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Haematology Analyzers- Something More Than the Simple Blood Cells

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Blood is a treasure of countless cells and molecules with enormous functions and structures. All of these are thoroughly being analyzed and utilized. Haematology analyzers have an inborn system to explore the blood cells. Day by day automated hematology analyzers are expanding their scope to unearth more and more characteristics of blood cells. From three and five part differential hematology analyzers, now capacity of these is extending into new realms. All the blood cells are in the arena not just to be count-in, but to show meaningful dynamics. This is likely to help in diagnosis and patients' service.

The advances in haematology in the last decades would not have been possible without discovering how to count the cells circulating in the blood. Manual counting over the time is replaced by automated counts. Laboratories today operate with versatile multi- parameter systems, ranging from single-channel instruments to continuous flow machines. The full utilization of their potential is likely to bring immense changes in haematology practice.

Improved technologies, attention to quality, new reagents and electronics, information technology and scientists' talent ensure a more profound and deeper knowledge of cell properties. Systems of electrical, electromagnetic, simple or complex optics or laser light scatter and absorbance at multiple angles, fluorescence, hydrodynamic focusing, automated cytochemistry, selective cell lysis and many others are the important technologies in this regard. Current analyzers are capable of measuring even minor immature or pathological cells populations.

Swirling world of growing technological progress has transformed the blood cell count. Automated haematology analyzers now extending to the extent where, in addition to haemoglobin estimation, red cell indices and cell counts, they are capable of utilizing blood components in varied ways. All this is likely to expand horizon of haematology as well as to ensure better patient management. [1]

Identification of platelet volume distribution curves led to introduction of new innovative platelet parameters. These are obtained from the measurement of platelet properties, such as volume, density and granulation or RNA content. [1] MPV expresses average size of platelets. Light scatter instruments, which use a double angle (small and wide) of signal detection, also measure a parameter called the mean platelet component (MPC), expressed in g/l, as a measure of the density of sphericized platelets (granule content). It is correlated with the activation status. From MPV and MPC, the mean platelet mass (MPM) can be calculated in pg. Another potentially helpful platelet size parameter is called the platelet- large cell ration (P-LCR). [2,3]

Low MPV thrombocytopenia, in particular, have a bone marrow hypo-productive origin. Thrombocytopenias with increased MPV depicts peripheral hyper-destructive origin, with the exception of rare congenital platelet disorders. A decreased MPV, in particular, is associated with hypoproliferative platelet disorder, in which the bone marrow does not ensure adequate production. Increased PDW and P-LCR undermines peripheral immune destruction of platelets.[3]

An increase in MPV and MPC represents a prognostic risk factor in coronary heart disease and acute myocardial infarction, or stroke. [1] Lower median values of MPV and PCT have been reported in HIV-infected, untreated patients, with an interesting correlation with the concentration of CD4+ lymphocytes, and represents a tool to monitor platelets' activation and disease

progression. [4]

While staining the platelets with fluorescent dyes, the younger platelet elements, can be identified. These are characterized by a more considerable amount of cytoplasm RNA than mature platelets, which decrease during maturation. Immature platelets are usually large. Their number is short and reflects recent activity of megakaryocytes. The proportion of immature platelets, out of total platelets' mass, can be expressed as immature platelet fraction (IPF%) or as a percentage of the so-called reticulated platelets, in analogy with reticulocytes (presence of RNA in the anucleated cytoplasm, the so called "reticulum"). These immaturity parameters are measured by some advanced systems,which have incorporated channels with specific fluorescent dyes for nucleic acid, such as oxazine. These can be measured in particular channels or can be derived through accurate volumetric separations. A laser light beam can discriminate between mature platelets and immature platelets, which have reacted with fluorescent dye or display peculiar light absorption.

Automated haematology analyzers are now capable of integrating measurement of erythrocytes sedimentation rate (ESR) with routine blood counts. Wetsergren method has several draw backs, including long analysis time, is considered a biohazard risk being an open system, requires high volume of blood, with a dedicated tube containing a specific anticoagulant, leading to an additional blood draw specific for ESR measurement only. Alternate methods for ESR estimation, use techniques like photometric rheology, capillary photometric kinetic technology or centrifugation. [5]

Haematology analyzers, which integrate measurement of ESR with routine complete blood counts, are now available. These analyzers measures ESR using near-infrared photometry to determine the degree of red blood cells aggregation that occurs in the first phase (rouleaux formation) of sedimentation. Erythrocytes are initially disaggregated by high blood flow velocity before abruptly coming to a halt. During this period, re-aggregation of erythrocytes is started again. In this technology photometric method is used to measure the degree of red blood cells aggregation. A change in aggregation state of erythrocytes leads to changes in the scattering effect of erythrocytes on the measurement beam. The light transmittance increases with greater aggregation. The degree of aggregation is obtained by measuring the light transmittance of a whole blood sample overtime. Data on the extent and rate of erythrocytes aggregation are collected within a span approximately 1.5 minutes. Subsequently, a mathematical model is applied to calculate one hour ESR result. For a single test approximately 160 ul of EDTA blood sample is required for simultaneous measurement of both blood complete counts and ESR.[5-7]

ESR measurement through these instruments show high correlation with the reference Westergren method with an acceptable positive bias and are less effected by varying Hct and MCV levels. Automated haematology analyzer's ESR estimation ensures high sample stability (upto 8 hours at room temperature and upto 24 hours at 2-8 ^{o} C). This circumvents inadvertent results when there is a delay in ESR estimation. [5,8]

Different principles have explored, in automated haematology analyzers, for malaria detection. Methods employed, in this regard, include detection of malarial pigment (hemozoin), analyzing depolarized laser light diffraction patterns, and detecting increased activation of monocytes. These techniques offer rapid checks for possible infections, although they rely on surrogate parameters for malaria detection. On the instrument's graphical representation, these abnormalities are presented as irregularities in the haematology scattergram, but they require some form of operator training and can be subjective in interpretation. [9]

Now automated haematology analyzers, with an ability to directly detect plasmodium infected blood cells, are also available. These analyzers employ fluorescence flow cytometry for blood cell counting and can detect malaria –infected red blood cells while performing a CBC. Infected red blood cells are identified as clusters on the scattergram, generating an "infected Red Blood Cell (iRBC)" flag. This flag indicates red blood cells with inclusions, signifying the presence of parasites in red blood cells, which the system automatically recognizes. In essence, malaria detection is based on a specific white cell differential by fluorescence scattergram during 1-minute CBC analysis. This is depicted on the side scattering and side fluorescence chart, similar to a conventional flow-cytometer and infected red cells appear as an additional cluster overlapping the existing RBC integration region.

These instruments stain plasmodium nucleic acids with Fluorocell M, along with a lytic solution (Lysercell M) . Infected red blood cells (iRBC) and white blood cells are detected by a violet semiconductor 405 nm laser beam.

Treatment of infected RBCs (iRBCs) with Lysercell M increase the fluorescence intensity of stained parasites nucleic acid by approximately 10-fold and reduces the size of iRBCs in a single-stage manner, facilitating the identification and quantification of ring forms, trophozoites and schizonts. These properties suggest that Lysercell M is useful for rapidly detecting iRBCs and accurately distinguishing the parasite development stages.[10] Parasitemia percentage is calculated by the ratio of infected and uninfected red blood cells (MI-RBC%) and absolute parasite density (MI-RBC#) expressed as parasites/ul. The data is automatically determined by analyzing scatter grams plotted three dimensionally with forward –side scattered light. iRBC are visible as a separate RBC population on scatter gram. [11,12] Unlike earlier surrogate methods of detection, this approach offers a specific indication of parasite presence in RBCs.[10,13]

Automated analyzers represents a revolution for reticulocytes, using dyes to bind reticulocytes RNA and flow cytometry to perform rapid and objective counts. The possibility to analyze thousands of cells per sample has reduced imprecision. As an absolute retics count is obtained, the correction for reduced haemoglobin is not required. [14,15]

The measuring principle of the system is based on flow cytometry combined with hydrodynamic focusing. Intracellular RNA is stained by Auromine O, which is fluorescent under argon laser light. The reticulocytes population is further subdivided into large fractioned retics (LFR), medium fractioned retics (MFR) and high fractioned retics (HFR). The percentage of reticulocytes is given as the sum of LFR, MFR and HFR. The immature ratics fraction (IRF) is calculated by MFR plus HFR.[14-16]

Two methods can be employed, in automated haematology analyzers, to assess reticulocytes' haemoglobin, i.e., reticulocytes haemoglobin content (CHr) and reticulocyte haemoglobin equivalent (RET-He). These appeared as rapid and cost-effective measure to detect iron deficiency, even before the anaemia develops .[17-20]

An additional interest about automated reticulocyte analysis is the introduction of a new parameter called immature reticulocytes fraction (IRF), based on reticulocytes nucleic acid content. IRF is an early and sensitive index of erythropoiesis. Increase in IRF precedes the reticulocytes percentage increase in predicting treatment response in iron deficiency anaemia and megaloblastic anaemia .[14]

Some haematology analyzers can quantitate the percentage of hypochromic and microcytic erythrocytes. In iron deficient erythropoiesis, a greater fraction of red blood cells (RBCs) is hypochromic rather than microcytic, and the microcytic/hypochromic ration shows diagnostic efficiency in the differential diagnosis with beta thalassaemia minor. The results are better than traditional discriminant factors or formulae. [21]

Various blood cell counters have incorporated methods capable of quantitatively measuring the proportion and absolute number of circulating elements such as nucleated red blood cells (NRBCs) and immature granulocytes. Reagents containing fluorescent dyes are generally used to identify NRBCs, on the basis of particular characteristics of size and DNA content. In neonates and premature babies, the NRBCs count helps to distinguish these cells, often present in large numbers, from normal white blood cells (WBCs),to correct the total and differential WBCs count.[22]

Cell population data (C.P.D) or positional parameters, gathered through automated analyzers, have gained considerable interest. The main investigations concern size and scatter properties of neutrophils, lymphocytes or monocytes as diagnostic and prognostic tools in infections and sepsis. Lymphocytes population data are also valuable for the differentiation of lymphoproliferative disorders and viral infections like infectious mononucleosis. [1]

The identification of blast cells, through automated analyzers, needs to address the issue of heterogeneity of their origin, cytological structure and morphology. They can be recognized through the increased intensity of fluorescence and varied chromatin density. The CellaVision and CytoDiff reclassification procedure, built in some automated hematology analyzers, has a diagnostic ability for predicting blasts close to that of manual microscopy.[23] According to the specific charecterisitcs analyzed by different technologies, the distributions that clusters of pathological cells assume, provide enough diagnostic information. The observations of scattergram and scatterplots allows to recognized different types of haematological malignant cells. [1]

Various methodologies have developed effective systems, through alarms' signaling, to identify atypical cells. Such flags trigger the microscopic review of a

blood smears stained with panoptic methods or with automatic blood smearers and strainers. The ability to identify specimen with blasts, immature granulocytes, nucleated red blood cells and atypical lymphocytes may vary from instrument to instrument but have improved over time. [1]

So many discoveries and innovations, generated through automated haematology analyzers, still need to be tested and applied on a larger scale. In red blood cells, hyperchromic red blood cells flagging for hereditary spherocytosis, percentage of microcytes, "low density haemoglobin "in iron deficiency, a semi-quantitative parameter, i.e., "Iron-Def" and many others are being introduced by different analyzers. Cell Population Data (C.P.D) representing cell volume (decreased neutrophil forward scatter light) and complexity of neutrophils (decreased neutrophil side scatter light) can be useful for screening Myelodysplastic Syndrome (MDS) using peripheral blood, but this still needs an acceptable validity. Many of the cell population datas (CPDs) of neutrophils, lymphocytes and monocytes may be considered useful as "sepsis parameters". Monocytes distribution width (MDW) seems to play a role to identify patients with sepsis. Role of Artificial Intelligence (A.I) , through Machine Learning Model and so many other algorithms is not very far away.[21] Full-field hemacytometry includes, even the analysis of non-hematic fluids, digital adds to the microscope , and the development of efficacious point of care devices. [1,3]

Sensitivity and specificity of the automated over conventional methods, which still are gold standard, needs to be elucidate fully. It can be assumed that these automated analyzers generated methods can be cost effective, less time consuming as compared to conventional methods. All these innovations, in automated hematology analyzers, ensure their utility with minimally trained staff, without specific sample preparations, free of observer-dependent viability and are available round the clock. This is likely to expand the utilization of automated haematology analyzers to unforeseen vistas. [3,12]

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