Automation in Hematology

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Key Words

Hematology analyzer · Automation · Blood cells

Summary

With increasing self-driven momentum, hematological diagnosis has developed from rather philosophical approaches in ancient Greece to the application of logical principles to investigating human blood within the last two centuries. The 20th century was a time of industrial standardization and thus a period in which established procedures operated by humans were perfected in hematology. In the 21st century, modern computers and network techniques are being integrated into a complex instrument, linked to each other mechanically and logically by control systems based on algorithms. Automation in hematology now permits comprehensive and highly precise diagnosis, with hardly any human help. This will be illustrated by four modern analysis systems. These diagnostic tools are not only of high importance for the treatment of patients but also for the screening of blood donors and blood products.

Schlüsselwörter

Hämatologie-Counter · Automation · Blutzellen

Zusammenfassung

Ein ständiger, sich beschleunigender Fortschritt in der hämatologischen Diagnostik führte von mehr philosophisch zu betrachtenden Denkansätzen im alten Griechenland zu logisch geprägten Grundlagen in der Methodik von Untersuchungsmethoden menschlichen Bluts in den letzten zwei Jahrhunderten. Das 20. Jahrhundert war vor dem Hintergrund industrieller Standardisierung das Zeitalter der Perfektionierung etablierter Verfahren in der Hämatologie, die von Menschen bedient wurden. Im 21. Jahrhundert wurden moderne Computer- und Netzwerktechniken in komplexe Gerätesysteme integriert, die untereinander mechanisch und logisch über algorithmisch geprägte Regelsysteme verbunden sind. Die Automation in der Hämatologie erlaubt heute, weitestgehend ohne menschliches Zutun, eine umfassende, hochpräzise Diagnostik, die am Beispiel von vier modernen Analysesystemen vorgestellt wird. Diese Diagnostik ist nicht nur in der Patientenversorgung von hoher Relevanz, sondern auch bei der Untersuchung von Blutspendern und Blutprodukten.

Introduction

Blood is one of the most complex organ systems in the human body. In the adult, it consists of about 45% solid components and 55% fluid components. Blood is formed from hematopoietic stem cells in the bone marrow. As required, red cells, white cells or platelets are formed and released into the or-

ganism. Together with plasma, these cells constitute the human blood. Blood as a whole is responsible for the most important functions of life, such as the transport of metabolic components, gas exchange, the immune defense and coagulation.

A state of health is characterized by homeostasis within the

cellular components and plasma and a normal relationship between solid and fluid components. Diseases are characterized by changes in individual blood parameters which are more or less typical for the underlying disease. It is therefore of greatest interest to be able to easily and rapidly measure these parameters at any time with high precision and accuracy to allow for a precise diagnosis.

History of Blood Testing

Inspection of blood was a basic principle of diagnosis from ancient times till the 16th century [1]. Blood inspection was an investigation of blood bled from arteries and was called hemiscopy or hematoscopy; the color and structure of the blood was investigated. In more modern times new concepts were developed, particularly by the Swiss doctor Paracelsus (1494–1541). These were closely linked to developments and progress in microscopy. These methods made it possible to examine cells for the first time.

Quantitative determination of the individual cellular components was first enabled in 1852 by the work of Karl Vierordt (1818–1884), a physiologist from Tübingen. He developed the first counting method, in which a specific blood volume were smeared onto a slide. The slide was then covered with a glass grid, and all 4.6 to 5.8 million erythrocytes per μl were counted [2]. In 1924, Neubauer published his net structure [3]. This led to the manual cell counts, which are still taken as gold standards in some areas. [4, 5]

Both venous and capillary blood was used as test material for the determination of leukocytes, erythrocytes, and platelets. Blood was isolated under optimized pre-analytical conditions; coagulation was prevented with the dipotassium salt of ethylenediaminetetraacetic acid (EDTA; about 1 mg/ml blood). Samples were prepared according to fixed procedures and then counted in the Neubauer counting chamber (improved model).

Erythrocytes, leukocytes, and platelets were visualized by selective staining and the lysis of interfering cells and then counted in a defined counting volume at 1,000-fold magnification. The precision and accuracy were highly dependent on the number of counted cells and, at a reasonable level of effort, were subject to fluctuations of up to 10%. Aside from the Neubauer counting chamber, other models (Bürker, Fuchs-Rosenthal, Thoma, Schilling, Türk) are still available, although these are only used routinely for special tests, such as examining cerebrospinal fluid [6]. The individual counting chambers differ in the number of squares and their marking and separation (course of the lines).

The first step towards automation was made in 1934 with the Moldavan capillary method [3]. A suspension of blood cells was illuminated in a dark field and counted in an optical chamber which converted the light impulses into electrical impulses.

The actual breakthrough in the development of hematological instruments suitable for routine work was achieved by Wallace Coulter in 1956 with his patent 'High speed automatic blood cell counter' [7]. The Coulter Model A was presented at the US National Electronics Conference in Chicago as the first nonoptical machine for counting blood cells. Coulter's cell counters are based on the principle of impedance measurements and, for the first time, enabled to count much higher cell numbers than the known manual methods. Nevertheless, there was still a long way to go to the first semiautomatic machines for routine work. The most important conditions for achieving this goal were the standardization of the methods and the elimination of methodological errors.

With this aim in view, the International Committee (later Council) for Standardization in Haematology (ICSH) was founded in 1963 at a congress of the European Society for Haematology [8] by Wallace Coulter and other leading scientists who worked in special committees on the standardization of the requirements for the methods. Some of the most important steps will be mentioned below:

- The optimization of the detector, with respect to the pulse volume and the reliable discrimination between genuine counted particles and background electrical noise. The signal to noise ratio should to be above 100:1.
- Correction of counting errors from spontaneously lysed cells during passage through the capillary opening. Optimization of the diluent, avoiding cell distortion [9].
- Attainment of constant volume flow in the suspension passing in real time through the opening.
- Avoidance of particle recirculation after passing through the opening.
- Optimization of the material for the machine lines and other materials. Development of reference counting instruments and calibrators [10–24].

These improvements were implemented and have led to the powerful analytical instruments which are currently available.

Modern Automated Systems

Blood count parameters have been measured automatically for more than 40 years. A variety of fully automated instruments are now commercially available. All instruments determine the hemogram, the differential blood count and the reticulocyte analysis in EDTA whole blood. Depending on the instrument, it may be possible to analyze cells from body fluids and bone marrow. All instruments can process the tests in either the manual or the automatic mode. The instruments are based on various measurement technologies used in different ways:

- impedance measurement,
- high frequency measurement,
- forward scatter (FSC) at different angles,
- fluorescence flow-through cytometry.

► Measuring transformer

Fig. 1. The principle of impedance measuring: Coulter method

Fig. 2. The principle of impedance measuring: Sysmex method.

Impedance Measurement

Impedance measurement (Coulter principle) was historically the first principle of measurement. This is based on the measurement of changes in resistance during cell passage through a small defined opening between two electrodes (fig. 1, 2). The cells have lower conductivity than the diluent [25, 26]. The resulting electrical impulse is proportional to the cell volume. The sum of the impulses from all cells in a fixed measurement volume is evaluated with a histogram. The resulting distribution curves are automatically discriminated according to the lower (platelets) or higher values (leukocytes) and then depicted graphically. The hematocrit is calculated as the sum of all impulses from the directly measured cell volume. The principle of hydrodynamic focussing (Sysmex) [9] was an important development of this concept. This almost totally pre-

Fig. 3. Hydrodynamic focusing with central stream for measuring blood cells and hematocrit.

Fig. 4. Sheath Flow RBC – Sysmex XT.

vented coincidence and gave a clean Gaussian curve for the erythrocytes (fig. 3). It also improved the separation of small erythrocytes and platelets. This measurement is volume based so that no special calibration by the user is needed (GE, Sysmex).

The development of hydrodynamic focussing enabled to avoid duplicated cell counting by coincidence. The sample stream is coated with a coat stream fluid (sheath stream). This reduces the diameter of the sample stream to cell size and isolates the individual cells. The cells are then passed through the electric field like a string of pearls and cannot be washed back by turbulence in the area of measurement (fig. 4) [27].

High Frequency Measurement

High frequency measurement provides an analysis of the internal structures of the cells. Either a special reagent (Sysmex) is added, or the measurements are performed on practically native leukocytes after completion of erythrocyte lysis (Beckman Coulter) [28]. The cells are exposed to a high frequency

field, and an impedance measurement is performed at the same time. The high frequency impedance impulse depends on the internal structures and volumes of the cells. In this way, immature granulocytes are separated from mature cells using special software (ACAS; Adaptive Cluster Analysis System), and much additional information on the cells is provided. This method is highly sensitive (detection of as few as 1% blasts/myelocytes).

Measurement by Scattered Laser Light

The measurement is performed with erythrocytes and platelets transformed to a spherical form (sphered erythrocytes; Patent Technicon, Bayer, Siemens) [29, 30] or treated with a surface active diluent to optimize their shape (Sysmex). There are three preconditions for exactly reproducible scatter signals when measuring and evaluating erythrocytes and platelets: i) formation of isovolumetric spheres or optimization, ii) a monochromatic light source, and iii) isolation of the blood cells in the measurement cell by hydrodynamic focusing (fig. 5).

Lauryl sulfate (SDS) is used to form isovolumetric spheres. Glutaraldehyde (Bayer) is used to fix the erythrocytes. The cell preparation is performed in isotonic solutions. The light source is a laser diode with defined wavelength. The cells are not only isolated individually but also separated by the detection of different contents of DNA/RNA (Sysmex). This is achieved by differential staining with fluorescent dyes for DNA/RNA. The double angle laser light scatter from individual cells is measured at several different angles (low and high angle ranges), depending on the manufacturer, and results then were processed.

This gives three distribution curves:

- the volume distribution of the erythrocytes, with the mean corpuscular volume (MCV) as mean,
- the hemoglobin distribution of the erythrocytes, with the cell hemoglobin concentration mean (CHCM = the directly measured mean cellular hemoglobin concentration) as mean (CHCM is only measured in instruments from Siemens),
- the platelet volume distribution, with the mean platelet volume (MPV) as mean.

The volume distribution of the platelets is log-normal, whereas the distribution of the erythrocyte volume and Hb concentration is Gaussian-normal. In a normal patient or donor sample, counting lasts for exactly 10 s. During this time, about 50,000 individual erythrocytes and about 3,000 platelets are counted simultaneously and morphologically evaluated (Siemens).

Measurement Technology of Reticulocyte Analysis

Reticulocytes are determined from EDTA whole blood, by bringing a blood aliquot in contact with a specific chromogen,

Fig. 5. The scattered light at different angles.

new methylene blue, or its derivatives, such as Oxazin 750. After staining of the so-called ‘reticulo-granular filamentous material’ (presumably ribosomal RNA), the cell suspension is measured in laser light. Scatter properties are measured at high and low angles, together with the absorption of the stained reticulocytes. Comprehensive reticulocyte analysis is possible with this combination of measurement signals.

Flow-Through Cytophotometric Analysis of Fluorescent Labeled Cells

A flow-through cytophotometer is an optical instrument used to investigate the morphological, genetic and biochemical properties of individual cells and particles suspended in fluid. The newest commercially available flow-through cytophotometers work with at least four different excitation wavelengths, thus allowing parallel measurement of up to 16 different parameters with 13 different fluorescences. A flow-through cytophotometer consists of three different technical components: the fluid components in which the cells or particles are transported, the optical component responsible for the detection of the fluorescent cells or particles, and the data processing system allowing visual representation of the signals together with evaluation and storage.

One of the most frequent uses of the flow-through cytometer is the quantification of cells with specific properties in complex fluids such as blood. This can be done indirectly (either with reference particles or with a reference measurement with a hematology counter) or directly (through a volume measurement).

Flow-through cytophotometry has now become a routine technique for many applications based on standardized procedures. The most frequent applications include standardized cell counting (cell culture, residual leukocytes in blood products, nucleated cells in cerebrospinal fluid, etc.) [31], tests of ploidy (pathology, microbiology, etc.) with DNA dyes (propid-

ium iodide, DAPI, Höchst dyes, 7-AAD, etc.) [32], and the immunological classification of leukocytes [33]. Antibodies coupled to fluorochromes are used in the fully automated immunological measurement of platelets [34–37], T-helper and T-suppressor cells [38, 39] and hematopoietic stem cells [40, 41]. PCR products can also be detected and quantified in flow-through cytometry using specific microparticles [42, 43].

The methods described above for counting and determining multiple properties of the cells are subject to permanent innovation, continuously extending the potential of these procedures. New high-precision and high-accuracy hematological analysis systems, such as Cell Dyn[®] Sapphire (Abbott GmbH & Co. KG, Wiesbaden, Germany), Coulter[®] LH 780 (Beckman Coulter GmbH, Krefeld, Germany), ADVIA[®] 2120 (Siemens Medical Solutions Diagnostics GmbH, Fernwald, Germany) and XE 5000, (Sysmex Europe GmbH, Norderstedt, Germany), offer a variety of possibilities according to the current state of the art.

Cell Dyn[®] Sapphire

The Cell Dyn Sapphire (Abbott GmbH & Co. KG, Wiesbaden, Germany) is the direct successor of the CD 4000. It is also used MAPSS (multi-angle polarized scatter separation) technology which had been introduced in the CD 3000 series and in which leukocytes are differentiated in a single measurement step. In each measurement, this system also determines the degree of vitality of the leukocytes and the nucleated red blood cells (NRBC; erythroblasts). In contrast to most competitive products, a full differential blood count is always performed. This clearly decreases the number of tests which have to be repeated [44, 45].

The Cell Dyn Sapphire clearly differed from competitive products with respect to the specific use of fluorescence laser differentiation (MAPSS technology). The resulting leukocyte differentiation is excellent. Abnormal cells (blasts, immature granulocytes, etc.), platelet aggregates and NRBC are always reliably detected, and the number of control measurements is minimized [46].

Platelet Analysis

Platelets are primarily analyzed by 2D optical measurement of scattered light. The optical measurement allows reliable detection of the platelet concentration, particularly in thrombocytopenic samples. The resistance channel also provides the platelet impedance count (PIC) which serves as a check of plausibility. The duplicate measurement of platelet concentration allows the reliable detection of a very wide variety of interferences. As an optional third measurement principle, the platelet concentration can be determined using FITC(fluoresceine isothiocyanate)-labeled, platelet-specific CD61 antibod-

ies and measurement of the FL1 fluorescence. This measurement allows highly precise measurement of thrombocytopenic blood in the context of decisions on transfusion, reliable exclusion of interference and the quantification of platelets in patients with giant platelets [47, 48].

Erythrocyte Analysis

The isovolumetric and spherically transformed erythrocytes are analyzed by impedance measurement and also by optical measurement. Morphological measurement is planned for the future.

Reticulocyte Analysis

Reticulocytes are fully automatically analyzed after selective setting. The isotonic blood dilution is incubated with RNA/DNA dye and then transported through the flow-through cuvette, using the differentiation approach. The green fluorescent dye (FL1) not only stains reticulocytes, but also parasitized erythrocytes such as those with plasmodia (in patients with malaria). The advantage of the fluorescent method is that it is highly reproducible.

The quotient of the number of immature reticulocytes to the total number of reticulocytes is designated as the IRF (immature reticulocyte fraction) or reticulocyte maturity index. An increase in the IRF is an early and specific indicator of the regeneration of erythropoiesis.

If the IRF is present as a clear peak in the histogram, this may indicate that the erythrocytes are infected with plasmodia. In such cases, the eosinophil scattergram must be carefully inspected as the monocytes with malaria pigment (hemozoin) exhibit atypical depolarization.

Coulter[®] LH 780

The hematology instruments of Beckman Coulter have a long tradition, starting with Wallace Coulter, the developer of the first hematological cell counter suitable for routine use and based on the impedance method. The newest development is the Coulter LH 780 (Beckman Coulter GmbH, Krefeld, Germany). This can be combined with the LH SlideMaker and the LH SlideStainer, giving the Coulter[®] LH 785 (Beckman Coulter GmbH), a compact automated hematological work-cell with integrated expert system [49, 50].

Cell Count in the Hemogram

The LH 780 is characterized by how simply and efficiently it is constructed. This includes the small number of reagents, a

Fig. 6. Typical leukocyte histogram for a normal finding, left displacement, chronic lymphatic leukemia (CLL), and giant neutrophils linked to vitamin B12 deficiency anemia.

total of four with cleaning solution and only two for the hemogram. For the erythrocyte and platelet count, the patient blood is only diluted approximately 1:800 with the diluent, and then the cell count is performed by the impedance procedure. To quantify the leukocytes, an aliquot of aspirated blood is lysed with a quaternary ammonium salt. The erythrocytes are destroyed by this process and the leukocytes shrink to the nucleus and the coupled structures, like the endoplasmic reticulum. The leukocyte count is then based on the analysis of the impedance of the cell nuclei. The lysate is also used to determine the hemoglobin content (cyanide-free oxyhemoglobin method). The MCV of each individual erythrocyte is measured and the hematocrit calculated from the erythrocyte count and the MCV.

In contrast to all other systems, the counting of the erythrocytes, platelets and leukocytes and the volume determination is performed in parallel in three independent capillaries, and the mean is reported as final result. If a measurement from one capillary differs from the measurement from the other two by more than 5%, this is excluded from the calculation of the mean and a warning is displayed. For cytopenic samples, the counting time is automatically prolonged, thus ensuring that statistically reliable results are produced. Platelet counting is linear between 0 and $3 \times 10^6/\mu\text{l}$ so that the measurements in the range relevant to transfusion (between 5,000 and 10,000/ μl) are correct and available without additional checks. Numerous publications demonstrate that the impedance measurement procedure for platelet counting in the LH 700 series is either equivalent or superior to optical counting in other systems [51, 52].

In addition, this technology is capable of detecting and correcting for interferences to leukocyte counting (such as large nuclear erythrocytes, giant platelets or platelet aggregates) even at the level of the hemogram, therefore the leukocyte count is not increased by these factors. In addition, the leukocyte histogram can serve as screening for the differential blood count, and characteristic curve shapes may indicate pathological changes (fig. 6) [53].

Fig. 7. VCS differential blood count of lymphocytes, monocytes, neutrophils, eosinophils, basophils and NRBC.

Leukocyte Differentiation

The principle of leukocyte differentiation with the VCS technology [54] is based on the simultaneous measurement of volume, conductivity and scattered laser light. The cells are hardly changed by the use of special reagents. The original size of the leukocytes, the internal physical and chemical properties (nucleus/cytoplasm ratio, granules, etc.), and the characteristic surface structure of each cell are maintained. Volume, conductivity and scattered laser light are measured simultaneously, permitting classification of the cells to the five leukocyte populations (lymphocytes, monocytes, neutrophils, eosinophils and basophils) and to nucleated erythrocytes. An aliquot of the aspirated blood is briefly lysed with a weak solution of formic acid and immediately neutralized with a weak base (fig. 7).

The data from 8,192 cells are displayed in a matrix of more than 16.7 million data points and then transferred in clusters to a 3D coordinate system, where the x-axis represents laser light scatter, the y-axis volume and the z-axis conductivity.

This gives a unique representation of the leukocyte populations in the 3D space, analyzed by density, contour and size with highly developed software. This 3D analysis is used to de-

Fig. 8. Acryl block with UFC technology.

termine the relative proportions of leukocyte subpopulations and NRBC. The absolute numbers can be derived from the leukocyte count in the hemogram. If the cell count in the sample is low, the counting time can be extended to a maximum of 19 s. This guarantees the analysis of a statistically significant number of events. This is a major advantage in comparison to methods counting all cells from a single diluted aliquot, irrespective of whether the count is 500 or 5,000 or 50,000 leukocytes/ μ l. It is important for sample organization that the NRBC are counted in each differential blood count as these samples then have not to be picked out and remeasured if NRBC are present.

As a complement to the illustration of all leukocyte subpopulations in the 3D VCS plot, six additional quantitative parameters can be given for each cell type. The obtained morphometric data are quantification of cell size, internal cell structure and granularity. This is therefore very similar to the manual procedure of microscopic cell evaluation. The concept was taken over from traditional erythrocyte analysis. The MCV stands for mean erythrocyte volume, and the erythrocyte distribution width provides information on the homogeneity or heterogeneity of the erythrocyte population. The differential blood count analogously represents means for volume, conductivity and scattered light (mean V, mean C, mean S) and the corresponding distribution widths (SD V, SD C, SD S).

ADVIA® 2120

The new analytical system ADVIA 2120 (Siemens Medical Solutions Diagnostics GmbH, Fernwald, Germany) is based on developments by Technikon. These were subsequently converted from Bayer to the product line ADVIA and now belongs to Siemens Diagnostics.

The ADVIA 2120 is a fully automatic hematology system. The hemogram and the differential blood count as well as the reticulocyte diagnosis are performed automatically and fully selectively from EDTA whole blood. Studies on cells from cerebrospinal fluid or bone marrow are possible.

The novel UFC® (= unfluidics circuits or components) technology with an acryl block in the center is the actual 'heart' of the instrument (fig. 8). All cell preparations are processed and measured within the block.

The measurement technology for calculating the blood count parameters and performing the reticulocyte analysis are based on double angle scattered laser light measurement on isovolumetric spherically transformed erythrocytes and platelets or cell nuclei [55] and on the measurement of scattered light signals (volume) and absorption (peroxidase activity) of stained and unstained leukocytes [56]. Reticulocyte analysis is performed by measuring scattered laser light in two angle ranges in combination with absorption measurement. This allows both the separation of unstained erythrocytes from RNA-stained reticulocytes and the subclassification of different stages of maturation. The reticulocyte indices are measured (MCVr, CHCMr) or calculated (ChR) [57].

Erythrocyte Analysis: 'Differential Red Blood Cell Count'

Measurements of scattered laser light in two angle ranges on sphered individual erythrocytes can be used for the very precise determination of the erythrocyte parameters MCV, MCHC (mean cellular hemoglobin concentration), MCH (mean cellular hemoglobin), EVB (Erythrozytenverteilungsbreite; erythrocyte dissemination range), and HVB (Hämoglobinverteilungsbreite; hemoglobin dissemination range). Using the MIE theory [58], the volumes and hemoglobin concentrations can be calculated from the scattered light signals. Calibration is performed with defined droplet emulsions from highly purified alkanes (e.g. heptane, nonane and others). The refractive indices of these substances and the volumes of the droplets are very similar to those of erythrocytes [59]. Specific properties corresponding to morphological changes, such as anisocytosis or anisochromasia of the red cells and the thrombocrit as well as anisocytosis of the platelets, can be determined. There have hardly been any studies on the clinical relevance of the platelet changes.

In patients being treated with recombinant erythropoietin (rHu-EPO) for renal anemia, the percentage of hypochromic erythrocytes for current monitoring can guarantee reliable and timely recognition of functional iron deficiency, allowing early adequate iron supplementation [60, 61]. The erythrogram, also known as the Tic Tac Toe, the RBC matrix or the 'Red Diff', illustrates the volume distribution and hemoglobin distribution and depicts the quantification of erythrocyte morphology in each sample (fig. 9).

Y - Axis:
Low Angle - Signal
2 - 3 □
(correspond Volume)

X - Axis:
High Angle - Signal
5 - 15 □
(correspond Concentration of Hemoglobin)

Fig. 9. Transformation of signals to volumes and Hb concentrations.

Hemoglobin Measurement

The total hemoglobin concentration in a patient sample is measured at 546 nm with a modified ICSH method [56] with lysis followed by oxidation with KCN.

Platelet Analysis

Platelets are determined simultaneously to erythrocytes in the same measurement channel and under the same technical conditions. A novel two 2D platelet measurement method with different angles [62] enables separation of platelets exactly from cell fragments. Giant platelets and microcytes/fragmentocytes can be determined, and as few as only 2,000 cells/ μl of platelets can be measured. The upper limit of linearity is $4 \times 10^6/\mu\text{l}$, which is not reached [63]. Platelets with a volume >20 fl are explicitly flagged and are contained in the reported cell concentrations. Reticulated platelets can also be quantitated. The method is in preparation.

Reticulocyte Parameters: Reticulocyte Concentration, Subclassification and Indices

The following reticulocyte parameters are reported: relative (%) and absolute reticulocyte concentrations, subclassification into weakly absorbing (mature), intermediately absorbing (maturing) and strongly absorbing (immature) reticulocytes, and the reticulocyte indices MCVr, CHCMr and CHr. The reticulocyte indices MCVr and CHr are of increasing importance in diagnosis. The MCVr is taken as a very early predictor of engraftment after bone marrow transplantation [30] or peripheral stem cell transplantation. The CHr is a reliable and early parameter of iron deficient erythropoiesis during rHu-

EPO therapy and an early indicator of successful iron supplementation [64, 65].

Leukocyte Count and Differentiation

The analysis is performed using scattered halogen light and absorption from peroxidase-stained leukocytes. The leukocyte suspensions prepared in this way are measured in the flow cell after hydrodynamic focusing. The scattered light (volume) and the absorption (peroxidase activity) are measured for each cell. Cluster analysis allows five cell categories to be clearly separated: neutrophils, eosinophils, monocytes, lymphocytes including basophils, and LUC (large unstained cells). Basophils are explicitly determined in a second leukocyte measurement channel and added to the differential cell count. Double angle scattered laser light measurement on cell nuclei after treatment with phthalic acid is performed in a selective basophil/segmented nuclei channel. Cluster analysis of the chromatin density and the volume can then be performed, separating and quantitatively determining basophils, polymorphs and mononuclear cells. An automatic warning is displayed if immature forms occur, such as immature granulocytes or blasts [29].

Measurement of Cells in Other Body Fluids

The ADVIA 2120 can be used to determine cells in normal and pathological cerebrospinal fluid, using a specific measurement channel with scattered laser light and special reagents [66]. This provides quantitative numerical results for leukocytes, erythrocytes and leukocyte differentiation into cell categories, which are reported in cytograms and special cluster analyses.

It will soon be possible to perform measurements in bronchoalveolar lavages, punctates and ascites using specially developed software [29].

Automated Preparation of Blood Smears

Financial pressures are continuously increasing, as are the demands for quality. In this context, it is necessary to minimize the use of all manual methods. This also includes preparing smear samples from blood for microscopy. The ABX (Radeberg, Germany) was commissioned by Siemens Diagnostics to develop the ADVIA® Autoslide, an instrument for preparing blood smears under standardized conditions. There are many possible specifications for staining smear preparations, e.g. Giemsa or Pappenheim. The angle of application of the dye spatula at constant pressure of application is changed automatically in proportion to the hematocrit and the leukocyte count.

Coupling of Several Instruments to Give a WorkCell

Continuous progress in the concentration of laboratory work led to the development of WorkCells. In this case, an ADVIA 2120 and an automated smear instrument were coupled through a sample tube. After completion of the blood analysis in the hematology analyzer, the decision to prepare a smear preparation by the staining machine was made on the basis of a specific algorithm.

Logistics and Quality Management

Aside from the purely mechanical linkage, the WorkCell is logistically optimized with the intelligent software Hemalink® (ABX). Multiple hematological analytical systems, including microscopy, can be coupled to each other. Central validation, graphical presentation of the results, quality management over several workplaces, and the use of an extensive control system with supportive options for decisions on subsequent clinical procedures are available [67].

Sysmex X-Class Hematology Systems

For some years, the measurement technology of the systems of the Sysmex X-Class (Sysmex Europe GmbH, Norderstedt, Germany) has been based on fluorescence flow-through cytometry, optimized for specific requirements. Depending on the instrument specifications of the X-Class, this method is used for the analysis of leukocytes, reticulocytes or platelets and for the determination of the exact erythroblast count. The X-Class systems were first brought to market in 1999 with the introduction of the XE-2100. This was followed by

the XT-2000i, XT-1800i and the XE-2100D, the first 6-part differential instrument. This series was completed in 2006, with the XS-1000i and XS-800i, the smallest members. The XE-5000 was introduced to the German market in April 2007. In addition to all the methods for cell counting in blood, this system also offers the possibility of counting white blood cells (WBC) and RBC and differentiation of WBC in other body fluids.

Leukocytes

Leukocytes are measured in a so-called DIFF channel. The erythrocytes are lysed and the WBC are perforated in such a way that a specific nucleic acid stain can enter the cell. The fluorescence intensity (SFL) of the RNA and DNA in the nucleus and cytoplasmic cell organelles, together with an analysis of the lateral scattered light, enables differentiation between normal cell classes and immature granulocytes. Activated, antibody-forming B-lymphocytes contain high concentrations of ribosomal RNA and therefore form a separate lymphocyte population (high fluorescence lymphocyte count, HFLC). Quantitative determination of immature granulocytes and the proportion of HFLC lymphocytes can support diagnosis of inflammation and sepsis and provide valuable information on the state of the disease (fig. 10).

In another measurement channel, the IMI channel, complete lysis of the erythrocytes takes place. All 'mature' leukocytes are lysed with a reagent, which is specific for their high content of phospholipids. Immature myeloid precursors are measured with the help of the electronic measurement signals 'High Frequency' (HF) and 'Direct Current' (DC). This provides additional information about immature cells, which can provide decisive hints for sepsis diagnosis and for the optimal time point for apheresis in peripheral stem cell transplantation (fig. 11) [68–75].

Erythroblasts

Because of their nucleus, it is technically difficult to separate NRBC from leukocytes. The hematology machines of the XE series are the only analyzers providing a special channel for counting fluorescent labelled erythroblasts and thus are able to detect NRBC down to the very low level of 20 cells/μl. In this measurement procedure, the erythrocytes are lysed, the NRBC nuclei are exposed and the leukocyte membranes are perforated in such a way that a specific fluorescent dye can stain the cells. The leukocytes are clearly more intensively stained in the nucleus and cytoplasm than are the erythroblast nuclei. There are two clearly separated populations in the diagram in the presence of NRBC and the count is very specific and precise, even at very low concentrations [76].

Fig. 10. XE-IG Master WBC-Diff Screen.

Reticulocytes and Reticulocyte Hemoglobin Equivalent (RET-He)

The RNA content of reticulocytes decreases with increasing maturity. After perforation of the membrane, the nucleic acids are stained with a specific dye in the reticulocyte channel. On the basis of the increased fluorescence signal (SFL), the reticulocytes are distinguished from mature erythrocytes and classified into three maturity classes (LFR, MFR, HFR = low, medium and high fluorescence reticulocyte). The specific evaluation of the FSC of the reticulocyte population results in measurement of the RET-He, a cheap real time parameter to monitor iron supply for erythropoiesis [77].

Differential Determination of Mature and Immature Platelets by Fluorescence Staining

The RNA concentration in platelets decreases with maturity, and this can be quantitated by fluorescence measurements. This allows strict separation of erythrocytes at similar cell volumes. For the determination of platelets (PLT-O) by optical fluorescence, the fluorescence intensity (SFL) and the FSC signal are evaluated simultaneously. Microcytes and fragmented cells can thus be technically distinguished from large immature platelets (high RNA content). Using special software, the IPF Master (IPF = immature platelet fraction), the fraction of immature platelets can also be recorded. This greatly improves the diagnosis of thrombocytopenia (increased platelet consumption or reduced formation) [78–82].

WorkCell, Work Area Manager SIS, and Case Manager

The combination of the hematology analysis system XE 5000 with the smear and staining machine SP-1000 (Sysmex), com-

Fig. 11. Measuring immature leukocytes with HF and DC in the IMI channel.

bined with a belt for sample transport and an instrument for automated microscopy with digital image processing (CellaVision™ DM 96 oder DM 8; Lund, Sweden) is the current acme in the commercially available hematology systems. Budget cuts in the health service on the hand and coupled to the increasing number of analytical parameters available in the laboratory on the other demand new and intelligent solutions which focus on the relevant information. The X-Class systems can therefore be equipped with the SIS (Sysmex® Information System). As an intelligent control system and in accordance with the patient data, the Case Manager software filters suggestions from the enormous number of 76 parameters offered by the XE-5000. Thus, at any time of the day or night, all findings can be processed in a standard manner and validated. The therapists receive specific indications of criteria for differential diagnosis, including critical findings of the patient. The same is true for blood donors.

Conclusion

In transfusion medicine, automated hematology systems are nowadays indispensable for screening of blood donors and for quality control of blood products. Especially the fast and accurate determination of leukocytes, platelets and erythrocytes (including hemoglobin) is of high importance for the release of blood donations as well as for the quality control of blood components. In some cases, the extended functions of modern hematology systems – e.g. automated leukocyte differentiation or reticulocyte analysis – are helpful or even necessary for diagnostics in transfusion medicine. For example, the preliminary investigations of donors for stem cell collections by apheresis or of patients undergoing therapeutic aphereses require such modern automated hematological analysis.

The automation of hematological analytical instruments has reached a high level. Results on the composition of blood and its cellular components can be provided very rapidly and more extensively and with better accuracy and precision than ever before. The new generation of fully automated instruments support medical analysis by the integration of sophisticated services based on algorithms.

A scenario of this sort is however counteracted by an essential characteristic of human beings – our talent for improvisation which is and will remain indispensable for further development. One could mention a practical example. For an opera-

tion on a 300 g premature baby, it is essential to know the platelet count. The only available analytical material is a capillary with 20 µl whole blood. This problem may be solved by returning to platelet counting in the Neubauer chamber – a technique which has been regarded a long time to be apparently antiquated. Counting all squares in the middle chamber may lead to an acceptable reduction in the error, which is unacceptable under normal conditions. This underlines that only the interaction between the sophisticated instruments of medical engineering and our talent for improvisation give acceptable results in apparently hopeless situations.

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