LOOKING BACK TO OUR ROOTS:
80 YEARS OF WINTROBE'S INDICES

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ABSTRACT

This year (2014) we are celebrating the 80th anniversary of Dr Maxwell Myer Wintrobe's pioneer works, one of the most important contributions in clinical laboratory and medicine. Red cell indices continue to provide an essential support to the diagnosis and classification of anaemia. The erythrocyte indices, such as mean cell volume, mean cell hemoglobin concentration, and mean cell hemoglobin, are called the Wintrobe's indices. Automation in hematology has progressed steadily since Wallace Coulter first applied electrical impedance technology to counting red cells and white cells. Technological advances being incorporated into hematology analysers since then are now allowing access to more cellular information than was ever available before through a 'simple routine complete blood count'. Current research is beginning to demonstrate that this information also has great potential to identify cellular changes that typically occur in several important medical conditions-bringing us all one step closer to using hematology analysers as more than just simple cell counters, but instead as powerful tools for the management of any medical condition that impacts the biology of blood cells. There are increasing amounts of data provided, which require specialist knowledge to interpret as well as to understand the limitations in the measurement of the parameter. Both laboratory scientists and clinicians need to keep up-to-date with new parameters and methods in hematology, implying a stronger collaboration between them to improve clinical decision-making.

Keywords: Anaemia, erythrocyte indices, red cells, hematology analysers.

MAXWELL MYER WINTROBE - THE PIONEER

In 2014 we are celebrating the 80th anniversary of Dr Maxwell Myer Wintrobe´s pioneer works, one of the most important contributions in clinical laboratory and medicine. Red cell indices, the Wintrobe's indices, continue to provide an essential support to the diagnosis and classification of anaemia. From the measurements of the average volume and hemoglobin (Hb) concentration of erythrocytes, the underlying aetiology of anaemia was brilliantly unveiled.[1-4] Anaemia is a disease itself, but it is often symptomatic of other illnesses; every clinician must deal with anaemia in daily practice, taking into account that concomitant anaemia makes the prognosis of the underlying disease worst for those patients.

His text book: *Clinical Hematology*, first published in 1942,[5] remains a prototype of excellence and for many years stood alone as the premier text in the field. He had written and edited the first six editions by himself, though always depending on the critical peer review of his talented colleagues at Utah University, with his wife Becky, as he said, his severest and most helpful critic.[6] '*Hematology, the Blossoming of a Science: A Story of Inspiration and Effort*' was published in 1985,[7] soon before his passing. Writing this book as part memoir, part

history, Dr Wintrobe realised that he could never cover the lives of all who had contributed to what he called 'the Golden Age of Hematology'. When he died in 1986, his distinguished career in medicine had spanned 60 years. His scientific achievements are recorded in >400 publications. He was a world leader in hematology, a role attested by a legion of honours, memberships, and activities in national and international scientific societies; elected to the National Academy of Sciences, he became the first chairman of the section of Human Genetics, Hematotogy, and Oncology.[6]

Wintrobe's method was based on manual measurements of Hb, hematocrit, and erythrocyte count. Reading his articles in the 1930s,[1-4] his concern for the accuracy of the methods and the reliability of the measurements as the first step of his work to classify anaemia was clear; then he brilliantly postulated the link between cell morphology to deduce the real aetiology of each type of anaemia. The erythrocyte indices, which include mean cell volume (MCV), mean cell Hb concentration (MCHC), and mean cell Hb (MCH; derived from precisely measured values of Hb and red blood cell [RBC] count), are collectively called the Wintrobe indices.[8] The hemogram is one of the more required tests by clinicians and the indices are still applied for classifying anaemia. The analysis nowadays is totally automated and the correct interpretation of the results is required to reunite the knowledge about the characteristics of the equipment and the clinical meaning of the results.[9]

THE BIRTH OF AUTOMATION

The first attempt in automation of blood cell counts was the introduction of the Coulter Principle. While under contract to the US Navy in the late 1940s, (though a patent was not awarded until the 20th October, 1953), Wallace H. Coulter developed a technology for counting and sizing particles using impedance measurements. The technology was principally developed to count blood cells quickly by measuring the changes in electrical conductance as cells suspended in a conductive fluid passed through a small orifice. Presently, most automated cell counters incorporate this technology, which is referred to as the Coulter Principle.

Using count and pulse height analyser circuits, the number of particles and volume of each particle passing through the aperture can be measured.

If the volume of liquid passing through the aperture can be precisely controlled and measured, the concentration of the sample can also be determined. A typical measurement using Coulter counter instruments takes <1 minute, as counting and sizing rates of up to 10,000 particles per second are possible. The accuracy of the size measurements can be better than 1%.[10]

Automation in hematology has progressed steadily since Wallace Coulter first applied electrical impedance technology to counting red and white cells. By the 1980s, most hematology laboratories were reporting a 7-parameter complete blood count (CBC) and three-part differential obtained from a single aspiration on a stand-alone, bench-top instrument. Eventually, this process was upgraded even further when it became possible to obtain these results without uncapping the sample.

Automated blood cell analysis is traditionally performed using impedance technology that allows the measuring of RBCs. Combined with the determination of blood Hb concentration, basic RBC indices - as defined by Wintrobe - can be calculated: MCV, MCH, and MCHC. An important overlooked fact is that an individual's red cell volume determination in various hematology analysers is dependent on the technology used, and that hematocrit value is calculated from the measured MCV. The most reliable red cell index with all hematology analysers is MCH, which is derived from precisely measured values of Hb and RBC.[8]

The Advance of Technology

In the 1990s, the analysers were capable of quantifying the heterogeneity in distribution of MCV (red cell distribution width [RDW]), adding remarkable useful information to MVC. In order to mathematically describe a set, two types of measures are necessary: centralisation measures (more abundant individuals) and measures of dispersion, giving insight into the heterogeneity of the population. MCV is the mean of the volumes of all erythrocytes; RDW refers to the variety of individual cells with different volumes present in the whole population so the contribution of marginal sized subsets to the calculated mean value can be assessed.[11]

Although RDW is generally understood to be a coefficient of variation of the distributions of erythrocyte volume, some hematology analysers calculate RDW from the direct measurement of

the width of the distribution.[12] Other technologies have been adapted to the automated analysers, enabling them to analyse the physiological and chemical characteristics of cells, for example, flow cytometry. Nowadays most of the counters in the hematology laboratory utilise laser, impedance, and/or flow cytometry. The basic purpose of the optics bench in a flow cytometer is to detect light from cells as they pass through a flow cell illuminated by a laser beam. This light can be scattered from the surface and internal structures of the cell. In addition to volume and conductivity, several additional cellular measurements can be obtained for each individual cell, including different angles of light scatter and pulse time. This information can be used in multiple combinations, allowing discrimination of different cellular elements.[13]

The American Legacy

The flow cytometric optical technology for RBC parameters measurement was first made available by the then Technicon Company in their H* series of instruments, later followed by the Advia® hematology analysers (Bayer Diagnostics, presently Siemens Healthcare Diagnostics, Deerfield, IL, USA). This optical technology enables the measuring, simultaneously and independently, of the cellular Hb concentration of individual erythrocytes as well as their volume, derived from light scatter by isovolumetrically sphered RBC according to the Mie theory.[14,15] Mie theory describes the mathematics of light diffraction by spherical objects; in this case the red cells are transformed into isovolumetric spheres. When using monochromatic laser light, diffraction is only a function of the size and the refractive index of the object (related to its internal structure).

In the red cell/platelet channel, red cells are converted to spheres. Using Mie theory, low-angle (2-3°) and high-angle (5-15°) light scatter is measured and mathematical models in the software use these scatter signals for calculating the size and Hb concentration (CHC) of individual red cells. Based in both measurements, a cytogram can be provided. In this graph, the size of individual red cells is indicated on the y-axis, while their Hb concentration is indicated on the x-axis. The whole RBC population can be classified considering the Hb concentration and cell volume, and four new RBC extended parameters have become available: hypochromic RBC with CHC <280 g/L, hyperchromic RBC, CHC >410 g/L,

microcytic with volume <60 fL, and macrocytic with volume >120 fL.

Technicon Instruments was the first manufacturer to offer a complete set of extended RBC parameters in their H*3 hematology analyser[16] and this explains why the majority of literature on these parameters was produced using this analyser and its successors of the Advia series (Siemens Healthcare Diagnostics). More recently, Abbott (Abbott Diagnostics, Santa Clara, CA, USA) introduced extended RBC parameters on the CELL-DYN Sapphire analyser. These are calculated from three-dimensional laser light scatter, applying the principles of the Mie theory, which is basically the same as in the Advia analysers.[17,18]

The Asian Wisdom

Over the past years, other manufacturers have started offering extended RBC parameters, although differences in the technology used make it impossible to directly compare the numerical values of RBC parameters derived from different instruments. Sysmex analysers XE 5000 and XN (Sysmex Corporation, Kobe, Japan) report RBC extended parameters, the percentages of erythrocyte subsets. In the reticulocyte channel hypochromic and hyperchromic erythrocytes are quantified by means of flow cytometry, but it is important to highlight that it is Hb content which is reported. Hypochromic red cells by Sysmex are those with an Hb content of <17 pg, while hyperchromic are those with Hb >49 pg.[19] RBCs are continuously produced in the bone marrow; because of the long lifespan of mature erythrocytes the values of the derived parameters are related to iron status in the last 2-3 months.

MCV is the mean of the volumes of all erythrocytes; RDW refers to the heterogeneity of volumes present in the red cell population. This is not the case for MHC. Calculated from RBC count and Hb, MCH represents the average; the percentage of subsets can give complementary information of the contribution of the cell with extreme values (hypochromic and hyperchromic cells) to the mean values. The percentages reflect how Hb is distributed in the individual cells, in a uniform way or not; if polychromasia is present, the fluctuations of iron availability to the erythron in the previous weeks can be highlighted.[11,20] Similar application of those technologies are being introduced by other companies (Mindray Biomedical Electronics, Nanshan, Shenzhen, China) on their analysers.[21]

In summary, technological advances being incorporated into hematology analysers since Dr Wintrobe´s days are now allowing access to more cellular information than was ever available before through a 'simple routine CBC'.[22,23] The added information potentially contained in the 'new extended hemogram' has proven its usefulness in certain clinical conditions: anaemia of chronic disease, functional iron deficiency, and monitoring the availability of iron during treatment with erythropoietin,[11,17,24-31] thalassaemia trait screening,[32,33] spherocytosis,[34,35] and latent iron deficiency.[36]

A LOOK INTO THE FUTURE

Current research is beginning to demonstrate that this information also has great potential to identify cellular changes that typically occur in several important medical conditions, bringing us all one step closer to using hematology analysers as more than just simple cell counters, but instead as powerful tools for the management of any medical condition that impacts the biology of blood cells.

Bone marrow produces 200×10^9 cells per day; the body loses the same number through senescence, maintaining the overall steady state in physiological conditions, but little is known about the life cycle of 120 days of RBC. Novel and promising research[37] has added new light on the relationship between Hb content, Hb concentration, and cell volume. Using a systems biology approach, the dynamics of circulating red cells can be analysed, linking the changes of volume and Hb concentration to red cell lifespan and removal. The mechanisms that regulate the number, size, and Hb concentration of normal red cells in circulation are not well understood. It has been established, however, that after their release from the bone marrow, red cells undergo a reduction in their volume and total Hb content.

Higgins and Mahadevan[37] used a theory from statistical physics, together with standard red cell indices derived from electronic cell counters and the information generated by flow cytometry, to develop a master equation for the maturation and clearance of red cells. Their mathematical model implies that the total number of red cells added to the circulation equals the number removed, and suggests that there is a threshold for the mean cellular Hb concentration below which most red cells are cleared from the circulation.[38] The model distinguishes the dynamics of red cell population in healthy subjects from those of patients suffering iron deficiency, thalassaemia, or anaemia of chronic disease.[37]

As has been exposed, manufacturers have developed hematology analysers that achieve good levels of precision and accuracy in cell counting through the examination and identification of thousands, not hundreds, of cells in each sample analysed. There are increasing amounts of data provided, which require specialist knowledge to interpret and understand the limitations in the measurement of the parameter. Both laboratory scientists and clinicians need to keep up-to-date with new parameters and methods in hematology, implying a stronger collaboration between them. Good laboratory practice ensures that reliable results of laboratory tests are reported to the clinician.[39] The modern clinical hematology laboratory is facing strong pressures to provide clinically relevant information that can help the clinicians to make a diagnosis in a fast, cheap, and useful manner, but we must not forget the words by Dr Wintrobe: "And yet, we must not stop exploring and measuring, for there is always more to learn."

Happy anniversary!

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