SEED Haematology

Sysmex Educational Enhancement and Development
October 2012

The red blood cell indices

The full blood count

The complete blood count (CBC) is central to clinical decision making. This makes it one of the commonest laboratory investigations performed worldwide. Whilst the definition of what constitutes an CBC is influenced by the number and type of parameters measured by different haematology analysers, the traditional red cell indices that are widely used to classify anaemias are common to all.

The laboratory approach to anaemia

Anaemia is an extremely common global healthcare problem. However, anaemia is merely a symptom which can result from a multitude of causes. Effective treatment is only possible if the underlying cause is correctly identified. To this end, several classification systems have been devised. The most useful and widely used classification system is based on the red cell indices.

The diagnosis of anaemia

Anaemia is generally defined as a reduction in haemoglobin (HGB) level below the lower limit of normal. The values that define the presence or absence of anaemia are both sex and age dependent. The haematocrit (HCT), also sometimes referred to as a packed cell volume (PCV), is a related parameter that likewise is reduced in anaemia.

The red cell indices

The red cell parameters that are generated by all automated haematology systems include HGB, HCT, red blood cell count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). MCV, MCH and MCHC are commonly referred to as the red cell indices. More recently red cell distribution width (RDW), an automated parameter providing information on the degree of variation of individual red cell size, has been used in conjunction with the traditional red cell indices in order to narrow down the possible causes of anaemia in an individual patient.

Impedance technology

The RBC, HCT and MCV are all closely interrelated as they are derived from information obtained from the passage of cells through the aperture of the impedance channel of an automated haematology analyser. The impedance technology is based on the principle that an electrical field, created between two electrodes of opposite charge, can be used to count and determine the size of cells. Blood cells are poor conductors of electricity. The diluent in which they are suspended as they pass through the aperture during counting is an isotonic solution which is a good conductor of electricity. Consequently, when the cells suspended in the diluent pass through the aperture between the electrodes, each individual cell will momentarily increase the impedance (resistance) of the electrical path between the electrodes. Each cell generates an electrical pulse, in proportion to its size.

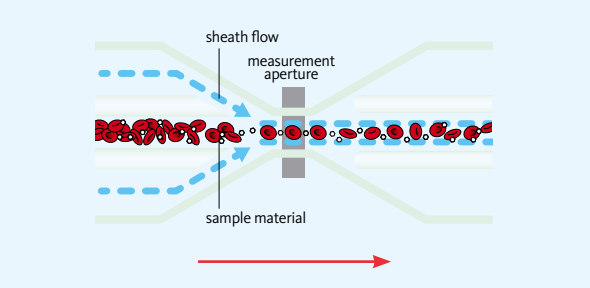

Fig. 1 *Impedance principle with hydrodynamic focusing*

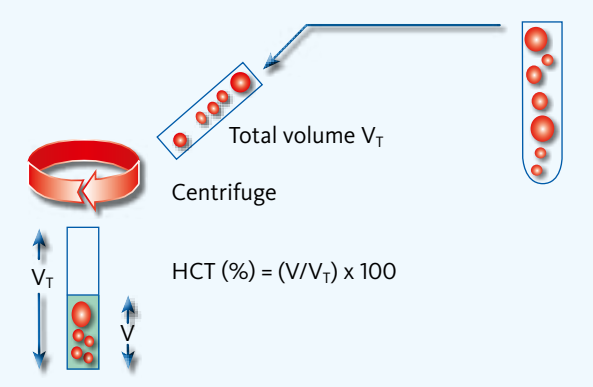

Fig. 2 *Schematic representation of how HCT is obtained using the centrifuge method.*

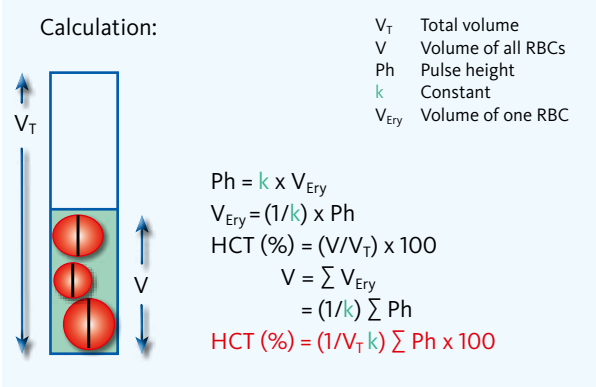

Fig. 4 *Formula for automated HCT measurement*

Red blood cell count

For analysers such as Sysmex that use the absolute counting principle, RBC is determined from the number of pulses generated in a specific volume of blood. The advantage of this approach is that end-user calibration is not required. Analysers utilising relative counting principles determine the RBC from the number of pulses generated in a fixed time period. Systems utilising relative counting are prone to errors due to aperture clogging and thus require regular calibration.

Haematocrit

HCT is a parameter that is a measure of the total or cumulative volume of red blood cells relative to the total volume of whole blood. This is also commonly referred to as the packed cell volume (PCV) and is expressed as a percentage value or as a fraction (unit L/L). Although the HCT and PCV are commonly used interchangeably, the International Council for Standardisation in Haematology has suggested that the term HCT rather than PCV should be used for the automated measurement. The measurement of HCT on automated haematology analysers has little to do with the actual packing of red cells. It is obtained using impedance technology whereby the passage of each individual cell through the aperture generates an electrical pulse that is assumed to be proportional to the volume of the cell. The HCT on a Sysmex analyser is obtained using the cumulative pulse height method. The HCT is obtained from the cumulative value of the individual cell pulse heights in the formula shown in Fig. 4.

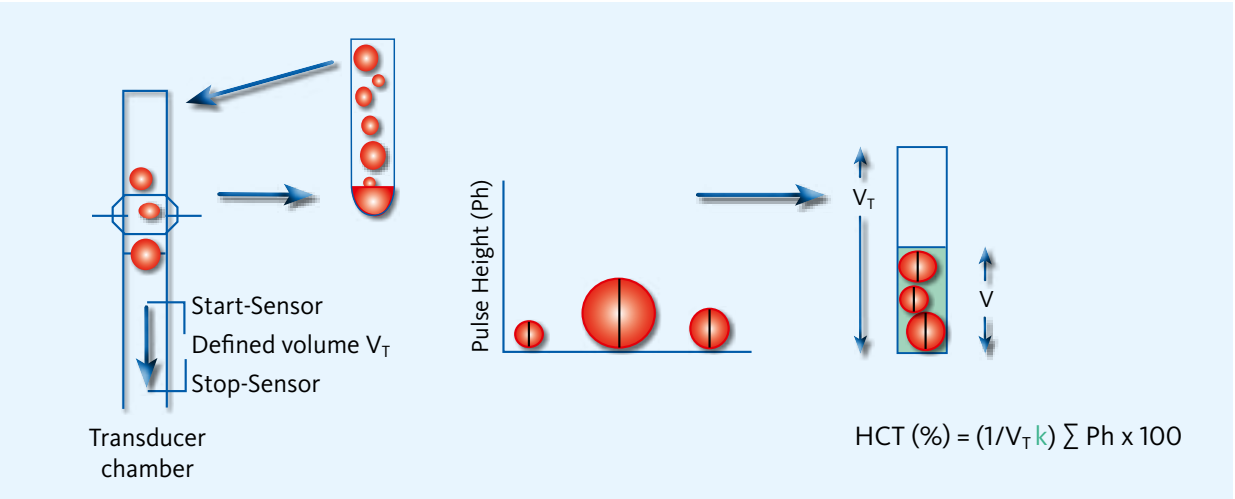

Fig. 3 *Schematic representation of automated HCT measurement using cumulative pulse height detection (for calculation see Fig. 4).*

Mean cell volume

The mean volume of red cells is calculated from the RBC and HCT using the following formula:

$$\text{MCV (fL)} = \frac{\text{HCT}}{\text{RBC}}$$

The normal reference range for MCV is age dependent. The words normocytic, microcytic and macrocytic are used to describe red cell populations with normal, low and high MCVs respectively.

Mean cell haemoglobin

The average amount of haemoglobin per red cell is calculated from the RBC and HGB using the following formula:

$$\text{MCH (pg)} = \frac{\text{HGB}}{\text{RBC}}$$

The normal reference range for MCH is age dependent. The MCH value tends to be proportional to MCV. The size of a cell is largely determined by the haemoglobin content. Cells that have a normal MCH are referred to as normochromic whereas those with low values are termed hypochromic.

Mean cell haemoglobin concentration

The MCHC is calculated from HCT and HGB using the following formula:

$$\text{MCHC (g/dL)} = \frac{\text{HGB}}{\text{HCT}}$$

The MCHC normal reference range is remarkably constant throughout life and generally has a very tight range with minimal variation expected. The MCHC, especially in older references, is also used to define normochromic and hypochromic red cell populations. An elevated MCHC is rare and virtually only occurs if cells are spherocytic or significantly dehydrated (see later).

Red cell distribution width

Automated analysers generate histograms by plotting the size of each individual cell, as determined from the pulse height generated as it passed through the impedance aperture. More than one cell population can be appreciated and typically platelets and red blood cells are displayed together. These two distinct cell populations are differentiated from each other by so-called size discriminators. The RDW is a quantitative measure of how variable the size of the individual red cells is. This is provided as an RDW-SD and RDW-CV.

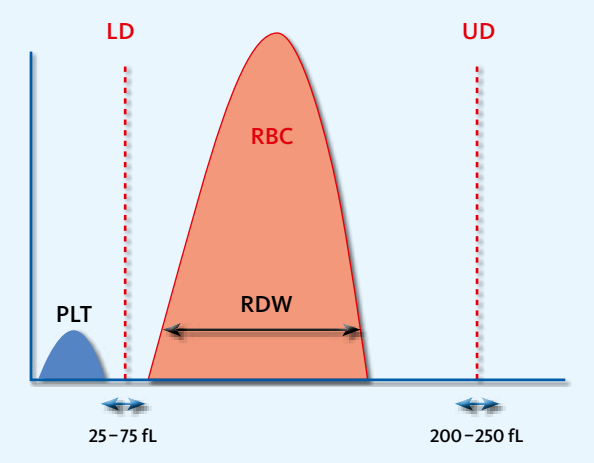

Fig. 5 *Red cell histogram illustrating RDW concept.*

The classification of anaemias using the red cell indices

The mechanisms by which anaemia occurs will alter the RBC indices in a predictable manner. Therefore, the RBC indices permit the clinician to narrow down the possible causes of anaemia. As the size of a red blood cell is dependent on its haemoglobin content, it follows that failure to produce haemoglobin will result in smaller than normal cells. Red cell microcytosis is classically seen in iron deficiency anaemia, thalassaemia (an inherited disease in which globin chain production is deficient), and anaemias associated with chronic infection or disease. Macrocytic cells occur when division of erythrocyte precursor cells in the bone marrow is impaired. The most common causes of macrocytic anaemia are vitamin B12 and folate deficiency, also referred to as megaloblastic anaemia. Normocytic anaemia may be caused by decreased production (due to malignancy or other causes of bone marrow failure), increased destruction (haemolysis) or blood loss. The RBC count is low, but the size and amount of haemoglobin in the cells are normal.

A low MCH indicates that cells contain too little haemoglobin due to inadequate production. Such cells are termed hypochromic. Iron deficiency is the commonest cause of a hypochromic anaemia. Typically low MCH cells will look pale when examined under the microscope.

The MCHC is the proportion of amount of haemoglobin in the red cell to the cell volume. Cells with too little haemoglobin are lighter in colour and have a low MCHC. The MCHC is low in microcytic, hypochromic anaemias such as iron deficiency, but is usually normal in macrocytic anaemias. As the cellular content of erythrocytes is almost exclusively haemoglobin, MCHC is also a reflection of the internal viscosity of a red cell. If the MCHC is too high, the cells will lose their capacity to deform and return to their original shape, an intrinsic requirement for red blood cells to be able to pass through the microcirculation repeatedly without being prematurely destroyed. Consequently, red cells have a natural maximum MCHC 'upper limit'. The MCHC is therefore hardly ever elevated for clinical reasons. The one exception is when red blood cells have become spherocytic due to loss of membrane. An elevated MCHC is most commonly observed in hereditary spherocytosis, a condition with decreased red cell survival caused by a structural protein defect in the cell membrane, but can also occur in acquired conditions such as immune-mediated haemolysis or severe burns.

The RDW is a quantitative measure of the variance in red blood cell size. A large RDW indicates abnormal variation in individual red blood cell size, termed anisocytosis, when viewed under the microscope. The RDW assists in the differentiation of anaemias that have similar red cell indices. It is typically used in the differentiation of iron deficiency anaemia and thalassaemia minor, both of which exhibit microcytosis and hypochromia with an overlap of MCV and MCH values. However, iron deficiency anaemia has an abnormally wide RDW, whereas thalassaemia minor does not.

A summary of the classification of anaemias using the red cell indices is shown in Tab. 2.

Are values obtained from different analysers always the same?

The answer is no. It is universally recognised that due to differences in technology, quantitative and qualitative information (e.g. flagging sensitivity) will not be 100% concordant between analysers from different manufacturers, or even different models from the same manufacturer. Consequently it is essential that patient results are interpreted in conjunction with a reference range that has been established on that specific make and model of analyser. Reference ranges from text books or alternate analysers cannot be used interchangeably without having validated that the values are suitable for use. This is however rarely done, and consequently questions about discrepancies of values obtained from different makes of analysers, in particular MCHC are a regular occurrence.

FAQ #1: Why are MCHC values obtained on a Sysmex analyser not identical to those from other analysers?

The MCHC obtained from Sysmex haematology analysers is derived from the HGB and HCT values as per the formula depicted earlier in the text.

HGB and HCT are both measured parameters on the Sysmex X-Class analysers. The validity of the MCHC value is therefore dependent on the accuracy of the HGB and HCT values. Both HGB and HCT are interdependent parameters that are measured with high precision. Consequently the MCHC has a relatively narrow normal range. A low MCHC is indicative of hypochromic red cells and is an early sensitive marker of

Morphological type of anaemia	Examples	MCV	MCH	MCHC
Normochromic normocytic	Blood loss Chronic disease	N	N	N
Hypochromic microcytic	Iron deficiency Thalassaemia Chronic disease (late stage)	↓	↓	N or ↓
Normochromatic macrocytic	Megaloblastic anaemia	↑	↑	N

Tab. 1 *Classification of anaemias using red cell indices*

developing iron deficiency. The MCH and MCHC will drop before the cells become microcytic. A raised MCHC is usually due to an analytical error hence the MCHC is commonly used to monitor the technical performance of an analyser. There are however three clinical exceptions:

1. Marked red cell spherocytosis (e.g. hereditary spherocytosis, severe burns, severe *Clostridium difficile* infection) – unusually high intracellular HGB concentration per individual red blood cell due to cell volume loss.
2. Cold agglutinin disease – here red blood cells stick together and result in a falsely low HCT.
3. Hyperlipidaemia or anything causing an unusual increase in plasma turbidity – falsely high HGB.

It should be noted that haematology analysers from different manufacturers apply different measurement principles for HGB and HCT. It is also important to note that whilst the MCHC is calculated from the HGB and HCT values, the HCT values, unlike Sysmex, are not measured by all manufacturers. The Beckman Coulter analysers measure the MCV which is used to calculate the HCT.

There are several factors involved that impact on the final MCV measurement. This is dependent on the shape and size of the aperture in the impedance channel, the presence or absence of hydrodynamic focusing and the osmolality of the sheath fluid. These factors collectively influence the degree to which individual cells become deformed as they pass through the aperture. Erythrocytes are known to change shape, from a biconcave discoid shape to an elongated, somewhat cigar-like shape, when exposed to rapid acceleration in a suspending fluid. With hydrodynamic focusing, as in the Sysmex X-Class (and pocH-100i) analysers, the degree of acceleration is significantly reduced. The height of the electrical pulse generated in turn is dependent on the cross-sectional area of the cell rather than true volume. The pulse height is used to calculate the individual cell volume based on a formula which contains a constant factor (Fig. 3). The determination of this constant factor is based on the assumption that all cells will deform predictably as they pass through the aperture.

This is however not the case as this deformability, over and above the factors already mentioned, is influenced by the internal viscosity of the cell, i.e. haemoglobin concentration.

It should also be noted that cells with a high internal viscosity (i.e. 'high MCHC' cells) are less deformable and therefore are overestimated in size and those with low internal viscosity (i.e. 'low MCHC' cells) are underestimated in size. As only 'high MCHC' cells are overestimated in size and only 'low MCHC' cells are underestimated in size, both 'normalise' the extremes of MCHC and therefore clamp down or narrow the true range of MCHC. Consequently, the MCHC has in the past generally been considered to be less useful as a true clinical parameter, but more useful as technical control parameter. The Sysmex MCHC however shows far less of this clamping effect, as the cells are less deformed in comparison to haematology analysers that do not use hydrodynamic focussing, and consequently reflects the true MCHC of the cells more closely, and hence a much wider normal reference range is to be expected.

Bull *et al.* (1996) demonstrated that the 'trueness' of the MCHC obtained from various analysers using the measured HCT (or MCV x RBC) as compared with HCT obtained using the reference microhaematocrit method (PCV) is dependent on the technology employed. They state that the square of the correlation coefficient (r^2) for each of the methods tested approximately reflects how close in percentage terms the analyser generated MCHC values is to the true MCHC value (manual method) as shown in Tab. 2.

Based on this, and other studies [2], it is evident that MCHC values obtained from analysers without hydrodynamic focusing correlate poorly with the actual MCHC of the red blood cell population in the sample and therefore cannot be used as a clinical parameter. Conversely, MCHC values obtained from analysers with hydrodynamic focusing (such as Sysmex) show much better correlation with manual methods and therefore are useful in the classification of anaemias.

Method	MCHC	MCV	MCH
Impedance without HDF (e.g. CELL-DYN 3000)	0.178	0.709	0.905
Impedance without HDF (e.g. Counter)	0.278	0.602	0.812
Optical with HDF and sphering (e.g. Bayer Technicon)	0.556	0.738	0.866
Impedance with HDF (e.g. Sysmex)	0.729	0.860	0.904

Tab. 2 *R^2 values of automated versus reference method red cell index values*

HDF = Hydrodynamic focussing

It is therefore to be expected that MCHC values obtained from a Beckman Coulter analyser will vary from those obtained on a Sysmex analyser. It should also be noted that red cell indices from one analyser are not interchangeable with those of another for the purposes of calculating the MCHC i.e. both HGB and HCT must be obtained from the same analyser if a manual calculation is being performed.

In view of these minor differences, the Sysmex haematology analyser 'Instructions For Use' (IFUs) state very clearly that each laboratory should set up its own reference range in accordance with CLSI (Clinical and Laboratory Standard Institute) guidelines.

In general, analysers that do not use hydrodynamic focussing (e.g. Beckman Coulter and the older CELL-DYN models) show the greatest 'clamping effect' and will have tighter MCHC normal ranges. Here the MCHC will best serve as a 'QC parameter' assessing the technical performance of the analyser.

In contrast, Sysmex analysers (impedance with hydrodynamic focusing) and the Advias (optical with hydrodynamic focusing and sphering) from Siemens show the least 'clamping effect' and here the MCHC will have a wider reference range and provide clinical value in the assessment of anaemias.

It is worth noting that the original textbook description of the categorisation of anaemias as normochromic or hypochromic was based on MCHC as at that time this parameter was calculated using the micro haematocrit and not an automated value. Some later descriptions however refer to MCH, probably due to the fact that the automated MCHC values are no longer considered to be universally reliable as a reflection of patient RBC status, but rather reflect good analyser performance.

FAQ #2: Why is the RDW value sometimes voted out?

In order for the analyser to calculate an RDW value from the red cell volume distribution curve, certain criteria have to be fulfilled. The RDW-SD measurement is performed at a relative height of 20% above the baseline. If either tail of the histogram curve fails to extend below this 20% mark, the RDW value cannot be calculated and will not be displayed. In so doing, the analyser is alerting the operator that something other than intact red cells, e.g. giant platelets or red cell agglutination, may be present and falsely distorting the red cell information. By suppressing the data, the operator is forced to review the sample to identify the cause of interference. Likewise, if the histogram has a double peak, which can happen when there is a dimorphic red cell population, the RDW will be voted out. In this case the RDW is not displayed as the analyser suspects that there are indeed two populations. In this case, physical inspection of the curve and noting the double peak will provide a lot more meaningful information that displaying an RDW value. Identifying the presence of dimorphism provides a lot more guidance as to what may be wrong with the patient than would a quantitative RDW value, which would be wide in this case. The list of possible causes of a double peak histogram is much narrower than possible causes of a wide RDW.

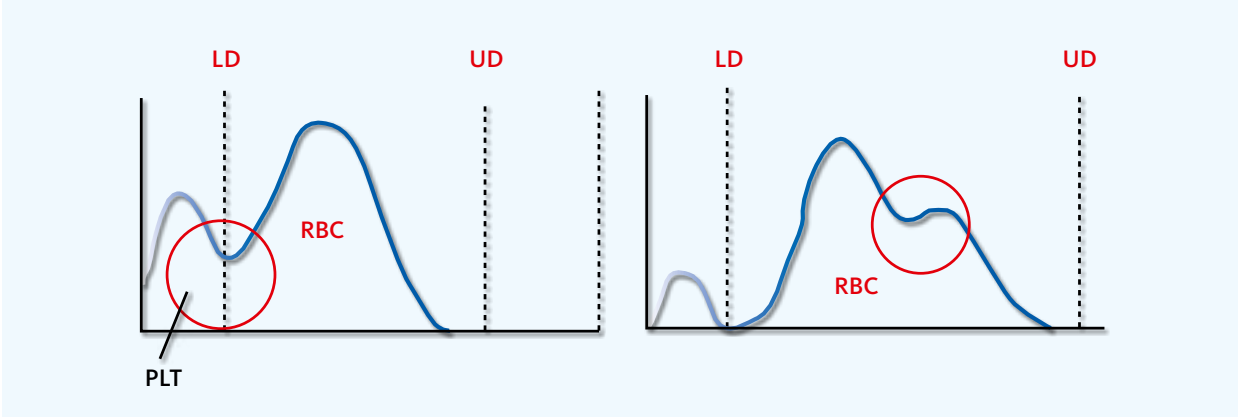

Fig. 6 *Red cell histograms showing examples of when the RDW value will be voted out.*

Take home message

The red cell indices are a very valuable guide to the classification of anaemia. This will in turn assist the clinician with the appropriate selection of further investigations in order to identify the underlying cause of the anaemia and facilitate appropriate treatment. The additional use of the reticulocyte count, and the associated RET-H_e parameter (available on Sysmex analysers equipped with a reticulocyte channel) will facilitate this further). Laboratories should always follow good laboratory practice principles and establish analyser specific reference ranges to ensure the appropriate interpretation of patient results as differences between analysers utilising different technologies are to be expected. Furthermore, it should always be remembered that when comparing results from different analysers that any time delays between measurements can have a significant impact on the results as cells tend to swell with time.

References

[1] ***Bull BS et al.*** *(1996): Red cell index or quality control parameter? In: McArthur JR, Lee SH, Wong JEL, Ong YW, eds. Haematology 1996, Educational programme of the 26th Congress of the International Society of Haematology. 1996:40.*

[2] ***Van Hove L et al.*** *(2000): Anemia diagnosis, classification, and monitoring using Cell-Dyn technology reviewed for the new millennium. Lab Hematol 6: 93 – 108.*

Compiled by
Dr. Marion Münster