC, most shape variations will not be reflected in numerical values of the CBC unless they are also associated with size changes or affect the hemoglobin content. Sickle cells (drepanocytes), hereditary ovalocytes (elliptocytes), stomatocytes, physiological echinocytes, acanthocytes, and most tear drops (dacryocytes) are of normal volume and hemoglobin content and thus are not detectable in the numerical output. There are some correlations that can be expected, though. Shistocytes and keratocytes (helmet cells), for example, can lower the MCV and increase the RDW but must be present in large numbers to impact these parameters. The macro-ovalocytes of megaloblastic anemia contribute to the elevated MCV, but the shape change is not evident in the numerical parameters. Target cells (codocytes), particularly in thalassemia, are hypochromic and can reduce the MCHC and perhaps the MCH, but not all hypochromic cells are target cells.

Step 6. As a final check, examine the RBC, HCT, MCH, and calculate the Rule of Three to ensure that the above interpretations are correct.

Red blood cell count

If the HB is elevated, the RBC is expected to be elevated also. This correlation helps to detect false elevations of the HB if the RBC is normal or decreased.

The RBC is not a good indicator of the degree of anemia, however, and thus is not a primary parameter for anemia assessment. To understand this, consider microcytic anemias. Though modern instruments measure the

MCV directly, the traditional calculation helps to make clearer why the RBC is not good for assessing anemia. Recall that MCV (fL) = HCT (%)/RBC (no power of 10) X 10. Mathematically, for the MCV to drop, either the HCT can fall or the RBC can rise disproportionately to the other. Physiologically, a true rise of the RBC is unlikely without a rise in HCT. But a disproportionate change does occur; that is, the HCT drops while the RBC, though dropping, does not drop proportionately to the HCT. So, the RBC number is elevated relative to the HCT. As an example, some patients with thalassemia (a microcytic anemia), will have a decreased hemoglobin, but a red blood cell count that is normal (i.e. disproportionately elevated), thus leading to the microcytic calculation. This example demonstrates that the RBC alone is not good indicator of the degree of anemia.

Hematocrit

The hematocrit is a calculated value on most modern instruments, rather than a measured value as when performed manually. Still, the traditional understanding of HCT as packed cell volume when blood is centrifuged is useful to understanding why the HCT is not as useful in anemia assessment as the HB. Two factors affect the packed cell volume, ignoring concerns for plasma trapping: the number of red blood cells and their size. The HCT can drop if the number of cells drops as in hemorrhage. The HCT can also drop when the size of individual cells shrinks as in microcytic anemias, whether the number of cells decreases or not. Typically, there will also be a decline in the red cell number as well, but, imagine for a moment, that just the cell size has decreased, while the number of cells remains constant. This would lead to a lower hematocrit than if the cells were of normal size. Thus, the influence of cell size on HCT means that it is not as good a measure of anemia as HB.

Mean cell hemoglobin

The MCH is the actual weight (amount) of hemoglobin in the average red blood cell expressed in picograms. It is dependent on the size of the red blood cells; small cells cannot contain as much hemoglobin as larger cells. Thus, the interpretation of the MCH always must take into account the volume of the cells, the MCV. It is expected that if the MCH is low then the MCV is also low, but hypochromia cannot be determined using the MCH. Thus, the MCH is not as useful, in most instances, as the

MCHC, which does correlate to visual assessments of hypochromia.

The Rule of Three

The traditional Rule of Three calculation is provided in Figure 1.

$$HB \text{ (g/dL) X 3} = HCT \text{ (\%) (+ or } - 2 \text{ \%)}$$

Figure 1. The equation for calculating the traditional Rule of Three is shown.

Stated another way, the hemoglobin number is 1/3 (i.e. 33%) of the hematocrit number. This is essentially a rearrangement of the MCHC calculation since the reference interval for MCHC (32-36%) includes 33%. Without the units, MCHC = HB/HCT X 100%. Substituting 33% for MCHC in this equation and rearranging:

33% = HB/HCT X 100%; multiply both sides by HCT

33% x HCT = HB X 100%; divide both sides by 33%
HCT = HB X 3

The Rule of Three holds when the MCHC is within the reference interval. Thus, using the Rule of Three is a double check on the MCHC. However, because the Rule of Three will NOT hold in instances of hypochromia, spherocytosis, or spurious results, it is a quick quality assessment to detect those circumstances.

Another Rule of Three, used somewhat less frequently, is provided in Figure 2.

$$HB = RBC \text{ (without powers of 10) X 3}$$

Figure 2. The calculation of the second Rule of Three is presented.

Stated another way, the RBC (without powers of 10) is 1/3 of the HB value. This is essentially a rearrangement of the MCH equation. The MCH reference interval is on the order of 26-34 pg, including the 30 value.

MCH (pg) = (HB (g/dL)/ RBC without units of 10) X 10

Substituting 30 for a normal MCH,

30 = (HB/RBC without the units of 10) X 10; multiply

both sides by the RBC (without powers of 10)

30 X RBC (without the power of 10) = HB X 10; divide both sides by 10

3 X RBC (without the powers of 10) = HB

Step 7. Use related test results when available, particularly reticulocyte parameters, to verify CBC findings and add diagnostic information.

A reticulocyte count is intended to provide information on the rbc output from the bone marrow. Under normal circumstances, about 1% of the rbcs die each day due to senescence and an equivalent number are released from the bone marrow as reticulocytes, though nearly fully mature. Passage through the spleen will remove extraneous membrane and nuclear remnants while enzymes continue to degrade residual ribosomes (RNA) that are stained or detected for reticulocyte counting.

For most laboratory test results, a rise above the reference interval is considered an indication of a pathological condition. Reticulocyte counts differ, in that rises in reticulocyte counts for anemic patients represent a normal physiological response to anemia. Meaningful diagnostic interpretation of reticulocyte counts during anemia is complicated by violations of the underlying principles that are used to determine the reticulocyte reference interval. Those principles are founded on the notion that individuals whose reticulocyte values are used to calculate reference intervals are: 1) not anemic and in particular, they have a normal RBC and 2) producing reticulocytes that will mature within one day (reticulocyte counts represent one day's output from the bone marrow). Violations of either of these principles complicate interpretation of reticulocyte counts as a measure of the rate of bone marrow rbc production as explained below. Modern hematology instruments can provide three parameters that are particularly pertinent to interpretation of the CBC and quality assessment including the relative reticulocyte count, the absolute reticulocyte count, and the immature reticulocyte fraction.

Relative reticulocyte count (%)

A manual reticulocyte count requires vital staining of the red blood cells before creating the blood film for microscopic examination. The stain precipitates the residual RNA so the reticulocytes are identifiable. The number of reticulocytes encountered while counting 1000 or 2000 red blood cells total (including the reticulocytes) is expressed as a percentage; that is, the reticulocytes are counted relative to the total rbcs. The use of a Miller disc can improve the counting ease.[2] The relative reticulocyte value is calculated by the equation shown in Figure 3.

Relative reticulocyte count (%) = number of reticulocytes counted / total number of red blood cells counted X 100%

Figure 3. The calculation of the relative reticulocyte count by microscopic counting is shown.

This manual method, even with the improvements provided by use of a Miller disc, is notoriously imprecise. Thus, the advent of modern instrumentation that can provide highly accurate reticulocyte counts has dramatically improved the clinical utility of the relative reticulocyte count.

The relative reticulocyte count can be increased when the bone marrow produces more than the ~1% new cells needed each day under normal conditions. But, the percentage of reticulocytes can increase if bone marrow output remains stable while the total number of rbcs decreases. Acute hemorrhage is a perfect example of this. On the day that a patient experiences an acute hemorrhage that is not replaced by transfusion, the number and rate of reticulocytes exiting the bone marrow that day would be normal. Yet, following the hemorrhage, the total number of rbcs declined. Since reticulocyte production continues at a normal pace in the immediate post-hemorrhage period, but the number of rbcs in circulation has declined, the percent of rbcs that are reticulocytes will increase from pre-hemorrhage levels, even though the output of reticulocytes has not yet increased. It will take several days for erythropoietin stimulation to raise the rate of reticulocytes exiting the marrow. Thus, in instances of erythropenia leading to anemia, the relative reticulocyte count can be falsely elevated by violation of the first principle underlying reticulocyte reference interval determination.

The second principle is violated in instances when the reticulocytes released from the bone marrow are less mature than normal. These reticulocytes are recognized on the Wright's stained peripheral blood film as having polychromasia. One of the effects of erythropoietin in

responding to anemia, as in the hemorrhage example above, is that reticulocytes are able to enter the blood stream from the bone marrow as younger cells. This response is evident within about 4 days, which is the fastest that red blood cells can be produced by the bone marrow. The lower the hematocrit, the earlier in their development that rbcs will leave the marrow. As a result, they do not mature (lose their RNA) in just one day. This means that the interpretation of the relative reticulocyte count will be falsely elevated by cells that require more than one day to mature.

Mathematical corrections for the violations of the underlying principles can be used to more accurately assess bone marrow production in instances of anemia, especially when accompanied by polychromasia. A corrected reticulocyte count (CRC) is calculated (Figure 4) to assess reticulocyte production when anemia is present without polcychromasia.

Corrected reticulocyte count (%) = relative reticulocyte count (%) X patient's hematocrit (%) / average normal hematocrit (%)

Figure 4. The formula for calculating a CRC is shown.

The average normal hematocrit will vary for men, women, and children but the middle value of the appropriate hematocrit reference interval can be used. Calculating the CRC essentially answers the question, "If this person had a normal hematocrit, what would his/her reticulocyte count be?" Note that because the person is anemic with a lower than normal hematocrit (and fewer rbcs), the CRC will always be lower than the patient's relative reticulocyte count.

The CRC value is NOT clinically assessed by comparison to the relative reticulocyte reference interval. The CRC is only used in instances of anemia when the bone marrow should be responding with increased production. Red cell production at a normal rate (i.e. within the reference interval) during anemia is inadequate for anemia compensation. Rather, the CRC value must be at least 3% to compensate for a mild anemia, assuming the cause were ended immediately, and even higher for more severe conditions, in order for the bone marrow to be judged as responding adequately to the anemia.

While the CRC corrects for the anemia principle violation, it does not fully address the issue of longer

living reticulocytes in the blood. An additional calculation (Figure 5), the reticulocyte production index (RPI), is needed to further correct for polychromasia.

Reticulocyte production index (%) = CRC / reticulocyte life span (days)

Figure 5. The calculation of the RPI is shown.

Reference tables are available that provide an estimate of the peripheral blood life span of cells as reticulocytes before they mature fully and are based on the patient's hematocrit.[3] Once again, since the RPI is only calculated when the life span is more than one day (and hence, a value above one), the RPI will always be lower than the CRC, and again, the clinical interpretation is not made against the relative reticulocyte reference interval. It is expected that even a mild anemia must respond with an output of at least 3% and more if the anemia is more severe. Some care providers will calculate the RPI whether there is polychromasia or not, since it always provides the most conservative estimate of bone marrow output.

Absolute reticulocyte count (retics/µL)

The absolute reticulocyte count can be calculated from the relative reticulocyte count and the RBC. An absolute reticulocyte count (retics/µL) can be calculated as shown in Figure 6. However, this value will lack accuracy due to the imprecision of the manual method for generating the relative reticulocyte count. Fortunately, modern instruments are able to provide highly accurate measured absolute reticulocyte counts. The instruments use varying methods, but they all stain the residual RNA and then detect it via flow cytometry. For anemic patients, comparing the results to the reference interval remains inadequate since the expectation is for increased reticulocyte production in anemia. With a reference interval of about $25\text{-}75 \times 10^3/\mu L$ (mean $50 \times 10^3/\mu L$), a response of about three times normal or $150 \times 10^3/\mu L$ is needed to demonstrate an effective bone marrow response in a mildly anemic patient. The instruments that perform an actual reticulocyte count also report a calculated relative reticulocyte count (see above).

Absolute reticulocyte count (retics/µL) = RBC (cells/uL) X relative reticulocyte count % / 100%

Figure 6. The formula to calculate an absolute reticulocyte count is shown.

A CRC and RPI can be calculated using the instrument

generated relative reticulocyte value and carry greater confidence in the clinical interpretation of the result since the original absolute count is highly reliable. The measured absolute reticulocyte count does not correct for the presence of polychromasia (i.e. shift reticulocytes), however. The next parameter to be discussed addresses this issue.

Immature reticulocyte fraction (IRF)

As has been generally described above, reticulocytes require two to four days to degrade their RNA and mature into typical biconcave red blood cells. Under normal circumstances, only the last day of their maturation occurs in the blood stream. However, in instances of anemia, younger reticulocytes and even nucleated red blood cells can be released into the circulation under the influence of erythropoietin. The presence of the so-called "shift reticulocytes" or "stress reticulocytes" that require more than one day in the blood to mature undermines the basic interpretation of a reticulocyte count as one day's output from the bone marrow. The RPI calculation accounts for this, but modern instruments are able to assess reticulocytes in an even more precise manner by calculation of the immature reticulocyte fraction (IRF).

Instruments are able to quantitate the amount of RNA in individual reticulocyte. The reticulocytes with the least RNA are considered "mature," while those with the most residual RNA are "immature." The IRF is reported as the percentage of the reticulocytes that are immature. Thus, the instrument report of an elevation of the IRF should correlate to the presence of polychromasia in the microscopic examination of the blood film. Of course, the greater sensitivity of the instrument can mean that a slightly increased IRF is reported without a report of polychromasia in the morphology, but typically, they are expected to occur together; the correlation should be expected and can be part of the quality check.

The instrument manufacturers continue to add additional rbc-related parameters to the suite of results they provide. As they are not provided routinely across instruments, they are not discussed here. However, when available, those values should be correlated to others. Examples include quantitative measures of the degree of hypochromia and microcytosis. These would naturally be expected to correlate to the morphological assessment, and in the case of microcytosis, to the MCV and even

RDW values.

Step 8. Interpret the red blood cell parameters for diagnostic significance and correlate with results of white blood cell and plt parameters.

The methodical interpretation and correlation of related parameters of the red blood cell values as outlined above provides confidence that the results are reliable for diagnostic interpretation. The clinical interpretation of the red blood cell parameters can then be correlated to the other findings in the CBC report. It may be that the patient's condition affects only the red blood cells as in mild iron deficiency. On the other hand, abnormalities of the rbcs, wbcs, and plts together can point to leukemia or other pathological conditions.

Applying the Steps for Interpretation of Red Blood Cell Parameters to Sample Cases

Case 1. A 36-year-old Caucasian woman was referred for out-patient testing by her family physician. The rbc results of the CBC are presented in Table 2.

Table 2. CBC results for Case 1.

	Results	Units	Reference Interval
RBC	3.3	$10^6/\mu L$	3.80-5.20
HB	7.5	g/dL	12.0-15.0
HCT	25	%	35-49
MCV	76	fL	80-100
MCH	22.7	pg	26.0-34.0
MCHC	30.0	%	32-36
RDW	17.5	%	11.5-14.5
Relative reticulocyte count	5.3	%	0.5-2.5
Morphology	Anisocytosis, poikilocytosis, hypochromia, and microcytosis		

Examine the CBC results following the steps described previously.

1. Interpret the HB value relative to the appropriate reference interval.

The decreased HB level indicates anemia.

2. Interpret the MCV relative to the reference interval.

The MCV is below the reference interval, so the patient's

cells are microcytic.

3. Interpret the MCHC relative to the reference interval.

The MCHC is below the reference interval indicating hypochromia.

4. Interpret the RDW relative to the reference interval.

The increased RDW reflects increased variation of red cell size or anisocytosis.

5. Examine the red blood cell morphology, if available, and correlate morphology with instrument parameters for consistency and quality purposes. Also review for additional diagnostic findings.

Hypochromia, microcytosis, and anisocytosis from the morphology report are consistent with the decreased MCHC and MCV and increased RDW respectively.

6. As a final check on the numerical parameters, examine the RBC, HCT, MCH, and calculate the Rule of Three to ensure that the above interpretations are correct.

Both the RBC and the HCT are decreased, together, with decreased HB, all point toward anemia. Notice the disproportionate decrease of the RBC and the HCT as compared to the HB, however, providing a good example of why these parameters are not good indicators of anemia. The RBC has not declined as much as would be expected by the Rule of Three (3 X 3.3 = 9.9 but the HB is only 7.2). In fact, this is a characteristic of microcytic anemias; the RBC (though below the reference interval) is high relative to the HB. And sometimes it can even be within the reference interval though the HB is low and the patient is truly anemic. The HCT is similarly affected because there are more cells, though small. The HB would predict an HCT of just 22.5 (7.5 X 3 = 22.5) but the patient's HCT was actually 25. MCH is low, which is consistent with low MCV; small cells cannot hold as much hemoglobin. The Rule of Three does not hold here due to hypochromia.

7. Use related test results when available, particularly reticulocyte parameters, to verify CBC

findings and add diagnostic information.

The relative reticulocyte count needs to be corrected because of anemia: 5.3% X (25% /42%) =3.2% (Here 42% is the middle value of the reference interval). The corrected reticulocyte count is higher than 3%, so her bone marrow is responding to the anemia, however, not adequately to prevent the development of anemia. The RPI does not need to be calculated here, as polychromasia is absent from the morphology report.

8. Interpret the red blood cell parameters for diagnostic significance and correlate with results of wbc and plt parameters.

Correlation with wbc and plt values would complete the analysis.

This 36-year-old Caucasian woman presented with a complaint of "feeling tired all the time" for the last two months. Physical examination was unremarkable except for pale conjunctiva. The results indicate a microcytic anemia which could explain her fatigue and the pale conjunctiva. Although the CRC is just above 3, the lack of polychromasia points to a non-regenerative anemia, likely iron deficiency. If untreated, the reticulocyte count would be expected to decline as the iron available for red cell production decreases and the patient becomes even more anemic. For this patient, the wbc and plts were normal and non-contributory to the diagnosis.

Case 2. The sample is from a 50-year-old man seen in the emergency department with a diagnosis of jaundice. CBC results are shown in Table 3.

Table 3. CBC results for Case 2.

	Results	Units	Reference Interval
RBC	2.50	$10^6/\mu L$	4.20-6.00
HB	14.5	g/dL	13.5-18.0
HCT	27	%	40-54
MCV	108	fL	80-100
MCH	55.8	pg	26.0-34.0
MCHC	54	%	32-36
RDW	18.0	%	11.5-14.5
Relative reticulocyte count	1.1		0.5-2.5%
Morphology	Anisocytosis, macrocytosis, and stomatocytes		

Recall that the order of completing the steps in rbc

analysis is not critical, but all must be completed.

1. Interpret the HB value relative to the appropriate reference interval.

The HB is within the reference interval so no anemia or erythrocytosis.

2. Interpret the MCV relative to the reference interval.

The MCV is elevated, thus macrocytosis is expected.

3. Interpret the MCHC relative to the reference interval.

The very high MCHC suggests spurious results, since it is higher than 42%. This triggers a jump to step 5 to examine the morphology; no spherocytosis is reported from morphology, supporting a conclusion that the result is spurious. Notice that the MCH is also very high [Step 6]. Finally, an instrument flag was generated indicating "Suspect lipemia."

Based on the equations for MCH and MCHC calculation, both MCH and MCHC can be falsely elevated when HB is erroneously elevated, pointing toward HB as a likely problem. Investigation of spurious results for HB assays would include lipemia, hemolysis, and hyperbilirubinemia. Visual inspection of the plasma can help to identify the cause. In this case, the Medical Laboratory Scientist noticed the milky appearance of the specimen despite the blood having been drawn under the fasting state. Therefore, the false elevation of HB is most likely caused by lipemia. Bilirubinemia cannot be ruled out, especially for a patient with a diagnosis of jaundice, but instruments are typically less sensitive to bilirubinemia than to lipemia.

The turbidity formed by increased level of triglyceride creates increased scatter of light, and increases the absorption in the spectrophotometric HB assay, thus resulting in a falsely elevated HB level. The parameters derived from HB, such as MCH and MCHC, are also erroneously affected. Various strategies are recommended by manufacturers to correct for lipemic interference, such as diluting samples 1:5 in saline, retesting, and then multiplying the HB value by 5 before reporting. In this case, the plasma of the original specimen was replaced with an equal amount of saline and the CBC was reanalyzed on the modified specimen. The new CBC results are available in Table 4. The results of the second analysis can then be analyzed diagnostically with all steps in usual order.

Table 4. CBC results on the saline replacement sample for Case 2

	Results	Units	Reference Interval
RBC	2.50	$10^6/\mu L$	4.20-6.00
HB	8.7	g/dL	13.5-18.0
HCT	27	%	40-54
MCV	108	fL	80-100
MCH	34.8	pg	26.0-34.0
MCHC	32.2	%	32-36
RDW	17.8	%	11.5-14.5
Relative reticulocyte count	1.1	%	0.5-2.0
Morphology	Anisocytosis, macrocytosis, and stomatocytes		

1. Interpret the HB value relative to the appropriate reference interval.

After correction, his HB level falls below the normal reference interval indicating anemia.

2. Interpret the MCV relative to the reference interval.

The anemia is macrocytic as shown by increased MCV.

3. Interpret the MCHC relative to the reference interval.

The MCHC drops within normal reference interval after correction, so his anemia is normochromic.

4. Interpret the RDW relative to the reference interval.

The increased RDW correlates to anisocytosis.

5. Examine the red blood cell morphology, if available, and correlate morphology with instrument parameters for consistency and quality purposes. Also review for additional diagnostic findings.

Anisocytosis and macrocytosis reported in the morphology are consistent with increased RDW and

MCV respectively. The normochromic conclusion from the MCHC is consistent with no mention of hypochromia on the morphology report since only abnormalities are mentioned. Notable poikilocytosis of stomatocytes is reported.

6. As a final check on the numerical parameters, examine the RBC, HCT, MCH, and calculate the Rule of Three to ensure that the above interpretations are correct.

Both the RBC and HCT are decreased and are consistent with anemia. His MCH is elevated due to the increased red cell size with adequate hemoglobinization of the cells. Since the MCHC is normal, the Rule of Three, predicting the HCT, holds. The Rule of Three, predicting the HB, also holds for this patient, because his cells are not very much larger than normal (MCV of 108 fL; in instances when the MCV is much higher, the Rule of Three prediction of HB may not hold because each large cell contains far more than the normal amount of hemoglobin).

7. Use related test results when available, particularly reticulocyte parameters, to verify CBC findings and add diagnostic information.

The relative reticulocyte count needs to be corrected because of anemia: 1.1% X (27/47)% = 0.63%, which is less than 3%. Therefore, his bone marrow is not responding adequately to anemia. An RPI is not needed because polychromasia is not mentioned in the morphology.

8. Interpret the red blood cell parameters for diagnostic significance and correlate with results of white blood cell and plt parameters.

Correlation with wbc and plt values would complete the analysis.

This 50-year-old man had been alcoholic for 5 years before this occasion when he developed jaundice. He was also diagnosed with hyperlipidemia type I 10 years ago. A macrocytic anemia not due to either Vitamin B12 or folic acid deficiency is frequently seen with alcoholism. Alcohol is a bone marrow suppressant resulting in the low reticulocyte count.

Case 3. A 65-year-old woman is referred for out-patient testing by an internal medicine physician. Her CBC results are provided in Table 5.

Table 5. CBC results for Case 3.

	Results	Units	Reference Interval
RBC	1.50	$10^6/\mu L$	3.80-5.10
HB	9.5	g/dL	11.5-15.0
HCT	16.5	%	34-44
MCV	110	fL	80-100
MCH	63.3	pg	27.0-34.0
MCHC	57.6	%	32-36
RDW	16.0	%	11.7-15.0
Relative reticulocyte count	12.3	%	0.5-2.0
Absolute reticulocyte count	181.5	$10^3/\mu L$	20-115
Immature reticulocyte fraction	19.5	%	2.0-16.0
Morphology	Spherocytosis, slight anisocytosis, clumps of RBCs, and polychromasia		

1. Interpret the HB value relative to the appropriate reference interval.

The hemoglobin is below the reference interval indicating anemia.

2. Interpret the MCV relative to the reference interval.

The MCV is elevated indicating macrocytosis.

3. Interpret the MCHC relative to the reference interval.

The MCHC is elevated, suggesting spherocytosis or a spurious result. Although spherocytosis is mentioned in the morphology, it is not likely the full explanation for the very high MCHC, since spherocytosis only causes the MCHC to increase slightly; a spurious cause is also likely. It is also an instance when the RBC is useful as a troubleshooting parameter, not a diagnostic one. Notice the strikingly low RBC count. The Rule of Three does not hold in this instance and the discrepancies between HB and RBC and/or HCT are obvious. The morphology provides a clue to the cause; clumps of red blood cells can lead to a falsely low RBC count. The MCH is falsely high because of the erroneously low RBC count, which leads to falsely low HCT and falsely high MCHC. This

constellation of results is typical in the presence of cold agglutinins. Warming the patient sample and on some instruments, diluting in warm saline for microsampling, can correct the problem. The results of the CBC after the patient's blood was warmed to 37 ˚C are presented in Table 6.

Table 6. CBC results for Case 3 after warming the sample to 37˚C for retesting.

	Results	Units	Reference Interval
RBC	3.3	10^6/µL	3.80-5.10
HB	9.5	g/dL	11.5-15.0
HCT	29	%	34-44
MCV	93	fL	80-100
MCH	29	pg	27.0-34.0
MCHC	33	%	32-36
RDW	16.0	%	11.7-15.0
Relative reticulocyte count	5.5	%	0.5-2.0
Absolute reticulocyte count	181	10^3/µL	20-115
Immature reticulocyte fraction	19.5	%	2.0-16.0
Morphology	Spherocytosis, slight anisocytosis, and polychromasia		

Results can be interpreted fully following the suggested steps in order.

1. Interpret the HB value relative to the appropriate reference interval.

The low HB level indicates anemia.

2. Interpret the MCV relative to the reference interval.

Her anemia is normocytic as shown by normal MCV.

3. the MCHC relative to the reference interval.

Her anemia normochromic as shown by normal MCHC.

4. Interpret the RDW relative to the reference interval.

The increased RDW predicts anisocytosis on the blood film.

5. Examine the red blood cell morphology, if available, and correlate morphology with instrument parameters for consistency and quality purposes. Also review for additional diagnostic findings.

Anisocytosis, predicted by the RDW, is seen on the peripheral blood film. Spherocytosis is also reported with polychromasia.

6. As a final check on the numerical parameters, examine the RBC, HCT, MCH, and calculate the Rule of Three to ensure that the above interpretations are correct.

Both RBC and HCT are decreased and are consistent with anemia. Both Rules of Three hold since the MCHC is normal.

7. Use related test results when available, particularly reticulocyte parameters, to verify CBC findings and add diagnostic information.

The RPI should be used to assess the reticulocyte response as polychromasia is seen from the morphology. CRC = 5.5% X (25% / 39%) =3.5%; RPI = CRC/2 = 3.5%/2 = 1.8%. Since the RPI is less than 3%, her bone marrow is responding, but inadequately, to compensate for her anemia. The increased absolute reticulocyte count also indicates the bone marrow is responding to her anemia, yet it is not responding enough to compensate, since 181 X 10^3/µL is less than 3 times 67.5 X 10^3/µL (the middle value of the reference interval). The increased IRF is consistent with the polychromasia seen on the peripheral blood film. Notice the significant decrease of the relative reticulocyte count on the warmed specimen compared to the unwarmed sample. Since the instrument calculates the relative reticulocyte count using the RBC in the denominator, the erroneously low RBC count from the first assay results in a falsely high relative reticulocyte count.

8. Interpret the red blood cell parameters for diagnostic significance and correlate with results of white blood cell and plt parameters.

Correlation with wbc and plt values would complete the analysis.

This 65-year-old woman was referred for multiple laboratory tests because she developed cyanosis of the

limbs in the winter. Her previous medical history was unremarkable. The titer of cold agglutinins was very high. Her anemia is likely mediated by cold agglutinins or cold autoantibodies. Although not expected to be active in vivo, they may bind complement in the extremities, especially with cold exposure. The spherocytosis is then caused by macrophages "biting" the rbcs covered by antibodies or complement, and removing some membrane. Polychromasia indicates an active bone marrow which is expected with a hemolytic process. The wbc and plt results were normal and non-contributory to this patient's diagnosis.

SUMMARY

For laboratorians, using a methodical approach to interpreting laboratory data like the rbc parameters of the CBC can ensure that spurious results are detected and corrected before final results are reported. In diagnostic interpretation, a methodical approach ensures that no significant aspect of a patient's laboratory findings is overlooked. Interpretation of the rbc parameters includes 8 steps (Table 1) that should be included in a complete review. A similar methodical approach to wbc and plt results can be coupled with the rbc analysis to ensure that conditions affecting more than one cell line are included in the diagnostic interpretation.

REFERENCES

1. Means RT, Glader B. Anemia: General considerations. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, Rodgers GM, editors. Wintrobe's Clinical Hematology 13th ed. Philadelphia: Lippincott, Williams and Wilkins; 2014.
2. Clark KS, Hippel TG. Manual, semiautomated, and point-of-care testing in hematology. In Keohane EM, Smith LJ, Walenga JM, editors. Rodak's Hematology: Clinical Principles and Applications. St. Louis: Elsevier; 2012.
3. Hillman RS, Finch, CA. Red Cell Manual, 7th ed. Philadelphia: F.A. Davis; 1996.